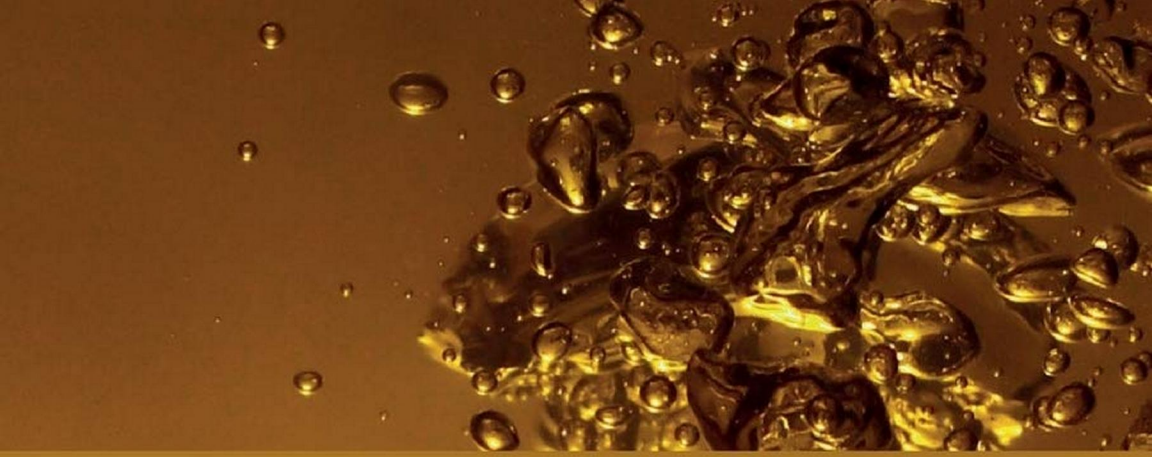

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LIQUID-PHASE EXTRACTION

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Liquid-Phase Extraction

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Liquid-Phase Extraction

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Chapter 1

Milestones in the Development of Liquid-Phase Extraction Techniques

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Chapter Outline

1.1 Introduction

Liquid-phase or solvent extraction is a venerable technique at least as old as recorded history [1]. It is generally employed as a sample preparation technique in which

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target compounds are transferred from one phase, the sample or sample-containing phase, to a liquid phase where further processing and/or analysis occurs [2]. For solvent extraction the receiving phase is a liquid, and the sample is either a gas, liquid, or solid material, which is at least partially soluble in the liquid phase. Typical samples are composed of target compounds of interest, or analytes, with the remainder of the sample referred to as the matrix for which detailed information is not required. The general purpose of solvent extraction, therefore, is the selective isolation of the target compounds from the sample with minimal matrix contamination. Solvent extraction is often employed as an initial step in sample preparation and, if required, is followed by additional sample cleanup procedures, including further solvent extraction steps (liquid-liquid partition) or complementary separation techniques.

The selective extraction of target compounds by contact with a solvent is due to the relative solubility of target compounds in the solvent compared with the matrix. For a liquid or solid, this process is generally referred to as trituration or leaching and for a gas as stripping. The isolation of the target compounds from their matrix requires a two (or more)-phase system and a mechanism for phase separation. This implies an additional restriction of low mutual solubility for the sample (or sample phase) and the extraction solvent. For solids a mechanical separation in which the solvent is displaced from the region of the sample matrix by decantation, filtration, centrifugation, or forced flow is typically used. For gases a common arrangement is to disperse the sample as bubbles in the extraction solvent that then migrate to the surface of the liquid and collapse having transferred soluble or reactive compounds to the extraction solvent. For liquid samples the processing steps involve active contact; agitation or dispersion of the sample and extraction phases; settling or condensation to recreate the two (or more)-phase system by gravity, centrifugation, or other means; and finally mechanical separation of the phase enriched in the target compounds from the phase (or phases) containing mainly matrix. For manual extraction the earlier processing steps typically require only simple apparatus available in most laboratories, while more sophisticated, specialized, and less common equipment is required for automation [3–5].

Liquid-liquid distribution is a common technique accompanying solvent extraction in which a dissolved substance is transferred from one liquid phase to another immiscible (or partially immiscible) liquid phase in contact with it. The driving force for the transfer is the difference in the solubility of the target compounds in each phase of the biphasic system. For compounds that exist in the same chemical form in both phases and have attained equilibrium in the biphasic system, the ratio of the compound in both phases is described by the partition constant. This can be formally defined as the ratio of the activity of species A in the extract $a_{A,1}$ to the activity in a second phase with which it is in equilibrium, $a_{A,2}$

$$K_D^{\circ}(A) = a_{A,1}/a_{A,2} \tag{1.1}$$

The value for K_D° depends on the choice of standard states, temperature, and pressure. Distribution isotherms are generally linear over a reasonable concentration range. This allows concentrations (mol/L) at low to moderate concentrations to be substituted for activities in Eq. (1.1) for the calculation of K_D°. Strictly speaking when concentrations are used in Eq. (1.1), the partition constant is referred to as the distribution constant K_D [2], but this distinction is rarely made in the literature. For compounds that can exist in more than one chemical form in at least one phase, the distribution ratio, D, is used in place of the distribution constant. It is defined as the total concentration of a compound in the extraction phase to its total concentration in the other phase, regardless of its chemical form. It is the appropriate form of the distribution constant when secondary chemical equilibriums in one or both phases exert partial control over the distribution process [6–9]. Common examples of secondary chemical equilibriums encountered in liquid-liquid distribution are ionization, ion-pair formation, chelate formation, micelle formation, and aggregation. The distribution ratio depends on the distribution constant for each equilibrium process and is thus influenced by a wider range of experimental conditions than for a single partition mechanism. The distribution ratio is also used in connection with continuous flow processes operating at a steady state and does not imply that the system has achieved equilibrium.

The fraction of a compound extracted, E, in a single-stage batch process depends on both the distribution constant and the phase ratio, V. The latter is defined as the ratio of the volume of extraction solvent, V_E, and sample solution, V_S, contained in the extraction device

$$E = K_D V/(1 + K_D V) \tag{1.2}$$

Extraction is favored by selecting conditions that result in a large value for K_D and a suitable phase ratio. Large values of the phase ratio ($V_E \gg V_S$) are favorable for the extraction of all compounds in a single-stage batch extraction but are rarely practical because the compounds are isolated in a too dilute solution. Typical experimental values for the phase ratio are closer to $V = 1$, and if K_D is sufficiently large, $V = 0.1$–1.0. For compounds with a moderate distribution constant, a more efficient use of extraction solvent compared with a single batch extraction is provided by multiple extractions. This utilizes a number of sequential extractions of the sample with fresh extraction solvent, typically with a fixed phase ratio in which case the fraction extracted is given by

$$E = 1 - (1/(1 + K_D V)^n \tag{1.3}$$

where n is the number of sequential extractions. When $K_D V = 10$, 99% of the compound is extracted with $n = 2$; when $K_D V = 1$, 99% of the compound is extracted with $n = 7$; and when $K_D V = 0.1$, 50% of the compound is extracted with $n = 7$.

Although large values of n favor exhaustive extraction, this approach is tedious, time-consuming, and labor-intensive for manual extraction. Automated batch methods, continuous flow methods, and countercurrent chromatography provide a more elegant option in this case [3, 4, 10]. Liquid-phase microextraction methods are characterized by an unfavorable phase ratio for exhaustive extraction ($V_S \gg V_E$). In this case the extraction conditions typically correspond to negligible depletion of the target compound concentration, and the extracted amount is independent of the sample volume [11, 12]. Calibration is required to relate the concentration of extracted target compounds to the sample concentration.

In a typical batch extraction process, equilibrium is not instantaneous. The extraction rate is controlled by the rate at which the compounds are transferred from the sample solution to the extraction phase [10–12]. For a sessile donor phase, mass transport occurs solely by diffusion and for an agitated donor phase by convection and diffusion. Diffusion is slow in liquids, and convection is a more efficient mechanism for mass transport. Extraction processes based on film formation are also more efficient due to the shorter distances for mass transport and the larger interfacial surface area. Mass transfer across the solvent interface in biphasic systems occurs only by partition except for surface-active compounds, which may adsorb at the liquid-liquid interface.

1.2 Techniques of Solvent Extraction

In this section, I describe some of the more important developments in the evolution of solvent extraction techniques at the laboratory scale and highlight some of the modern trends that continue this evolution today. Throughout analytical chemistry, measurement techniques are being repurposed to reduce sample requirements and reagent consumption, to increase automation, and to move from laboratory to point-of-collection analysis. These trends are often associated with lower costs and respond to the pressure to analyze an increasing number of samples to provide more detailed information for informed decisions. The environmental impact of analytic methods is a further consideration. These goals are being met by downsizing extraction processes and automating methods where possible. Obtaining a representative sample is the main constraint governing the scale of extraction techniques. Batch sample processing and continuous flow methods afford different routes to automation. The increasing improvement in sensitivity of analytic instruments has reduced sample size requirements for analysis. The fraction of an extracted sample utilized for the analysis provides an indication of further potential cost and time savings possible by downsizing the sample preparation procedure and may eliminate or simplify intermediate sample processing steps, such as solvent evaporation.

1.2.1 Liquid-liquid extraction

Classical liquid-liquid extraction has simple equipment requirements, mostly separation funnels, flasks, and vials. Sample volumes from the microliter to liter range are easily accommodated. Mixers, shakers, and centrifuges of different types facilitate agitation, settling, and phase separation. Multiwell plates and liquid handlers are useful for parallel sample processing for high-throughput sample processing [4, 5]. Exhaustive extractions require a large distribution constant or multiple extractions with a moderate distribution constant. For compounds with unfavorable distribution constants, further optimization of the extraction solvent is one route to success. Secondary chemical equilibriums can be exploited to obtain more favorable distribution constants for specific compounds. For weak acids and bases, manipulation of the sample pH can be used to suppress ionization [7]. For strong acids and bases, formation of ion-pair complexes is more useful [8]. For neutral compounds in general, adding a salt to an aqueous sample solution prior to extraction, termed salting out, can be effective for increasing the fraction extracted [6].

To assist in the determination of metal ions, the formation of solvent-extractable hydrophobic chelate complexes is a well-established approach. The use of organic reagents in chemical analysis began in the early 1800s but was generally focused on gravimetric methods and spot tests. Around the middle of the 1900s, chelating reagents for the extraction of metals were developed and significantly advanced the selective detection of low concentrations of metal ions [6, 13–15]. Since many of these metal-chelate complexes are colored and their development overlapped with the growing availability of spectrophotometric instruments in analytic laboratories, this synergy resulted in a peak for applications of the spectrophotometric analysis of metals as extractable chelates in the 1960s, which was followed by a steady decline as atomic spectrometric instruments became generally available. The extraction of metal chelates still remains relevant today for laboratories with limited capital resources and for specialized applications requiring matrix simplification or preconcentration for trace analysis by atomic spectrometric and other instrumental techniques.

1.2.1.1 Continuous Liquid-Liquid Extraction

Continuous liquid-liquid extraction is an option to enhance the fraction of target compounds extracted when the sample volume is large, the distribution constant is unfavorable, or the rate of extraction is slow. Numerous continuous extractors for lighter-than-water or heavier-than-water solvents have been described [16–20]. Fig. 1.1 is a representative example of an all-glass apparatus for continuous liquid-liquid extraction. Generally, either the lighter- or heavier-density organic solvent is vaporized, condensed, and allowed to percolate through the

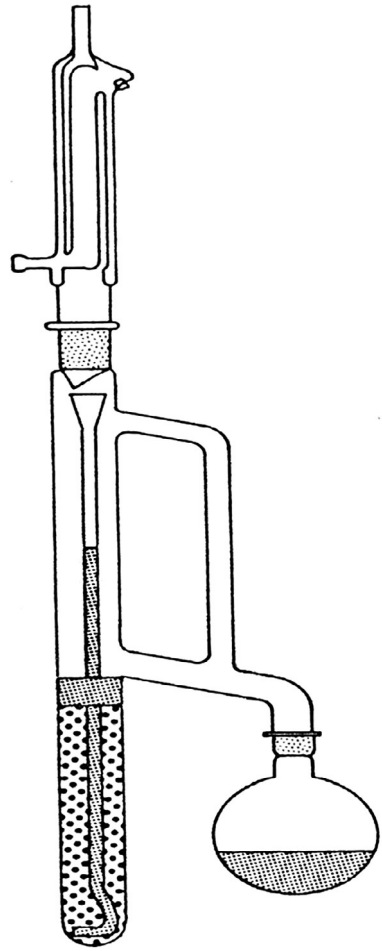

Fig. 1.1 All glass apparatus for continuous liquid-liquid extraction with an extraction solvent lighter than water.

aqueous sample for the required time. In this way, the exhaustive extraction of target compounds can be performed in a continuous and progressive manner with a fixed volume of organic solvent. Large-scale, variable sample-volume, and on-site extractors with different degrees of automation have also been described [21–23]. The efficiency of the extraction process depends on several variables: the viscosity of the phases, the magnitude of the distribution constant, the phase ratio, the interfacial surface area, and the migration velocity of the extraction phase moving through the sample solution.

1.2.1.2 Multistage Countercurrent Distribution

The countercurrent distribution apparatus is an example of a discontinuous, multistage extraction system based on liquid-liquid partition. Discontinuous processes are characterized by a number of separate equilibration steps followed by transfer of either or both liquid phases for further interaction with fresh liquid phase. Martin and Synge introduced automated machines for countercurrent distribution in the 1940s subsequently improved upon by Craig and others in the 1950s [24–26]. The popular all-glass machine developed by Craig and Post consists of a battery of units (or cells) mounted on a horizontal axis. Movement about the axis provided gentle mixing of the phases, placed the glass units in position for phase separation by gravity, and allowed decanting the upper (or lower) phase to complete a single operating cycle. The time for each operation within a cycle and the number of transfers were programmable. Apparatuses with up to 1000 cells were developed, although machines with 50–250 cells were more common.

The standard method of operation in a countercurrent distribution is referred to as the fundamental procedure. At the conclusion of the agitation and settling steps of a cycle, one phase (usually the upper) is transferred quantitatively to the next cell of the apparatus, where it is brought into contact with a fresh volume of the stationary phase. Simultaneously, a fresh portion of upper (or lower) phase is introduced into the first cell of the apparatus, and the extraction cycle repeated. The alternate equilibration and transfers with introduction of fresh mobile phase at each transfer are repeated until the initial portion of mobile phase has reached the last cell in the apparatus. The distribution is then complete and the cells emptied individually or grouped according to their content. If an appreciable section of the apparatus contains no sample components after completion of the fundamental procedure, a recycle option can be utilized to increase the resolving power of the apparatus. In this case the mobile phase from the terminal cell of the apparatus is reintroduced into the first cell, and the operating cycle continued. The process is stopped, in principle, when the leading boundary of the fastest-moving component is about to overtake the trailing boundary of the slowest component. At this stage, all the cells of the apparatus contain sample components, and the potential resolving power is fully utilized. The recycle mode can be combined with the single withdrawal procedure (elution countercurrent distribution) in which the resolved components are removed continuously in the mobile phase with fresh mobile phase added to replace the withdrawn phase. Variations of the single withdrawal procedure include the double or alternate withdrawal of the upper and lower phases and the continuous feed of sample solution to the first cell of the apparatus to maximize the sample loading. In the absence of changes to the distribution constant during the separation, simple theory allows prediction of the location of sample components (cells containing maximum concentration of target compounds) and their distribution over neighboring cells (peak width) [27–29].

The technical development of countercurrent distribution apparatuses had already ended by the 1980s, and most had been decommissioned by the 1990s. There are no reported applications today. There were a number of reasons for the demise of countercurrent distribution as a separation technique. Shortly after developing the countercurrent distribution apparatus, Martin and Synge introduced liquid-liquid chromatography, which had inherent advantages in speed and performance, and only required simple apparatus easily scaled to different sample sizes. This eventually led to the commercial development of high-performance liquid chromatography. Almost in parallel, Ito began the development of several smaller and more efficient devices for countercurrent chromatography in the 1970s and Murayama and Nunogaki centrifugal partition chromatography in the 1980s [30–33]. These devices differ from the countercurrent distribution apparatus in the use of hydrostatic forces created by planetary motion or spinning about a central axis to stabilize the liquid stationary phase, while the mobile phase is continuously pumped through it. Countercurrent chromatography is a continuous process and does not employ distinct separation stages. In addition, it was found that most of the applications traditionally performed by countercurrent distribution could be transferred to countercurrent chromatography without difficulty. Countercurrent chromatography remains an important option for preparative liquid-phase separations and extractions with equipment available from several manufacturers. Compared with column chromatography, it does not employ a solid stationary phase or support and has unique advantages whenever sorptive surface interactions render column separations impractical.

1.2.1.3 Liquid-Liquid Chromatography

The introduction of liquid-liquid chromatography using a physically adsorbed liquid on a porous support as a stationary phase by Martin and Synge in the early 1940s commenced a frenzy of activity in liquid chromatography culminating in the development of high-performance liquid chromatography in use today. The introduction of chemically bonded stationary phases in the 1960s and countercurrent chromatography a decade later signaled its rapid demise. Today, liquid-liquid chromatography is occasionally used as a method to determine liquid-liquid partition constants but has few other applications [34–36]. The overriding problem in liquid-liquid chromatography is erosion of the stationary phase by action of the mobile phase. This can be ameliorated to some extent using solvent generated liquid-liquid chromatography [34, 37]. In this approach, one of the phases from an equilibrated liquid-liquid biphasic system is applied as the mobile phase to a solid support, which is preferentially wetted by the other phase of the biphasic system. The support is usually silica when the stationary phase is aqueous or a polar organic solvent and a chemically bonded sorbent when the stationary phase is a

low-polarity solvent. The stationary phase solvent typically fills the pores of the sorbent. To adjust the phase ratio of the column, a sorbent with a different specific pore volume or surface area is required. Solvent-generated stationary phases allow more stable and reproducible systems to be prepared compared with conventional methods, for example, loading the column from a solvent in which the stationary phase is soluble followed by displacing the solvent and excess stationary phase from the column with mobile phase saturated with stationary phase. It is the slight mutual solubility of the two phases and its fluctuation with the column operating conditions that makes these systems unstable with reference to chemically bonded phases. A further inconvenience is that the sample solution must have a similar composition to the mobile phase, or otherwise the sample loading must be small, to minimize erosion of the stationary phase. The concept of liquid-liquid chromatography is continued today in matrix solid-phase dispersion [38, 39]. Here the sample is intimately mixed with an abrasive sorbent to form a free-flowing powder, loaded into a disposable column, and the target compounds recovered by solvent elution. Sample matrix is partially retained by the sorbent affording a sample suitable for direct analysis or after additional cleanup.

1.2.1.4 Steam Distillation-Solvent Extraction

Simple distillation and steam distillation are the two most common distillation techniques used to isolate volatile organic compounds [16]. Solvent extraction and gas-phase stripping techniques are generally inefficient methods for the isolation of polar semivolatile compounds from water due to the low extraction efficiency of water-immiscible solvents on the one hand and their slow mass transfer to the gas phase in stripping techniques on the other hand. Steam distillation in which live steam is blown continuously through the sample or by boiling water and the sample together is an attractive alternative. In either case, the volatile organic compounds are entrained and carried along with the steam at a rate proportional to their relative partial pressure at the temperature of the distillation. Low-molecular mass and reasonably volatile compounds can be efficiently isolated in a small volume of condensed steam. Since a number of compounds form low-boiling azeotropes with water, this helps to extend the mass range of the compounds that can be isolated by steam distillation. For large sample sizes, phase separation occurs concurrently with steam distillation after condensation, but for small sample sizes, solvent extraction is required for adequate recovery of the target compounds. The ability of the solvent to extract the target compounds from the condensed water, therefore, will also influence their recovery. A small-scale steam distillation continuous liquid-liquid extraction apparatus based on a macroscale version designed by Likens and Nickerson [40] is shown in Fig. 1.2 [41, 42]. The small-scale apparatus can handle 10–100 mL of aqueous solution or 1–20 g of solid

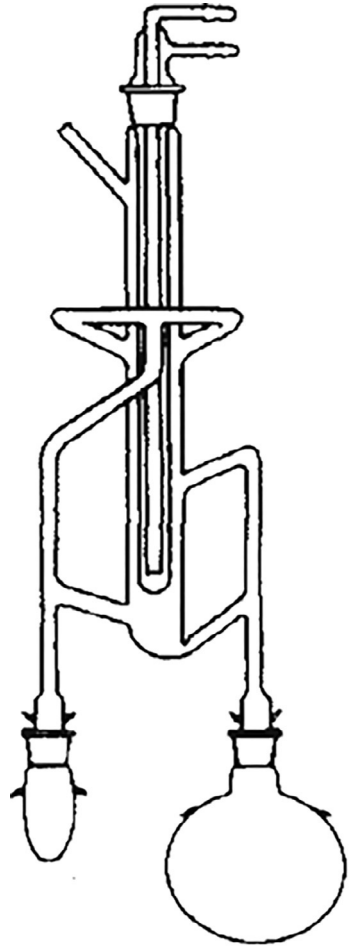

Fig. 1.2 Small-scale apparatus for continuous steam distillation-solvent extraction with a lighter-than-water extraction solvent (simply switching the position of the two flasks converts the apparatus to heavier-than-water extractor). *(Reproduced with permission from Jayatilaka A, Poole SK, Poole CF, Chichila TMP. Simultaneous micro steam distillation/solvent extraction for the selective isolation of semivolatile flavor compounds from cinnamon and their separation by series-coupled column gas chromatography. Anal Chim Acta 1995; 302:147–162.)*

material blended with water with extraction of the steam distilled compounds into 1 mL of organic solvent. The sample is placed in flask A and the extraction solvent into flask B. At the start of the extraction process, the phase separator C is charged with a mixture of water and extraction solvent; the organic solvent reflux is started; and shortly afterward, steam is generated by heating flask A. The steam and organic

solvent vapors are mixed and condensed in the upper part of the apparatus and return continually to the phase separator where they are recycled to the appropriate flasks. The concentration of extracted material continues to increase over time until a steady-state condition is reached for the target compounds after which the steam distillation is stopped and the apparatus allowed to cool. This technique is widely used for the extraction of semivolatile compounds such as essential oils and flavor and fragrance compounds from plant and food matrices. It is a selective isolation technique since recovery depends on the rate of distillation of the target compounds, their distribution constants for the solvent extraction stage, and the total time of the extraction process.

1.2.1.5 Solvent Sublation

Solvent sublation, a nonfoaming flotation technique, was introduced by Sebba in 1962 as an alternative technique for mineral floatation [43]. Flotation was already a well-established technique for extracting mineral particles by foam flotation but was considered to have limited possibilities for the isolation of organic compounds [44]. Solvent sublation is a bubble separation technique in which surface-active or surface-hydrophobic compounds are adsorbed on the bubble surface of an ascending gas stream and then collected in a layer of water-immiscible solvent placed on top of the aqueous sample solution. Solvent sublation requires only a simple apparatus consisting of a flotation column, a vertical glass tube with a sintered glass disk at the bottom below which a controlled flow of air or nitrogen is introduced. The sintered glass disk breaks up the gas flow into a stream of small bubbles that ascend through the aqueous sample solution collapsing at the water-solvent interface. Compounds adsorbed at the bubble surface are transported to the solvent layer and discharged at the water-solvent interface. This results in thin films of water being dragged into the solvent phase and then returned as water droplets to the sample solution. If the target compounds have a higher affinity for the extraction solvent compared with the sample solution, they will be concentrated in the extraction solvent, which typically is of a smaller volume than the sample solution. The rate of extraction is controlled by the bubble transport mechanism, the release of the transported compounds at the solvent-water interface, and the diffusional transport of compounds between the aqueous phase and extraction solvent driven by the concentration gradient. Efficient extraction by solvent sublation implies that the experimental conditions are optimized to promote mass transfer by bubble transport compared with diffusional transport [45]. It also implies that the adsorption of compounds at the bubble surface is favorable for transport to the solvent layer.

Solvent sublation techniques are generally used for the extraction of surface-active compounds. By utilizing complexing agents and carrier surfactants, its

extension to a wider range of sample types including metal ions is possible [45–47]. Aqueous two-phase flotation introduced in 2009 facilitates applications to compounds of higher water solubility of interest in biotechnology and for product isolation from fermentation broths and plant materials [48]. Flotation complexation extraction introduced around 2000 facilitates the extraction of polar compounds by a complexing or ion-pair reagent in the receiving phase for the efficient extraction of compounds of low solubility in water-immiscible organic solvents [49, 50]. At the water-solvent interface, collapse of the bubbles results in a rapid decrease of the surface area and transfer of the target compounds to the organic solvent. Normally, if the organic solvent provides a poor solvating environment for the target compounds, they are rapidly returned to the aqueous phase, and the extraction process is unfavorable for their isolation. In the presence of a reagent that forms a more soluble complex with the target compounds, their concentration continues to build up in the extraction solvent, and the rate of mass transfer is enhanced by bubble transport.

1.2.1.6 Salting-Out Assisted Liquid-Liquid Extraction

Salting-out assisted liquid-liquid extraction is a type of homogeneous liquid-liquid extraction based on the salting-out effect of a water-miscible organic solvent by the addition of substances capable of inducing phase separation from an aqueous solution [51–54]. It employs the spontaneous formation of a biphasic system and simultaneous extraction of target compounds into the separated organic solvent-rich phase. It was originally utilized in the early 1970s to enhance the selectivity of instrumental methods for metal analysis, but within a decade, it was being employed for the isolation of organic compounds and, more recently, has emerged as a popular option for automated high-throughput analysis of pharmaceutical compounds in biological fluids. Salting-out assisted liquid-liquid extraction is well suited to the analysis of polar compounds not efficiently extracted by conventional water-immiscible organic solvents but is not limited to polar compounds only. Typical organic solvents for salting-out assisted liquid-liquid extraction are acetonitrile, acetone, ethanol, isopropanol, dioxane, and tetrahydrofuran [51, 55–58]. Phase separation can be induced by a change in temperature, ion-pair formation, the addition of a third solvent, the addition of sucrose, or the addition of an inorganic salt. For practical applications, only salt addition and to a lesser extent sucrose addition are important. In general terms, salting out is the phenomenon observed when the solubility of a nonelectrolyte compound in water decreases with an increase in the concentration of a salt. The opposite phenomenon, salting in, is also observed in liquid-liquid extraction, but need not concern us here. The effectiveness of a salt at inducing phase separation is largely determined by the anion and tends to parallel the lyotropic (Hofmeister) series [51, 55, 59]. Small, multiply charged anions with a high charge density such as sulfate, phosphate, and carbonate

salts of magnesium, calcium, and potassium are typically used while many early reports employed sodium chloride, although this is not expected to be as efficient as the salts just mentioned. A comprehensive understanding of the salting-out effect has yet to emerge, but it is generally explained by a combination of electrostatic repulsion and enhancement of the hydrophobic effect. In selecting a particular salt for an application, the cardinal guiding principles are as follows: (1) the salt should have low solubility in the water-miscible organic solvent; (2) the salt should have high water solubility; and (3) the ability of the salt to precipitate hydrophobic substances should be high (based on the lyotropic series). The separated organic phase typically contains little water or salt, but if beneficial, the salt identity or concentration can be varied to optimize the composition of the organic solvent-rich phase. Salting-out assisted liquid-liquid extraction is the enabling technique incorporated in the QuEChERS method for the multiresidue analysis of pest control compounds in foods and environmental samples [60].

Since the solvents used for salting-out assisted liquid-liquid extractions are water-miscible and the target compounds are fully dissolved in the aqueous solution before salting out, no lengthy mechanical mixing is needed to promote efficient extraction. The high salt and organic solvent concentration typically employed effectively precipitate proteins in biological fluids prior to phase separation. Also less solvent is typically consumed compared with conventional liquid-liquid extractions, and tedious evaporation and reconstitution steps can be avoided simplifying the work flow. The organic solvent-rich phase is often relatively clean avoiding the need for subsequent sample cleanup with the majority of the salts, particle residues, and polar matrix components retained in the aqueous phase.

1.2.1.7 Aqueous Two-Phase Extraction

Aqueous two-phase extraction was developed in the 1950s by Albertsson and coworkers for the isolation of biopolymers, cells, microorganisms, and similar samples generally incompatible with extraction by organic solvents [61, 62]. Aqueous two-phase systems form when two, usually, water-soluble polymers or a water-soluble polymer and a salt at high ionic strength are mixed at a concentration (or temperature) above a critical value. As the polymers are mixed, they form large aggregates that induce phase separation into two aqueous phases with a different composition. The two layers are water-rich with different polymer or salt concentrations. The relative effectiveness of salts in promoting phase separation follow the Hofmeister series with alkali metal salts containing sulfate, phosphate, and citrate ions most effective [63]. Typical water-soluble polymers are poly(ethylene glycols), poly(vinyl alcohols), and dextrans of different mass ranges. Interfacial tension is extremely low in these systems affording a large interfacial contact area resulting in efficient mass transfer.

The basis of separation in aqueous two-phase extraction is the selective distribution of compounds between the two water-rich phases controlled by their interactions with the polymer and salt components of each phase. Generally, small molecules are somewhat evenly distributed between phases, macromolecules exhibit a wide range of distribution constants, and particles are typically diverted to one phase or the interface [64–66]. The distribution constants for macromolecules can be adjusted by altering the concentration and mass range of the polymers, the type of ions and their ionic strength, and pH for ionizable compounds. Denaturation or the loss of biological activity is not usually a problem. In recent years, the application range of aqueous two-phase extraction has been extended by substituting surfactant micelles [66] or ionic liquids [67] for conventional polymers as the phase-separating medium. This has facilitated applications to small molecules and macromolecules. For ionic liquids, it is necessary to distinguish water-soluble ionic liquids useful for aqueous two-phase extraction from water-immiscible ionic liquids suitable for traditional liquid-liquid extraction [68, 69]. Only ionic liquids miscible with water near room temperature can be considered for the formation of ionic liquid-based aqueous two-phase extraction since only these ionic liquids are capable of forming two-water rich phases.

1.2.1.8 Micelle-Mediated Extraction

At low concentrations of surfactants, the surfactant molecules tends to accumulate at the water-air interface causing a reduction in the interfacial tension but has little effect on the solubility of other compounds in the aqueous solution. As the surfactant concentration increases and a critical point surpassed, the critical micelle concentration, self-assembled aggregates (micelles or vesicles) form homogenously throughout the solution. These aggregates have favorable absorption properties for a wide range of compounds.

Micelles are dynamic structures composed of surfactant monomers with their hydrocarbon chains packed into a central core surrounded by the polar head groups. Compared with conventional solvents, they are spatially heterogeneous (the core region is hydrocarbon "like" and largely anhydrous, and the surface region is polar and solvated by water); on account of their small size and shape, they have a high surface-to-volume ratio (interfacial solvents); and their size, shape, and aggregation number depend on their immediate environment (ionic strength, ion type, pH, etc.) [70–73]. The spatial heterogeneity of micelles has fueled speculation that solutes of different polarity are localized in different regions of the micelle while other models suggest a more homogeneous environment for all compounds and that the solubility regime might be different for low sample concentrations typical of the extraction of dilute solutions and other applications, such as detergency, where larger concentration regimes are common. This aspect of the extraction property of micelles remains

unclear and generates conflicting arguments. Although the mechanism may be unclear, the fact that micelles have useful solvating properties that facilitate the extraction of a wide range of compounds is not in doubt.

For the purpose of extraction, micelle formation must be accompanied by a phase separation mechanism. This is achieved by the formation of a coacervative phase of relatively low volume with extracted compounds in equilibrium with a surfactant-poor phase of a larger volume [71–74]. Coacervates are water-immiscible, surfactant-rich liquid phases produced from colloidal solutions by the action of dehydrating agents. In the 1970s Watanabe and coworkers introduced cloud-point extraction in which the coacervate phase was separated from a micellar solution containing charge neutral (nonionic and zwitterionic) surfactants by a change in temperature to above the critical cloud-point temperature [75, 76]. Clouding is the result of the efficient dehydration of the hydrophilic portion of micelles occurring at elevated temperatures. Early work by Hinze and coworkers [77, 78] was instrumental in popularizing cloud point extraction, which remains the most widely used form of micelle-mediated extraction today. At a later time, coacervate extraction was expanded to include charged surfactants (anionic or cationic) with phase separation induced by a change in solution pH, the addition of an electrolyte, or the addition of a water-miscible organic solvent in which the micelles have low solubility. Typical applications of micelle-mediated extraction include the isolation of metal chelates with appropriate reagents for chelate formation [76, 79], metal nanoparticles [80], and organic environmental contaminants and biologically active compounds [72, 81]. Coacervate extracts are highly viscous and may require dilution for convenient handling. They are generally compatible with most instrumental techniques except for gas chromatography, where column contamination necessitates additional sample cleanup.

1.2.1.9 Liquid-Membrane Extraction

A liquid membrane consists of a supported or unsupported liquid phase that serves as a permeable conduit between the sample solution (donor phase) and extraction solvent (acceptor phase). Bulk liquid membranes are simply formed by interspersing a layer of immiscible solvent between the acceptor and donor phases [82]. They are characterized by slow mass transfer and long extraction times. More important today are supported liquid membranes introduced by Andunsson in the 1980s [83]. Typically, these consist of a microporous polymeric membrane in which the pores are impregnated with an organic solvent. The organic solvent is immobilized in the membrane pores by capillary forces. Typical porous membrane supports include poly(propylene), poly(tetrafluoroethylene), and poly(vinylidene difluoride). Common solvents for the extraction of aqueous samples include undecane, dihexyl ether, trioctylphosphate, and *n*-octanol. Low mutual solubility in the sample and acceptor

phase, moderate volatility, and low viscosity are the general properties required of the membrane liquid phase.

The membrane is typically formatted as a flat surface or hollow-fiber configuration for extraction and used in either the two- or three-phase mode. In the two-phase mode, the acceptor phase and the liquid phase for the membrane are generally the same. Contact between the liquid phases occurs through the membrane pores, and the chemistry of the extraction process is the same as for conventional liquid-liquid extraction. Efficient extraction requires a relatively large value for the distribution constant. For two-phase extraction, the technique is referred to as micromembrane liquid-liquid extraction allowing supported liquid membrane extraction to be generally used for three-phase systems [83–85]. Two-phase extraction techniques are easily interfaced with gas chromatography when the acceptor phase is an organic solvent [86].

In the three-phase technique, enrichment does not depend on the distribution constant directly. The liquid membrane typically contains an organic solvent that is different from both the sample solution and the receiving phase. The conditions for the donor phase are adjusted to favor transport through the liquid membrane and the conditions for the acceptor phase to minimize back extraction into the liquid membrane. To facilitate transport through the membrane pores filled with organic solvent, weak acids and bases are neutralized, metals are converted to neutral complexes, and ions are paired with oppositely charged species forming ion pairs. Reagents used for this purpose are also known as carrier molecules or ions and may be present in the liquid membrane solvent only, in the donor phase only, or in both [85, 87]. At the donor-membrane solvent interface, each species is converted to a form providing efficient extraction and transport through the liquid membrane. At the membrane-acceptor interface, a competitive reaction occurs that renders the species unfavorable for transport through the membrane back to the donor phase. The trapping mechanism employed to minimize back extraction of the target compounds is critical for the success of the extraction. For example, a weak acid in the donor phase might be neutralized by a change in pH and rendered more soluble in the membrane solvent and at the membrane solvent-acceptor interface rendered ionic by choosing a higher pH to minimize its back extraction. For metals, the formation of a neutral chelate to promote transport from the donor to acceptor phase and then formation of a more stable charged species in the acceptor phase to minimize back extraction are used [85, 88]. When practical a relatively small static volume of acceptor phase is utilized to enhance the concentration enrichment.

Automation of the extraction process is possible by pumping the donor phase over the surface of the liquid membrane with a stagnant acceptor phase [89]. Using a solvent segmented donor phase allows continuous renewal of the liquid membrane solvent resulting in improved system stability and allowing the use of more polar solvents for the membrane solvent [90].

1.2.1.10 Liquid-Phase Microextraction

At the turn of the century, interest in the further development of liquid-liquid extraction techniques was waning as it seemed to be incompatible with the new interest in green chemistry principles, further possibilities for automation seemed limited, and from effective competition in the form of solid-phase extraction techniques [91, 92]. This situation changed quickly, inducing a frenzy of research activity with the development of small-scale extraction techniques that addressed a litany of disadvantages and not just those related to scale. This research-driven area became known as liquid-phase microextraction beginning with single-drop microextraction in the middle 1990s [10, 12, 91]. In its simplest form the extraction solvent was suspended as a single drop from the tip of a microsyringe needle immersed in an agitated sample solution or suspended in the headspace above the surface of the sample. The high sample-volume-to-extraction-solvent-volume ratio favors high concentration enrichment factors but low absolute sample recovery. For similar reasons the extraction rate is generally slow and equilibrium rarely attained for typical sampling times. Small organic solvent drops at the tip of a needle or other support are somewhat unstable and may become dislodged during the extraction process. In an attempt to improve the robustness of the single-drop microextraction technique, hollow-fiber liquid-phase microextraction was proposed in 1999 [92–94]. This technique is a scaled-down version of liquid-membrane extraction (see Section 1.2.1.9). The acceptor phase is placed inside the lumen of a short length of a microporous polymeric hollow fiber with an internal diameter of about 0.5 mm. The membrane pores are impregnated with organic solvent and the fiber bundle either immersed in the stirred sample solution or suspended above it in the headspace. Either the two-phase (acceptor solvent and membrane solvent are the same) or three-phase (membrane solvent is different from the sample solution and acceptor phase) modes are used for sampling. Electromembrane extraction introduced by Pedersen-Bjergaard and Rasmussen in 2006 facilitated faster extraction and higher selectivity for ionizable compounds [94, 95]. In this technique a potential difference between the sample solution and acceptor phase is used to drive the ionized compounds through the liquid membrane. Solid-drop liquid-phase microextraction, introduced by Yamini and coworkers in 2007, provided an alternative approach to tackle the potential problem of the instability of suspended microdroplets in an agitated solution. In this case an appropriate small volume of an immiscible organic solvent is spread on the surface of a stirred aqueous sample solution followed by collection of the drop now formed in the vortex depression created by rapid stirring of the sample solution. The extraction solvent was collected by cooling the sample solution to solidify the drop and removing it with a microspatula [96]. The extraction solvent must have a melting point near room temperature to facilitate its recovery as a sold drop.

Of equal importance in the evolution of liquid-phase microextraction techniques was the development of dispersive liquid-liquid microextraction by Assadi and coworkers in 2006 [97]. This technique employs a ternary solvent system comprising a water-immiscible extraction solvent, a disperser solvent miscible with both water and the extraction solvent, and the aqueous sample solution [92, 97–99]. A few percent of the extraction solvent dissolved in a disperser solvent with a total volume of a few hundred microliters, typically, is rapidly injected into the aqueous sample solution forming an emulsion (a cloudy solution consisting of microdroplets of extraction solvent uniformly dispersed throughout the sample solution). Centrifugation is typically used to isolate the extraction solvent by sedimentation. If the extraction solvent is heavier than water, then it forms the lower layer after sedimentation and is easily removed by a microsyringe. Lighter-than-water extraction solvents require an extraction vessel shaped to facilitate isolation of the extraction solvent in a low-volume orifice. Formation of an emulsion creates favorable conditions for the extraction typically reducing the extraction time and enhancing preconcentration factors. The function of the disperser solvent is to promote droplet formation and to enlarge the contact surface area between the extraction solvent and sample solution.

Progress in liquid-phase microextraction has been so rapid that a large number of derivative methods have been developed from the three basic techniques described earlier. Some of these may have advantages for particular applications, while others claim to simplify sample handling or facilitate automation. These issues are not discussed here but are described in the complementary chapters in this book.

1.2.1.11 Segmented Continuous Flow Extraction

Early success in the automation of liquid-liquid extraction is represented by the air-bubble segmented continuous flow systems of the late 1950s eventually resulting in the introduction of commercial instruments, such as the Technicon AutoAnalyzer [100, 101]. Extraction was implemented as a module consisting of a coiled glass tube through which a concurrent flow of aqueous sample solution (or slugs of sample solution inserted in the aqueous phase) and organic solvent were combined in some cases with prior air-bubble segmentation [102]. These systems were quite complex and consumed a relatively large amount of solvent. They fell out of favor for extraction with the development of flow injection analysis in the late 1970s [103–105]. The sample as a continuous aqueous stream or well-defined volume in an aqueous carrier stream is directed toward a segmenter where it is brought into contact with the stream of organic solvent emerging from the segmenter as a single flow of alternate aqueous and organic solvent zones. These enter an extraction coil where extractable compounds are transferred to the organic solvent and then move to a phase separator, which recreates two separate continuous streams of organic solvent and aqueous solution. A typical manifold for two-phase liquid-liquid extraction is shown in

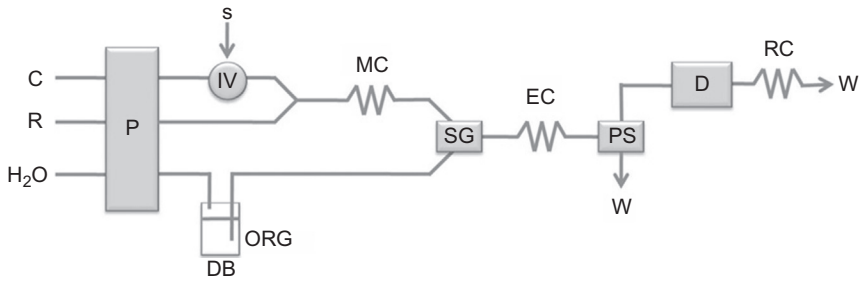

Fig. 1.3 A flow injection analysis manifold for liquid-liquid extraction. Abbreviations: *C*, carrier; *R*, reagent; *P*, propulsion unit; *S*, sample; *IV*, injection valve; *MC*, mixing coil; *DB*, displacement bottle; *ORG*, organic solvent; *SG*, segmenter; *EC*, extraction coil; *PS*, phase separator; *D*, detector; *RC*, restrictor; *W*, waste. *(Reproduced with permission from Silvestre CTC, Santos JLM, Lima JLFC, Zagatto EAG. Liquid-liquid extraction in flow analysis: a critical review. Anal Chim Acta 2009; 652: 54–69.)*

Fig. 1.3 [104]. The instrumentation for flow injection analysis is reasonably mature today with multiple options for core components and numerous manifold designs to accommodate different extraction principles [104, 106]. Current research is aimed at further miniaturization including the development of microfluidic, lab-on-a-chip, and lab-on-a-valve platforms [89, 107, 108].

In contrast to air-bubble segmented continuous flow systems, flow injection analysis involves the use of smaller-diameter tubing and does not employ air-bubble segmentation. Favorable mass transfer during extraction results from the presence of a wetting film at the tube wall. The nonwetting phase is a dispersed phase whose segments are completely surrounded by the wetting phase. The frictional drag at the wall and solvent-water interface of the dispersed phase results in convection forces that enhance mass transfer between phases. The rate of extraction depends on the tube and coil diameters, the phase ratio, segment length, and flow rate. Equilibrium is not necessarily achieved for a given combination of operating parameters, but the fraction extracted should be reproducible if the experimental conditions remain unchanged.

In 1970 Tanimura et al. [109] introduced an alternative droplet-based automated separation system based on liquid-liquid partition called droplet countercurrent chromatography. The droplet countercurrent apparatus consists of a collection of narrow-bore glass tubes held in a vertical position and connected head-to-toe by capillary tubing (Fig. 1.4) [110–112]. The inner surface of the tubes is treated to minimize wetting of the tube wall by the mobile phase to promote droplet formation before completely filling the tubes with the stationary phase. The mobile phase is then pumped at a steady rate into the apparatus where it enters the head or bottom of the first tube depending on its relative density. A steady stream of droplets about

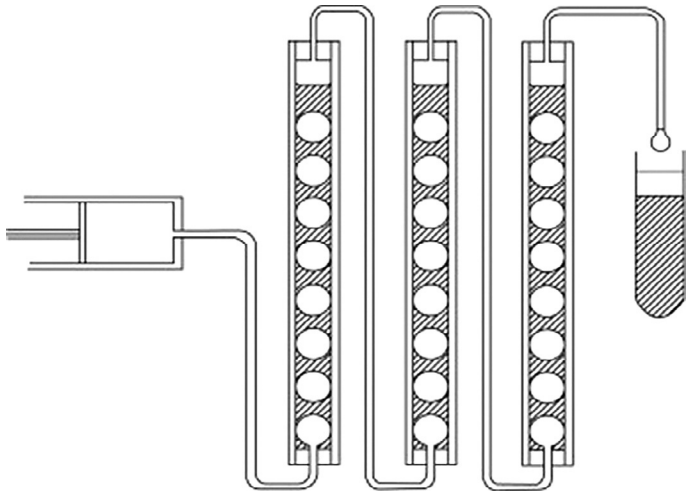

Fig. 1.4 Apparatus for droplet countercurrent chromatography in the ascending droplet mode (mobile phase of lower density than water).

as large as the tube diameter is formed and maintained by the continuous delivery of the mobile phase. The ascending or descending series of proper-sized droplets serves to divide the stationary phase into distinct segments minimizing mixing of the stationary and mobile phases along the length of the column. When a droplet reaches the end of the tube, it is delivered through the capillary connection to the next tube where droplets are reformed and so on, until all tubes have been traversed and the extracted components in the mobile phase are collected as individual fractions separated in time. The large number of droplets affords an efficient partition process, but the method is time-consuming and the conditions required for droplet formation restrict the choice of solvent systems. By the 1990s droplet countercurrent chromatography had fallen from favor and increasingly replaced by more compact and efficient countercurrent apparatuses employing hydrostatic forces to immobilize the stationary phase, while the mobile phase moved through it as a continuous stream. Different versions of this process are in use today known as high-speed countercurrent chromatography [30–33].

1.2.2 Gas-liquid extraction

Solvent-containing impingers and bubblers, Fig. 1.5, have been used extensively to isolate semivolatile organic compounds, reactive gases, and polar compounds not easily recovered from solid adsorbents from the gas phase [10, 113]. Typical applications are found in sampling atmospheric aerosols, stack gases, engine exhaust, and

Fig. 1.5 Solvent extraction of gases using a bubbler (*left*) or impinger (*right*).

personal and workplace air monitoring where they are sometimes used in combination with high-volume filters and denuders for removing particles [114, 115]. Impingers do not insure intimate mixing of the gas as it passes through the solvent but are suitable for extracting gases and vapors with a high affinity for the solvent or for compounds that react readily with a solution of a suitable reagent. Specific examples include the extraction of acidic gases by water, volatile amines and ammonia by acidified water [114], carbonyl compounds with an acidified aqueous solution of 2,4-dinitrophenylhydrazine [116, 117], and isocyanates by reactive trapping [115, 118]. Bubblers provide a more efficient mixing of the gas and solvent but are typically operated at lower gas flow rates. They are generally used to extract similar compounds as those identified for impingers. Outside the chemistry field, liquid-containing impingers have been used since the early 1900s for the efficient sampling of bioaerosols (bacteria, viruses, spores, pollen, etc.) [119, 120]. Liquid-containing impingers minimize dehydration of biological agents preserving their activity compared with solvent-free techniques.

Denuders were introduced in the 1950s of which there are numerous designs, the most common being annular and concentric tubular types. They exploit the significant difference in diffusion between gases and particles during passage through the device. The gas molecules diffuse to the wall and are trapped by adsorption or absorption at the coated wall surface, whereas particles pass through and are collected separately on a filter or by impaction. Gas diffusion scrubbers were introduced in the 1980s and contain a porous membrane permeable to gases and volatile

compounds that diffuse through the membrane and are trapped on the opposite side in the liquid phase [121, 122]. Denuders and gas diffusion scrubbers are smaller and more convenient for active sampling in field and personal air monitoring situations.

1.2.3 Solid-liquid extraction

Solids present a challenge for solvent extraction since the matrix may not be fully penetrated by the solvent, and the recovery of target compounds may depend on processes independent of the solvent. The rate and extent of extraction often depend on characteristics of the matrix, properties of the target compounds, and the distribution of target compounds within the matrix. The processes contributing to the transfer of the target compounds from a solid modeled as a porous particle coated with a layer of swollen organic material to the bulk extraction solvent are illustrated in Fig. 1.6 [11, 123–125]. Substances adsorbed at the outer surface of the particle are relatively easy to extract if the solvent competes effectively for adsorptive sites at the particle surface. The same compound residing within the organic layer of the matrix may be extracted slowly if the rate determining step is the diffusion of the compound to the particle surface in an interior pore followed by its mass transport by diffusion through the pore network of the particle to the region containing the extra particle extraction solvent. Modern methods of extraction attempt to effectively accelerate the extraction process by using higher temperature and/or pressure as described later.

Solid samples are usually processed initially to enhance the rate of extraction and to facilitate an exhaustive extraction [9, 126–129]. Typical procedures are drying, grinding, homogenization, and sieving. Air-drying is suitable for plant materials and freeze-drying for tissue and food commodities. Freeze-drying, the vacuum removal of solid water by sublimation, is a slow process but results in a sample that is easy to work with and is more convenient for temporary storage. Volatile compounds are lost, however, due to the long time the sample is held under vacuum. Additional drying can be achieved by adding or grinding the sample with a desiccant, such as sodium sulfate or diatomaceous earth, which also acts as a dispersion medium. Water-containing matrices may inhibit the extraction of target compounds by solvents of low polarity [127, 129]. Milling, chopping, and homogenization methods are used to reduce the particle size and to increase the surface area of the sample placed in contact with the extraction solvent. Samples containing a large amount of fats usually require solidification prior to grinding by adding dry ice or using a cryogenic mill. Sieving commonly follows grinding to obtain a sample with a uniform particle size range. Grinding decreases the diffusion distance for target compounds within the sample, and sieving unifies the average diffusion distance. Sample handling considerations dictate that practical particle sizes, typically around 150 μm (60–120 mesh), are larger than suggested by theory. In practice, the adopted size range depends mainly on the matrix type. Homogenization provides more

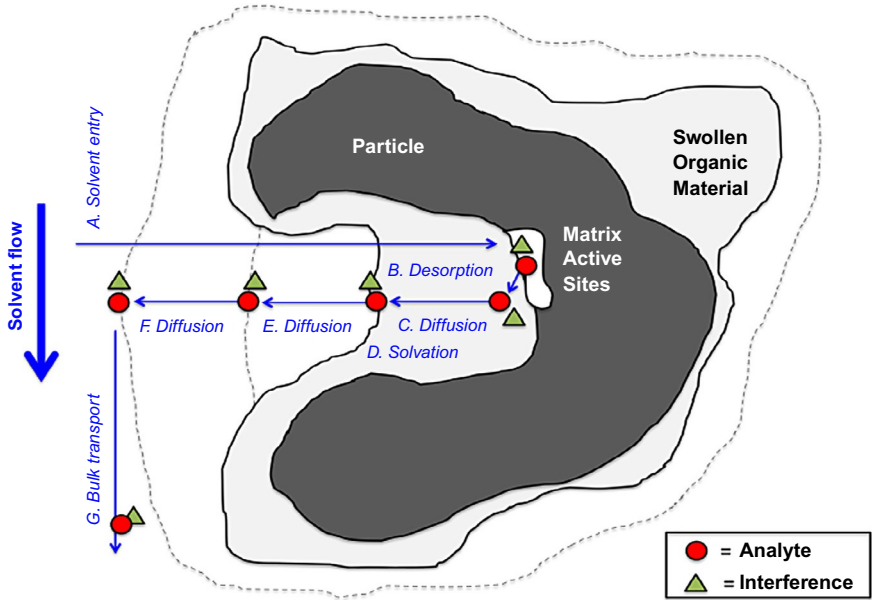

Fig. 1.6 Processes contributing to the extraction of target compounds modeled as a porous solid coated with a layer of swollen organic material. (A) infusion of extraction solvent into the particle pore network; (B) desorption from matrix active sites; (C) diffusion of target compounds through the layer of swollen organic material; (D) solvation of target compounds at the matrix-solvent interface; (E) diffusion through the static extraction solvent occupying a portion of the particle pore volume; (F) diffusion through the layer of stagnant extraction solvent outside the particle; and (G) transport through the interstitial space controlled by flowing extraction solvent. *(Reproduced with permission from Subedi B, Aguilar L, Robinson EM, Hageman KJ, Bjorklund E, Sheesley, RJ, Usenko S. Selective pressurized liquid extraction as a sample-preparation technique for persistent organic pollutants and contaminants of emerging concern. Trends Anal Chem 2015;68:119–132.)*

efficient contact between solvent and solid and promotes higher recovery of target compounds [129, 130]. Biological tissues of a small size are homogenized in a probe-like, high-speed homogenizer with the addition of water as required. Large-scale meat and vegetable samples are usually homogenized with a water-miscible organic solvent in a blender to simultaneously commute and extract the sample. Processing aids like dry ice or diatomaceous earth are used to homogenize awkward samples, such as high-fat samples.

Traditional methods of solid-liquid extraction provide poor sample utilization. All measurements require a representative sample, and for inhomogeneous samples, this has tended to favor sample sizes of 50–100 g, requiring 200 mL or more organic solvent for efficient extraction. At the other end of the sample preparation cascade,

modern instruments for the determination step typically utilize <100 μL of extract. This inevitably necessitates a tedious solvent reduction step that increases the costs of the analysis and stands in opposition to the principles of green chemistry [124, 125]. Similar considerations underpin developments in liquid-phase microextraction (Section 1.2.1.10). Modern methods of solvent extraction tend to favor smaller sample sizes, 1–10 g, and solvent volumes, 10–20 mL, but, in terms of balancing the requirements of obtaining a representative sample and extract utilization, are still relatively large.

1.2.3.1 Shake Flask Extraction

Shake-flask methods are suitable for target compounds of high solubility in the extraction solvent and for which matrix interactions with target compounds are relatively weak [128–130]. It will be beneficial if the sample is a porous solid or semi-solid. The solid and extraction solvent are placed in a suitable vessel and gently rocked from side to side or more vigorously agitated to promote mixing of the sample and solvent using automated laboratory devices for a prescribed time. At the end of the extraction time, the solid and liquid phases are separated by decantation, centrifugation, or filtration. Shake-flask methods are the least efficient of the liquid-solid batch extraction methods, and ultrasound- or microwave-assisted extraction approaches are selected for difficult-to-extract matrices

1.2.3.2 Soxhlet Extraction

The Soxhlet apparatus was first described in 1879 and remains the technique that more recent methods are compared with to demonstrate their suitability [127–134]. It is the technique specified for the extraction of solids in numerous regulatory and official methods of analysis. Soxhlet extraction works best for solids that can be commuted to a free-flowing powder, for target compounds of high solubility and matrices of low solubility in the extraction solvent, for target compounds with a low vapor pressure with respect to the boiling point of the extraction solvent, and for extraction solvents of low viscosity. The operating principle of the classical Soxhlet extractor can be illustrated with respect to Fig. 1.7. The extraction solvent or solvent mixture is vaporized, condensed, and allowed to percolate through the solid sample contained in an extraction thimble. The return of the solvent to the boiling flask is discontinuous, working on the siphon principle, and returns only when a certain volume of solvent has accumulated in the extraction chamber. Soxhlet extractors are available for milligram to kilogram sample sizes and for extractions either at room temperature or near the boiling point of the solvent. Typical conditions employ 10 g of sample, 50–200 mL of solvent, and a total extraction time of 1–6 h (or overnight) involving multiple extraction cycles. The main disadvantage apart from the long extraction time and large volume of extraction solvent is that the extracted

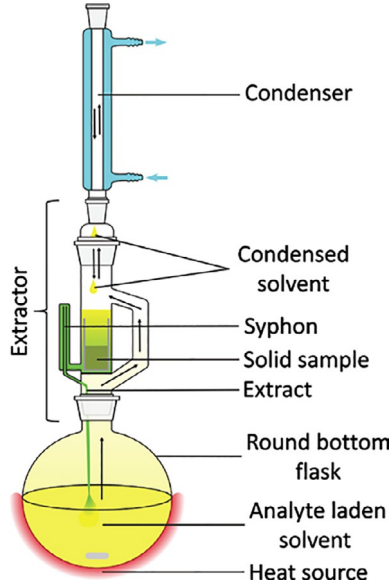

Fig. 1.7 Typical all-glass apparatus for laboratory-scale Soxhlet extraction.

compounds must be stable at the solvent boiling point, since eventually they are accumulated in the boiling flask. Conveniently, there is no need for a separation step, such as filtration, at the end of the extraction process.

Automated solvent extraction was developed to address some of the disadvantages of the classical Soxhlet apparatus [132, 134]. It recognizes the poor extraction kinetics at low temperatures by commencing the extraction process with the sample in its thimble suspended in the boiling solvent rather than in contact with the condensed solvent, as is normally the case. At a suitable time the extraction thimble is raised above the boiling solvent and rinsed with condensed solvent, in a similar manner to the conventional extraction process, except that a much shorter time is required. Finally the sample is concentrated by in situ distillation with collection of the sample solvent for reuse or disposal. The total time for extraction is reduced by a factor of 4–10, largely a result of the immersion step; solvent consumption is reduced perhaps by about half; and banks of extractors can be left to perform their duties unattended. Other advanced extractor designs include high-pressure, ultrasound-assisted, and microwave-assisted extractors [134].

1.2.3.3 Ultrasound-Assisted Extraction

Sonic treatment, either by immersion in a sonic water bath or by horn and probe devices, is commonly used to accelerate the extraction rate for coarse granular solids

and to disrupt the cellular structure of biological samples releasing the cell contents [130, 135–138]. Probe devices provide higher energy and better targeting of the sample, while sonic water baths allow several samples to be extracted in parallel. However, in the latter case, the extraction efficiency may depend on the location of the samples within the sonic water bath. The application of ultrasound waves to a liquid medium establishes a series of pressure waves that propagate through the liquid creating a cycle of bubble formation and collapse known as cavitation. The implosion of cavitation bubbles generates microturbulence with high-velocity interparticle collisions and results in the acceleration of mass transport within porous particles. The interaction of sample particles with collapsing bubbles causes particle size reduction and disruption of cellular material. Associated with bubble collapse are increases in local temperatures and pressures, which favor enhanced solubility and diffusivity and improved penetration and transport within particles, respectively. Since these are local effects, the slow increase in bulk temperature favors the use of ultrasound methods for the extraction of thermally labile compounds. The recovery of target compounds is frequently similar to Soxhlet extraction but depends on the sample type. Compared with Soxhlet extraction, it is faster, typically employs less solvent, and generally extracts fewer matrix interferences. It is often the method of choice for initial screening studies as a fast and uncomplicated sample processing technique. On the other hand, separation of the solid residue from the extraction solvent and rinsing steps are required at the end of the extraction adding to the sample processing time.

1.2.3.4 Microwave-Assisted Extraction

Microwave-assisted extraction was introduced in 1975 for the acid digestion of samples for metal analysis. This remains a major application of microwave technology. For the solvent extraction of organic compounds, applications started to appear in the 1980s using domestic microwave ovens and accelerated after 1986 with the introduction of the first purpose-designed instruments for microwave-assisted solvent extraction. Microwaves are nonionizing radiation that interacts with matter causing molecular motion such as the migration of ions and rotation of molecular dipoles. The absorption of microwave energy is roughly proportional to the relative permittivity of the sample or solvent and results in a rapid increase in temperature. There are two general approaches to microwave-assisted solvent extraction [127, 129, 133, 139, 140]. Target compounds contained in a microwave-absorbing sample surrounded by a solvent of low permittivity (low polarity solvent) can be extracted by expulsion from the hot sample into the (relatively) cold solvent. Alternatively, if the solvent is microwave-absorbing (extraction with a polar solvent), then heat is produced throughout the extraction system and flows from the solvent to the sample with contributions from the sample if it is also microwave-absorbing.

Two types of microwave-assisted extraction apparatus are commercially available: closed extraction vessels with controlled temperature and pressure capability and focused microwave ovens operated at atmospheric pressure. In a sealed extraction vessel, the internal temperature may be several times higher than the atmospheric pressure solvent boiling point. The method of heating is probably less important than the increased pressure that allows higher extraction temperatures compared with systems at atmospheric pressure. Under these conditions the surface tension and viscosity of the extraction solvent are reduced, favoring penetration of the matrix and enhancing mass transfer. The higher temperatures also increase the solubility of target compounds and weaken matrix interactions with the target compounds, allowing fast and more complete extractions with a smaller solvent volume. Compared with Soxhlet extraction, microwave-assisted solvent extraction requires minutes rather than hours and utilizes less solvent, perhaps by an order of magnitude. Microwave-assisted extraction is not appropriate for the extraction of temperature-labile compounds for which ultrasound-assisted extraction is usually a better choice. Viscous solvents may also exhibit lower extraction efficiency. Since it is necessary to wait while the vessels cool down to room temperature after the extraction and before proceeding with the analysis and the extract and sample have to be separated by decanting, centrifugation, or filtration, full automation is not provided. Initial capital costs are high compared with conventional extraction approaches, and although solvent consumption is significantly reduced, a solvent reduction step is still usually required.

1.2.3.5 Pressurized Liquid Extraction

Pressurized liquid extraction is based on the same principles as closed-vessel microwave-assisted solvent extraction with microwave energy replaced by a conventional heat source operating in a totally automated environment [125, 126, 129, 133, 140]. The first instruments for pressurized-liquid extraction were introduced by Dionex in 1995 under the trade name Accelerated Solvent Extraction (ASE). The apparatus is shown in Fig. 1.8 [140]. The solid sample is placed in a stainless steel vessel and brought to operating pressure (>100 bar) by pumping solvent into the vessel. The vessel is heated to a selected temperature (50–200°C) and maintained at the selected temperature for a selected time before relieving the pressure by allowing the solvent to escape to a collection vessel through a static valve. The remaining extract is flushed from the vessel by fresh solvent followed by a nitrogen gas purge. The duration of the static extraction time is important. A longer static extraction time favors solvent absorption by the matrix and increased penetration of solvent into sample interstices. Repeating the static extraction with fresh solvent in a cyclic fashion under full automation provides an option for difficult to extract samples. The final method is a balance between static time, the number of cycles, and an

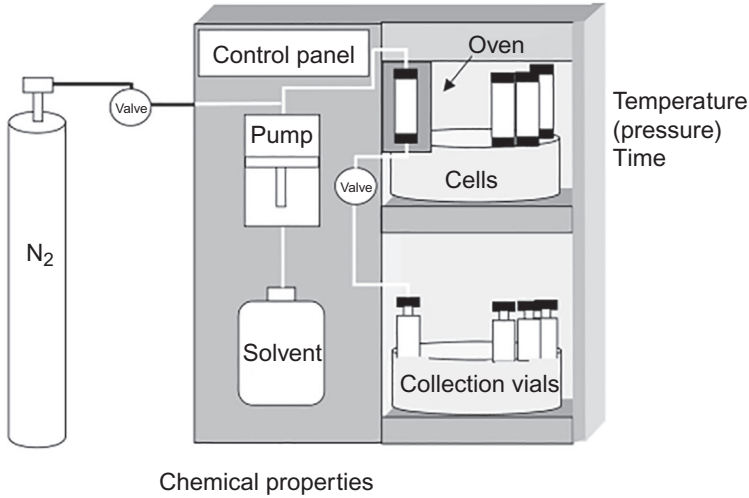

Fig. 1.8 Apparatus for pressurized-liquid extraction. *(Reproduced with permission from Camel V. Recent extraction techniques for solid matrices—supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction; their potential and pitfalls. Analyst 2001;126:1182–1193.)*

acceptable total extraction time. Solvent consumption is low (15 mL for a 10 g sample); extraction time is short (typically <20 min per sample). Extraction with extreme temperature conditions sometimes results in complex extracts unsuitable for final analysis without further cleanup. In-cell cleanup uses an extraction vessel packed with separate portions of sample and cleanup sorbent [141]. When water is the extraction solvent, the technique is referred to as pressurized hot water extraction [124, 129, 142]. At modest pressures and elevated temperatures below its critical temperature, liquid water acquires properties closer to those of typical organic solvents and is considered a suitable alternative to organic solvents for developing green extraction processes.

1.3 Solvent Reduction Methods

Traditional methods of solvent extraction result in the isolation of target compounds in dilute solutions that generally require preconcentration prior to analysis. Large volumes of solvent are typically evaporated by a rotary evaporator, Kuderna-Danish evaporative concentrator, automated simple distillation apparatus, or rotary or centrifugal concentrators [143–145]. Small solvent volumes can be handled by the gas blowdown method. Rotary evaporators are available in most laboratories and are convenient for solvent evaporation under reduced pressure. Volatile compounds

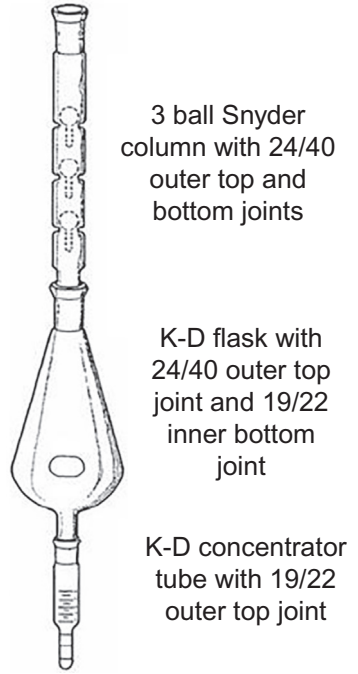

Fig. 1.9 Kuderna-Danish evaporative concentrator for solvent evaporation.

are lost by entrainment in the solvent vapors. Uncontrolled expulsion of extract from the flask due to uneven evaporation is one of the main reasons for preferring automated and specialized evaporative concentrators. The Kuderna-Danish evaporative concentrator, Fig. 1.9, is generally operated at atmospheric pressure under partial reflux conditions. Condensed vapors in the three-ball Snyder column are returned to the boiling flask, washing down organics from the sides of the glassware; the returning condensate also contacts the rising vapors and helps to recondense volatile organic compounds. Although the Kuderna-Danish concentrator provides a slower rate of evaporation than rotary evaporators, it generally provides higher recoveries of trace organic compounds [143, 146]. Samples are usually concentrated to 5–10 mL in the first stage and then transferred to a micro Kuderna-Danish evaporator or to a controlled-rate evaporative concentrator. The Kuderna-Danish technique is time-consuming and requires constant attention to monitor solvent evaporation in the first stage and to avoid evaporation to dryness in the second stage. The addition of a small volume of a low-volatility solvent, referred to as a keeper, can assist in stabilizing the recovery of target compounds [144]. Evaporative concentrators rely on the application of heat or vacuum for solvent evaporation and vortex or

centrifugal forces to maintain stable evaporation conditions. These instruments represent the state of the art for automated, controlled rate solvent evaporation.

The manual gas blowdown method is suitable for the evaporation of volatile solvents of <25 mL. A gentle stream of gas is passed over the surface of the extract contained in a conically tipped vessel or culture tube partially immersed in a water bath or heating block [143]. The rate of solvent evaporation is a function of the gas flow rate, the position of the gas inlet tube relative to the surface of the refluxing solvent, the water bath or heating block temperature, and the solvent surface area. At gas flow rates that are too high, target compounds may be lost by nebulization. High-purity nitrogen or helium gasses are typically used to minimize sample contamination. They can be further purified using chemical scrubbers and particle filters if contamination is suspected [147]. Automated systems working on the gas blowdown principle allow the evaporation of sample volumes up to 200 mL in parallel endpoint detection, temperature control, and different mechanisms to stabilize the evaporation process. Whether gas blowdown, vacuum, or heat-based automated solvent reduction devices are employed, they are likely to provide more consistent results than manual methods and increase laboratory productivity. Capital costs may appear high, but they represent value for money in a busy laboratory that handles many solvent extracted samples.

1.4 Applications of Classical Liquid-Liquid Extraction

1.4.1 Bioassay-directed screening techniques

For the initial screening of complex samples, fractionation into subgroups containing compounds with similar properties is often a good starting point when neither the composition nor target compounds have been identified. In a general screen, fractionation may be combined with bioassay, microbial toxicity, cell survival, or chemical group identification tests to isolate fractions for more detailed studies. A simple approach is the sequential extraction of an aqueous solution by water-immiscible organic solvents of increasing polarity [148]. In other cases an activity-directed test can be utilized to identify target fraction(s) for further chemical analysis in which the activity test is used to direct the sequence of sample preparation steps [149–151]. Fig. 1.10 is an example of a bioassay-directed fractionation scheme based on liquid-liquid extraction for the isolation of molting hormone from whole insects (desert locust) [152, 153]. Methanol was identified as a suitable solvent for the initial extraction of molting hormone from anaesthetized insects. Insoluble material was discarding and the methanol extract diluted with water and extracted with *n*-hexane to remove fats and pigments. Little activity was lost to the hexane phase as the molting hormone was a polar compound based on its high solubility in methanol.

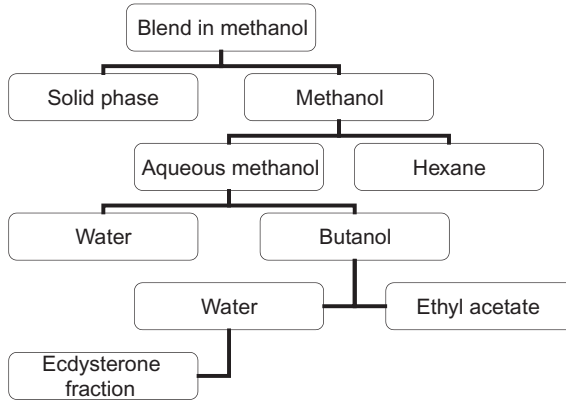

Fig. 1.10 Bioassay-directed liquid-liquid extraction of molting hormone ecdysterone (2β, 3β, 14α, 20, 22, and 25-hexahydroxy-5β-cholestan-7-en-6-one) from the desert locust *Schistocerca gregaria*. Methanol extract enrichment factor 3.45; methanol-water-hexane distribution constant is large and the enrichment factor 2.50; butane-water distribution constant 5.3 and enrichment factor 5.33; ethyl acetate-water distribution constant 3.20 and enrichment factor 3.00. Total enrichment factor for solvent extraction and liquid-liquid distribution cleanup was 138.

Extraction of the methanol-water phase by hexane reduced the quantity of biologically inactive material contained in the methanol extract. The methanol-water phase was concentrated and partitioned between *n*-butanol and water for the selective isolation of molting hormone from water-soluble coextractants. This was followed by a water-ethyl acetate partition to remove moderately polar coextractants. The transfer of molting hormone to the organic solvent phase (*n*-butanol) from water and then reextraction into water in the ethyl acetate-water partition system were important steps in minimizing matrix interference in the subsequent chemical identification of the molting hormone as ecdysterone (2β, 3β, 14α, 20, 22, or 25-hexahydroxy-5β-cholestan-7-en-6-one). The concentration of ecdysterone in the desert locust is in the ng/g range, but a sensitive bioassay provided adequate direction for the optimization of the extraction system. The figure legend indicates the enrichment factor obtained at each partitioning step and the partition constant for ecdysterone at each stage.

A general scheme for the fractionation of samples into groups with similar chemical identities is illustrated by Fig. 1.11 [154]. The sample is dissolved in a water-immiscible organic solvent, in this case methylene chloride, although other solvents can be used if considered advantageous based on sample solubility. By liquid-liquid partition with an aqueous solution of different pH, the sample is fractionated into neutral compounds, basic compounds, and acidic compounds. The acidic compounds can be further subdivided into strong and weak acids using aqueous sodium carbonate

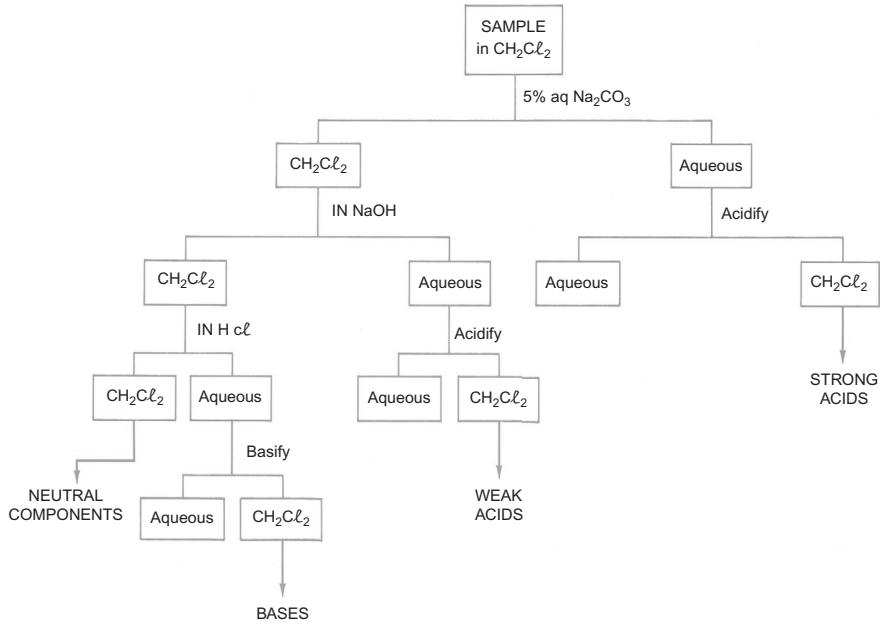

Fig. 1.11 General scheme for the fractionation of samples soluble in a water-immiscible organic solvent by liquid-liquid distribution. Aqueous solutions of different pH are used to isolate neutral, base, weak acid, and strong acid fractions.

and sodium hydroxide solutions. Ionizable compounds can be manipulated in this way since in the neutral form, they are soluble in organic solvents, while in the ionic form, they have low solubility. Neutral compounds are not affected by changes in pH, but further fractionation is possible using functional group selective reagents. Sodium bisulfate solutions can be used to isolate aldehydes and hydrazide reagents (Girard's reagent T) for the isolation of ketones. The aldehydes and ketones form water-soluble complexes extractable from the methylene chloride solution of neutral compounds separately from methylene chloride and subsequently back extracted into an organic solvent after hydrolysis of the complexes. The neutral fraction can also be separated into polar and nonpolar neutral compounds by distribution between dimethyl sulfoxide and pentane.

1.4.2 Extraction of drugs from biological fluids

Fig. 1.12 provides a general scheme for the isolation of pharmaceutical compounds from whole blood, urine, and feces [5, 7, 8, 155–157]. Basic drugs are typically extracted at pH > 7 from whole blood, while acidic drugs are usually extracted at pH < 5 from plasma or serum. Proteins are typically precipitated prior to

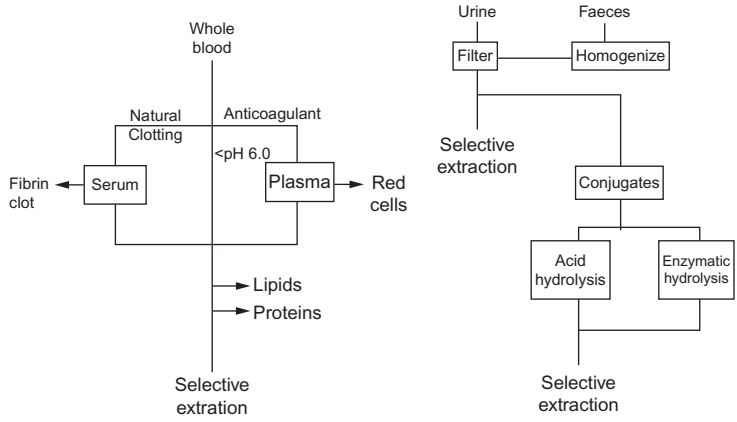

Fig. 1.12 General scheme for the isolation of drugs from biological fluids. *(Reproduced with permission from Poole SK, Dean TA, Oudsema JW, Poole CF. Sample preparation for chromatographic separations: an overview. Anal Chim Acta 1990;236:3–42.)*

liquid-liquid extraction by the addition of a polar organic solvent followed by centrifugation or by hydrolysis with a proteolytic enzyme. Lipids can be removed in a subsequent step by extraction with a nonpolar solvent. The lipid- and protein-free sample is then suitable for the selective isolation of drugs using appropriate water-miscible organic solvents with favorable distribution constants, changes in pH, ion-pair formation, etc. The protein and lipid concentration in urine is significantly lower than in plasma, and direct extraction of the filtered sample is possible for many drugs. Urine contains significant amounts of bound and conjugated drugs that are generally not extractable without prior acid or enzymatic hydrolysis.

1.4.3 Extraction of lipids from animal tissue

The Folch method [158, 159], a common modification described by Bligh and Dyer [160], is one of the most enduring solvent extraction techniques in use since the 1950s for the isolation of lipids from animal tissues and biological fluids. Lipids are a diverse group of biologically important compounds consisting mainly of low-polarity neutral compounds (acylglycerides, sterols, sterol esters, waxes, and hydrophobic pigments) involved in energy storage and more polar compounds (free fatty acids, phospholipids, and sphingolipids) utilized in the construction of cellular membranes. Lipids are also found bound to carbohydrates and proteins as glycolipids and lipoproteins, respectively, with multiple roles in cell development. The purpose of the Folch and Bligh and Dyer methods is the quantitative isolation of total lipids in a single fraction allowing the lipid content to be expressed on a mass basis [161, 162]

and, in more recent times, as a class fractionation technique for the comprehensive speciation of lipids in lipidomics [163, 164].

The Folch and Bligh and Dyer methods utilize binary mixtures of chloroform and methanol (in different ratios) for the extraction of lipids followed by the addition of water for formation of a two-phase system. The spontaneous dissociation into two phases results in the partitioning of polar biological molecules (e.g., proteins and carbohydrates) and salts into the water-rich phase and neutral compounds of lower polarity, including most of the lipids, into the organic solvent-rich phase. The critical feature for the success of this method is the partial miscibility of the chloroform in the water-rich phase and water in the organic solvent-rich phase. This ensures all biomolecules are solubilized in one of the phases with only insoluble particles of cellular debris remaining. A number of solvents have been proposed to replace chloroform by more environmentally friendly solvents with limited success [165]. A possible exception is the Matyash method that uses a ternary solvent mixture of methanol-methyl *tert*-butyl ether-water [166]. The Folch, Bligh and Dyer, and Matyash methods generally exhibit only small differences for the extraction of major lipid classes but more obvious differences in the extraction of low-abundance lipids. This provides the rationale for preferring one method over another for specific applications or alteration of the relative solvent composition of the classic methods to achieve sample-specific goals.

The Folch method employs a ternary solvent system of chloroform-methanol-water 8:4:3 (v/v) in which the approximate composition of the water-rich layer is 3:48:47 chloroform-methanol-water (v/v) and the chloroform-rich layer 86:14:1 (v/v) chloroform-methanol-water [167]. Cavity formation in the water-rich layer is less favorable than for the chloroform-rich layer resulting in the concentration of low-polarity compounds in the chloroform-rich layer. Polar interactions of a dipole-type and hydrogen-bonding interactions typical of water-soluble biomolecules and ionic interactions favor distribution to the water-rich layer. The earlier features and favorable solubility for lipids go a long way to explaining why the venerable Folch partitioning method has remained a valuable frontline method for the isolation of lipids. A comparison with the chloroform-water system indicates that methanol plays an important role in the distribution mechanism. The Folch ternary solvent system has distribution properties unlike those established for a large database of (>60 aqueous and totally organic) biphasic solvent systems.

1.4.4 Extraction of pesticides from agricultural commodities

Over 1000 compounds are registered for pest control purposes with the potential to contaminate agricultural commodities and processed food [168, 169]. For many of these pesticides and their common metabolites, maximum residue limits have been

established in several countries and require the development of effective analytical methods to establish compliance with local regulations. For such a large chemical inventory, single-residue methods are not practical for surveillance purposes, and this has accelerated the development of multiresidue methods capable of detecting large numbers of pest control compounds spanning different chemical classes from a single sample [168–170]. An early example of a multiresidue regulatory method was the Mills method developed in the 1960s when the dominant class of pesticides was relatively low-polarity compounds, such as organochlorine pesticides [171]. Pesticides from low-fat commodities were isolated by blending samples with acetonitrile for high-moisture commodities and acetonitrile-water for dry commodities; the acetonitrile extract was diluted with water, if required, and the low-polarity pesticides reextracted into a hydrocarbon solvent (petroleum ether). The recovery of polar pesticides was generally poor by this method. In the 1970s new methods were developed to extend the polarity range of covered pesticides to include organophosphorous and organonitrogen compounds in which samples were typically homogenized with acetone for the initial extraction. In what became known as the Luke method, the acetone extract was diluted with water and reextracted with a moderately polar organic solvent, such as methylene chloride or mixtures of petroleum ether and methylene chloride [172]. To promote phase separation, sodium chloride was added to the water-containing phase during the liquid-liquid distribution step. In the 1980s environmental and health concerns had come to the fore, and the use of large volumes of chlorinated solvents was considered undesirable. Two general approaches emerged based on either the selection of alternative solvents for the liquid-liquid distribution or solid-phase extraction cleanup, for example, in what became known as the Luke II method [173, 174]. For liquid-liquid distribution the methylene chloride solvent was generally replaced by ethyl acetate or mixtures of ethyl acetate and a hydrocarbon solvent [175–177]. Some polar pesticides have limited solubility in ethyl acetate, and coextracted matrix components could be a problem complicating cleanup procedures. The late 1980s saw the emergence of salting-out methods for phase separation of samples extracted with acetone or acetonitrile, although work with organic solvents of low water miscibility continued [178–180]. Acetone was simply too miscible with water for effective phase separation using the salting-out approach in the absence of the addition of a low-polarity solvent, while salts such as magnesium sulfate and sodium sulfate and sugars were shown to be effective for acetonitrile-water phase separation. In 2003 the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was introduced with a view to streamlining the processes based on the salting-out technique and quickly became the general method of choice in pesticide residue laboratories [59, 169, 170, 181–183]. This approach is based on a small-scale extraction using acetonitrile, salting-out and dehydrating of the acetonitrile phase by magnesium sulfate and sodium chloride, and cleanup of the acetonitrile extract by dispersive solid-phase extraction.

Modern multiresidue methods typically use mass spectrometric detection compared with element-selective or functional group-selective detectors that dominated the determination step until the turn of the century. Another change is that today, about an equal number of pesticides can be determined by gas or liquid chromatography, while in earlier days, gas chromatography was used almost exclusively in pesticide residue methods. These changes are reflected in contemporary extraction methods that need to address problems associated with the coextracted matrix, such as ion suppression/enhancement in liquid chromatography-mass spectrometry [169, 170] and matrix-induced response enhancement in gas chromatography [184]. Pesticide residue methods have been streamlined for speed, parallel sample processing, lower costs, and reduced solvent use, conditions that favor higher sample throughput and lower per-sample costs desirable for screening programs.

1.5 Conclusions

Notwithstanding more than a century of application and development, liquid-phase extraction techniques remain relevant today. Its demise has been reported many times in the past as competition arose from complementary techniques. Its inherent simplicity and capability to adapt extraction processes to emerging trends, however, have maintained its position in the front line of extraction methods. The focus on miniaturization of sample processing techniques is a current trend compatible with the basic principles of liquid-liquid extraction and occupies those focused on research in sample preparation at present. In this chapter, I have focused on events in the past leading to the present-day situation, while other chapters in this book will look to the contemporary situation and predictable future trends in liquid-phase extraction techniques.

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Chapter 2

Solvent Selection for Liquid-Phase Extraction

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Chapter Outline

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2.1 Introduction

Liquid-phase extraction employs solvents for the purpose of isolating target compounds by their favorable solubility in the extraction solvent relative to the sample matrix and to provide transport to relocate the target compounds to a region separated from the matrix. When the sample matrix is itself a liquid or a liquid-soluble solid or gas, liquid-liquid distribution in which a solution of the sample and the extraction solvent are of low mutual solubility can be used to isolate target compounds from other compounds or the sample matrix by the higher affinity of the target compounds for one solvent compared with the other. Given that the universe consists only of matter in the form of gas, liquid, or solid, the number of potential liquids that might be used for extraction purpose is enormous. Consequently, it is necessary to devise guidelines to reduce the potentially overwhelming number of liquids that could be utilized for extraction to a manageable number of preferred liquids with a wide range of extraction properties to facilitate method development for different target compounds hosted in different sample matrices. The purpose of the studies described in this chapter is to provide some guidance on this issue.

2.2 Physical Properties of Common Solvents

The liquid range for a solvent is defined by its melting point and normal boiling point as a temperature range. This range must incorporate the temperature used for the extraction. Although according to this description most compounds can behave as solvents, it is only those that are liquid at room temperature that tend to be classed as such. Additional favorable properties include a high flash point, high vapor pressure, and low toxicity for safety reasons; chemical stability both with respect to shelf life and reactivity with target compound functional groups; low corrosivity; and good thermal stability if the solvent is to be recycled by distillation [1, 2]. Real solvents generally differ from an ideal solvent due to the need to accept compromises among the desirable properties. For example, a high vapor pressure reduces environmental and safety concerns but is unfavorable if the solvent is to be recycled by distillation. A low vapor pressure may be undesirable for microextraction techniques due to volume changes caused by evaporative losses but less of a concern for typical laboratory-scale extraction methods.

Some common solvents used for laboratory-scale extraction and their physical properties are summarized in Table 2.1 [3–6]. For two-phase systems a density difference is required for efficient demixing after agitation. If the density of the two phases is similar, then phase separation is unlikely, or centrifugation will be required. Solvents of low viscosity provide more favorable mass transfer properties and more

TABLE 2.1 Physicochemical Properties of Solvents Commonly Used for Extraction at Atmospheric Pressure and 20°C

Solvent	Boiling Point (°C)	Density (g/mL)	Viscosity (cP)	Surface Tension (mN/m)	Refractive Index	Dielectric Constant
Acetic acid	117.9	1.0492	1.139(25)	27.40	1.3716	6.15
Acetone	56.29	0.7900	0.36	23.32	1.3586	20.89
Acetonitrile	81.60	0.7822	0.35	19.10	1.3441	37.5
Benzene	80.1	0.8765	2.284	28.80	1.5011	2.282
n-Butyl acetate	126.11	0.8796	0.74	25.09	1.3942	5.01
n-Butan-1-ol	117.5	0.8097	2.98	24.57	1.3993	17.51
Butan-2-ol	107.7	0.8016	4.21(15)	22.98	1.3959	16.68
n-Butyl chloride	78.44	0.8862	0.45	23.75	1.4021	7.39
Carbon tetrachloride	76.72	1.59	0.969	27.00	1.4598	2.238
Chlorobenzene	131.69	1.1058	0.80	33.28	1.5248	5.62
Chloroform	61.15	1.4892	0.57	27.16	1.4458	4.81
Cyclohexane	80.72	0.7785	1.0	24.98	1.4262	2.02
o-Dichlorobenzene	180.48	1.3058	1.32	26.84	1.5514	9.93
1,2-Dichloroethane	83.35	1.253	0.79	32.23	1.4448	10.36
Dichloromethane	39.75	1.326	0.44	28.12	1.4241	8.93
Diethyl ether	34.6	0.7134	0.245	17.06	1.353	4.20
Dimethyl acetamide	166.1	0.9415	2.14	32.43	1.4384	37.78

Continued

TABLE 2.1 Physicochemical Properties of Solvents Commonly Used for Extraction at Atmospheric Pressure and 20°C—cont'd

Solvent	Boiling Point (°C)	Density (g/mL)	Viscosity (cP)	Surface Tension (mN/m)	Refractive Index	Dielectric Constant
N,N-Dimethylformamide	153.0	0.9487	0.92	36.76	1.4305	38.25
Dimethyl sulfoxide	189.0	1.1004	2.24	43.54	1.4793	46.68
1,4-Dioxane	101.32	1.0336	1.37	34.45	1.4224	2.25
Ethanol	78.3	0.7893	1.200	22.39	1.3614	24.6
Ethyl acetate	77.11	0.9006	0.45	23.75	1.3724	6.02
Ethylene dichloride	83.48	1.253	0.79	32.23	1.4448	10.6
Ethylene glycol	198.93	1.1088	19.9	48.90	1.4318	37.0
Formamide	111	1.1334	3.75	59.10	1.4472	109
Heptane	98.43	0.6837	0.42	20.30	1.3876	1.92
Hexane	68.7	0.6594	0.31	17.91	1.3749	1.88
Isopentyl ether	83.8	0.8	1.01		1.408	2.08
Methanol	64.7	0.7913	0.59	22.55	1.3284	32.70
2-Methoxyethanol	124.6	0.9646	1.72	31.8	1.4021	16.93
Methyl *t*-butyl ether	55.2	0.7405	0.27	19.4	1.3689	2.6
Methyl ethyl ketone	79.64	0.8049	0.43	24.0(25)	1.3788	18.51
Methyl isobutyl ketone	117.4	0.8008	0.58	23.64	1.3957	13.11
Octan-1-ol	194.4	0.8270	7.36	25.24	1.4295	10.3

Propan-1-ol	97.20	0.8037	2.3	23.70	1.3856	20.33(25)
Propan-2-ol	82.26	0.7854	2.4	21.79	1.3772	19.92
Propylene carbonate	241.7	1.2006	2.50	40.9	1.4210	64.9
Pyridine	115.25	0.9832	0.95	36.88	1.5102	12.4
Tetrahydrofuran	66	0.888	0.55	27.31	1.4072	7.58
Toluene	110.62	0.8669	0.59	28.53	1.4969	2.38(25)
Triethylamine	89.5	0.7276	0.363	20.66(25)	1.4010	2.40
Trifluoroacetic acid	71.8	1.5351	0.93	13.63(24)	1.285	39.5
2,2,2-Trifluoroethanol	172.4	1.384	1.74	21.11	1.291	8.55
2,2,4-Trimethylpentane	99.24	0.6919	0.50	18.77	1.3914	1.94
Water	100.0	0.9982	1.00	72.8	1.3330	80.1
o-Xylene	144.41	0.8802	0.81	30.03	1.5054	2.57

readily penetrate the pore structure of solid samples. Capillary forces at the entrance of micro- and mesopores resist solvent penetration requiring either external pressure or higher temperatures to overcome these forces. Low interfacial tension facilitates mass transfer across the phase boundary, and for favorable extraction kinetics the solvent should possess good wetting characteristics for solid and semisolid samples. A low interfacial tension facilitates the disruption of solvent droplets with low agitation and is also associated with emulsion formation in two-phase liquid systems. This can be counteracted by selecting solvents of low viscosity with a high density difference.

2.2.1 Impurities

Chemical compounds, including those that are liquid at room temperature and potentially useful as solvents, are sold based on a specification including an indication of purity. For research-grade chemicals, this is typically <98% (w/w) and for high-purity solvents identified for specific applications <99.9% (w/w). Common impurities are starting materials carried over from the manufacturing process, by-products of the reaction used for the synthesis, and contaminants acquired from packaging and acquired during use. Likely, impurities are different for each solvent type, while contaminants may be more commonly distributed across all solvent classes due to their ubiquitous presence in the laboratory environment or common use in packaging materials [3, 6, 7]. Laboratory grade solvents are typically purified by filtration, distillation, and/or adsorption chromatography, which may not be effective for impurities with similar physical and chemical properties to the solvent. Most solvents contain traces of water that can only be minimized further by adsorption or chemical means [6]. Solvents may also contain small amounts of compounds intentionally added to a solvent to extend its shelf life or stabilizers to inhibit autodecomposition reactions and in some cases intentional contamination, such as the addition of a denaturant to ethanol, to comply with country-specific laws and regulations. Chloroform may contain amylene and 1% (v/v) ethanol to minimize the formation of phosgene and hydrogen chloride, and dichloromethane may contain amylene or cyclohexene to minimize formation of hydrogen chloride. Ethers, such as 1,4-dioxane and tetrahydrofuran, may contain 2,6-di-*t*-butyl-4-methoxyphenol to minimize peroxide formation. Diethyl ether may contain 2% (v/v) ethanol for the same purpose. Solvent purity is typically assayed by evaporation (residue limit), refractive index and spectroscopic measurements for reagent grade chemicals, and gas and liquid chromatography for high-purity solvents [3, 6–12]. Reference spectra for most common solvents and their impurities can generally be found in searchable mass spectral databases [7]. Mass spectrometry is the main resource for identifying typical organic impurities. Optical emission spectroscopy is commonly used for metal identification and ion chromatography for anion identification. For minor components and additives, nuclear magnetic resonance spectroscopy can be useful.

2.2.2 Solvents forming biphasic systems with water

Water holds a unique position in liquid-liquid extraction as the most frequently used solvent for forming two-phase systems [13]. This is understandable as water is a ubiquitous component of biological and environmental samples favoring the selection of water as a general solvent for handling these sample types. Water is the most cohesive of common laboratory solvents favoring the formation of two-phase systems with a wide range of low-polarity organic solvents. Any organic solvent capable of competing with water for polar interactions is generally miscible with water forming a homogeneous solution. Solvents of higher mass are generally less soluble in water, and two-phase systems can be formed if they contain only a single polar functional group. The mutual solubility of water and some common organic solvents used for liquid-liquid extraction is summarized in Table 2.2 [14]. Solvents that exceed a maximum mutual solubility, often taken to be 10% (v/v), generally show poor selectivity and are more likely to form emulsions with some sample matrices but might still be useful for some applications. Mutual solubility is a limiting factor for the

TABLE 2.2 Mutual Solubility of Organic Solvents and Water (% w/w) at 20–25°C

Solvent	Solubility of Solvent in Water	Solubility of Water in Solvent
n-Hexane	0.014	0.010
n-Heptane	0.0003	0.010
2,2,4-Trimethylpentane	0.0002	0.006
Cyclohexane	0.050	0.040
Benzene	0.18	0.073
Toluene	0.052	0.033
Chlorobenzene	0.050	0.040
Dichloromethane	1.60	0.24
Chloroform	0.815	0.056
1,2-Dichloroethane	0.87	0.35
Ethyl acetate	8.7	3.3
Butyl acetate	0.68	1.20
Diethyl ether	6.89	1.26
Methyl t-butyl ether	4.6	1.50
Propylene carbonate	17.5	8.3
n-Octanol	0.058	3.82

identification of solvents for microextraction techniques where the volume of the extraction phase is only a small fraction of the sample volume. Ternary and quaternary solvent mixtures containing water as one solvent can form biphasic systems but are little used for shake-flask extraction. An exception is Folch partition for the isolation of neutral lipids from animal tissues [15]. Tissue samples are homogenized in a mixture of chloroform and methanol and the solvent phase diluted with water to an approximate composition of chloroform-methanol-water (8:4:3). This results in phase separation from which lipids are isolated from the chloroform-rich layer with an approximate composition chloroform-methanol-water (86:14:1). Ternary and quaternary solvent mixtures containing water are widely used in high-speed countercurrent chromatography for the preparative-scale separation of mixtures [16]. High-speed countercurrent chromatography utilizes centrifugal forces to hold one phase (the stationary phase) in place, while a second immiscible phase (the mobile phase) is passed through it. The Arizona scale defines a range of varied biphasic solvent systems composed of mixtures of *n*-heptane-ethyl acetate-methanol-water with the binary solvent systems ethyl acetate-water (most polar) and *n*-heptane-methanol (least polar) at the extreme ends of the scale [17]. A series of ternary and quaternary solvent compositions of intermediate polarity occupy distinct points within the scale.

2.3 Solvent Classification Methods

Many early attempts at solvent classification were based on solvent polarity scales defined by solute-solvent interactions with a single compound or group of compounds employing spectroscopic, kinetic, or equilibrium measurements for the most part to determine the effect of the solvent on some observable property of the compound(s) [5, 18–20]. Katritzky et al. collected reports for 184 solvent polarity scales and organized them into four categories: (i) solvent effects for equilibrium and kinetic rate constants for chemical reactions, (ii) fundamental physicochemical and spectroscopic properties of solvents, (iii) spectroscopic and other properties of probe compounds dissolved in solvents, and (iv) miscellaneous multiparameter approaches [18]. The general conclusion from this and similar large-scale studies of this kind was the limited scope for success in connecting these scales to a unique solvent property reasonably defined as polarity. The latter now can be thought of as the capability of a solvent to participate in all recognized intermolecular interactions. Given that these interactions are several and varied in intensity for individual solvents, the usefulness of a single summative scale for solvent classification in itself is questionable. In addition, the general theory now used to model solvent interactions has moved away from considering solvents as a continuum (bulk solvent properties) to one of discrete systems characterized by pairwise solute-solvent and

solvent-solvent interactions with solvent differences described by multiparameter models [21, 22]. Thus the concept of solvent polarity while easily understood is confronted by the difficulty of defining a unique scale for its measurement and the lack of a suitable compound(s) that could be defined as a reference for such a scale. The modern approach to solvent classification is the use of multiparameter scales aligned with individual intermolecular interactions.

Some multiparameter approaches for solvent classification with a focus on physicochemical solvent properties are worthy of mention before taking up solvent classification based on intermolecular interactions. These early methods were generally based on a group of characteristic bulk solvent properties treated as variables with chemometric techniques employed to reduce the dimensionality and/or cluster the data according to a particular metric [19, 23]. An early and still relevant example is the approach of Chastrette et al. [24], which employed eight solvent properties (molar refraction, dipole moment, Hildebrand's solubility parameter, refractive index, boiling point, Kirkwood function, highest occupied molecular orbital energy, and lowest unoccupied molecular orbital energy) and principal component analysis to assign each of 83 liquids into nine selectivity groups summarized in Table 2.3. The

TABLE 2.3 Classification of Solvent Properties by Chastrette et al. [24] Using Multivariate Statistical Treatment of Physicochemical and Quantum Chemical Parameters

Group	Solvent Type	Typical Solvents	Number of Solvents
I	Aprotic dipolar	Acetonitrile Acetone Ethyl acetate Dichloromethane	14
II	Aprotic highly dipolar	Dimethyl sulfoxide *N,N*-Dimethylformamide Pyridine	9
III	Aprotic highly polarizable dipolar	Hexamethylphosphotriamide	2
IV	Aromatic apolar	Toluene Benzene	8
V	Aromatic polar	Chlorobenzene *o*-Dichlorobenzene	12
VI	Electron-pair donor	Triethylamine Diethyl ether Dioxane	10

Continued

TABLE 2.3 Classification of Solvent Properties by Chastrette et al. [24] Using Multivariate Statistical Treatment of Physicochemical and Quantum Chemical Parameters—cont'd

Group	Solvent Type	Typical Solvents	Number of Solvents
VII	Hydrogen bonding	Methanol Ethanol Pentan-2-ol	19
VIII	Hydrogen bonding strongly associated	Formamide Water Ethylene glycol	5
IX	Miscellaneous	Carbon disulfide Chloroform Aniline	4

general group labels are in reasonable agreement with empirical knowledge, but some solvent assignments were considered unlikely based on expected family behavior, for example, trifluoroacetic acid in Group I, benzyl alcohol and octan-1-ol in Group V, and cyclohexane and tetrahydrofuran in Group VI. This illustrates the generally observed deficiency of classification schemes based in large part on the physicochemical properties of bulk solvents as variables. Gramatica et al. [25] used various structural, empirical, and topological descriptors and a combination of the *k*-nearest neighbor and neural network methods to classify 152 liquids into five groups. For 82 of the 83 solvents in Chastrette's classification, 24 were identified as aprotic polar solvents, 16 as aromatic apolar and lightly polar solvents, 9 as electron-pair donor solvents, 25 as hydrogen-bond donor solvents, and 5 as aliphatic aprotic apolar solvents. Compared with Chastrette's group assignments, groups I–III were combined into a single group (aprotic polar), Groups IV and V (aromatic apolar and lightly polar), and Groups VII and VIII (hydrogen-bond donors). A new class of aliphatic aprotic apolar solvents was identified containing alkane, cycloalkane, carbon tetrachloride, and tetrachloroethylene. Solvents considered misclassified by Chastrette's method are now logically relocated to groups that seem more appropriate. However, the classification scheme is probably too broad for solvent selection for extraction and the expanded (152 solvent data set) leaves too many solvents in overlapping groups. Several of the molecular descriptors lack an obvious connection to the solvation mechanism, and further analysis in terms of intermolecular interactions is impossible. The multiparameter methods described in the succeeding text get around this problem and have been more widely adopted for solvent selection for extraction.

2.3.1 Hildebrand's solubility parameter

Hildebrand's solubility parameter, generally referred to as the total solubility parameter, δ_T, is defined as the square root of the energy of vaporization per unit volume [4, 5, 26]. It provides a measure of the cohesive energy density of a solvent and is one of several approaches utilized as a surrogate measure of solvent polarity. The original solubility parameter concept was developed from assumptions of regular solution theory in which the principal interactions between molecules were dominated by dispersion forces. Hansen extended the solubility parameter model to polar solvents by partitioning the total solubility parameter into three partial contributions defined as δ_D for the contribution of nonspecific intermolecular interactions related to dispersion forces, δ_P for the contribution of polar interactions attributed to permanent dipole-permanent dipole interactions (orientation), and δ_H for the contribution from all remaining specific intermolecular interactions including hydrogen bonding [27]. The total solubility parameter and the partial solubility parameters are simply related by the expression $\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2$. In other cases the partial solubility parameters were expanded to partition δ_P into dipole-dipole, δ_O, and dipole-induced dipole, δ_{IN}, (induction) interactions and δ_H into hydrogen-bond proton donor, δ_a, and hydrogen-bond proton acceptor, δ_b, interactions [27–31]. This results in equations with different numbers of terms to describe solvent properties. For volatile solvents the total solubility parameter is generally accessible by experiment, but the partial solubility parameters are deduced from empirical models leading to numerically different values for the same notional parameter. This can cause confusion when compiling partial solubility parameter constants from different sources where different calculation methods for the same term have been employed. In addition, there is no general agreement that a particular method is the correct method.

The partial solubility parameters are an indication of the capability of the solvent to enter into specific intermolecular interactions; the larger the value, the stronger the interaction. The larger the difference in the partial solubility parameters for two solvents, the more likely they will be immiscible, but there does not seem to be any specific difference that could be utilized for the purpose of identifying immiscible solvent pairs. For maximum solubility the partial solubility parameters δ_D, δ_O, and δ_{IN} (or δ_P) for the solute and solvent should be similar and the values for δ_a and δ_b complementary. The approximate nature of the partial solubility parameters for polar solvents and the difficulty of assigning a specific method for their calculation have contributed to declining interest in this approach for solvent selection for extraction. A still relevant application is the extraction of polymers and studies of polymer interactions and solubility, particularly for polymers of low polarity [31, 32].

2.3.2 Solvent selectivity triangle

The solvent-selectivity triangle classification of solvents is based on the calculation of three solvent-selectivity factors, x_i, and a solvent polarity index, P' [33–35]. The former characterize the solvent's capability to interact with solutes by orientation and proton donor/acceptor interactions x_n, x_e, and x_d, respectively. The original solvent classification was based on Rohrschneider's compilation of experimental gas-liquid partition constants for several probe compounds in 80 common solvents [36]. Three of the probe compounds were defined as prototypical compounds for specific intermolecular interactions: nitromethane, ethanol, and dioxane for orientation, proton donor, and proton acceptor interactions, respectively. The effect of solute size and solute-solvent dispersion and induction interactions arising from solvent polarizability was subtracted from the experimental partition constants for the prototypical compounds by multiplication by the solvent molar volume and referencing this quantity to the value for a hypothetical hydrocarbon with the same molar volume for each prototypical compound. The polar distribution constants obtained in this way were then corrected empirically to have a zero value for the interactions of the prototypical compounds with a saturated hydrocarbon solvent. These residual values were stated to arise from inductive and entropy effects not fully accounted for by the calculation method [37]. The polarity index, P', was then defined as the sum of the logarithmic polar distribution constants for the prototypical compounds and the selectivity factors as the polar distribution constant for each prototypical compound divided by the polarity index. In this way the sum of the three selectivity factors will always equal one, while their individual values will be different for solvents of different selectivity. Typical values for P', x_n, x_e, and x_d for some common solvents are summarized in Table 2.4, which includes corrected values from [38] for some solvents considered to have poor experimental values in Rohrschneider's original partition constant compilation.

The selectivity of each solvent is represented by a point with three coordinates plotted on the face of a triangle with each side of the triangle as the axes for the individual selectivity factors. Located at the three apexes of the triangle are the prototypical compounds (or the molecular interactions assigned to each prototypical compound). When plotted this way, Fig. 2.1, the solvents are clustered into eight selectivity groups. Solvents located in the same group have similar selectivity, and solvents located in other groups have different selectivity. Typical examples of common solvents and their selectivity group membership are shown in Table 2.5. Solvent selection for extraction commences by selecting a solvent from each selectivity group with suitable physicochemical properties for the extraction technique employed. For liquid-liquid extraction, representative solvents from each

TABLE 2.4 Solvent Polarity and Solvent-Selectivity Factors for Common Solvents Using the Solvent-Selectivity Triangle

	Polarity	Selectivity Factors		
Solvent	(*P′*)	x_e	x_d	x_n
Acetic acid	6.13	0.39	0.31	0.30
Acetone	5.1	0.35	0.23	0.42
Acetonitrile	5.8	0.31	0.27	0.42
Benzene	2.7	0.23	0.32	0.45
n-Butan-1-ol	3.9	0.59	0.19	0.25
Chlorobenzene	2.7	0.23	0.33	0.44
Chloroform	4.31	0.31	0.35	0.34
Dichloromethane	4.29	0.27	0.33	0.40
Diethyl ether	3.15	0.53	0.13	0.34
Dimethylacetamide	6.45	0.41	0.20	0.38
N,N-Dimethylformamide	6.31	0.40	0.21	0.39
Dimethyl sulfoxide	7.29	0.40	0.22	0.37
1,4-Dioxane	5.27	0.37	0.23	0.40
Ethanol	4.40	0.52	0.19	0.29
Ethyl acetate	4.4	0.34	0.23	0.43
Ethylene glycol	6.9	0.43	0.29	0.28
Formamide	9.6	0.36	0.33	0.30
Methanol	5.1	0.48	0.22	0.31
2-Methoxyethanol	5.71	0.41	0.22	0.36
Octan-1-ol	3.23	0.58	0.17	0.25
Propan-1-ol	4.13	0.54	0.19	0.27
Propan-2-ol	3.91	0.57	0.17	0.26
Propylene carbonate	6.1	0.31	0.27	0.42
Pyridine	5.43	0.42	0.22	0.36
Tetrahydrofuran	4.28	0.41	0.19	0.40
Toluene	2.68	0.28	0.27	0.45
Triethylamine	2.19	0.66	0.08	0.26
2,2,2-Trifluoroethanol	7.55	0.40	0.33	0.27
Water	10.2	0.37	0.37	0.25
p-Xylene	2.55	0.28	0.26	0.45

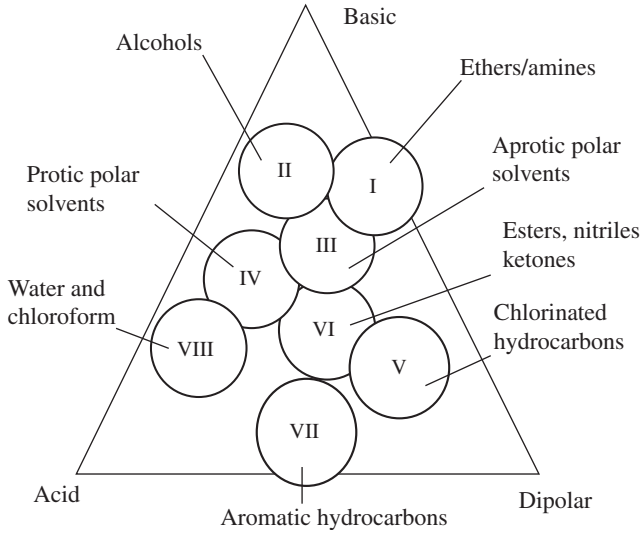

Fig. 2.1 Solvent-selectivity triangle based on the selectivity factors for the prototypical compounds nitromethane (dipolar), ethanol (hydrogen-bond acidity), and 1,4-dioxane (hydrogen-bond basicity). Because of solvent complementarity, basic solvents should be located close to the acidic apex of the triangle and vice versa for acidic solvents. Representative solvents from the different selectivity groups are indicated in Table 2.5.

TABLE 2.5 Classification of Solvents Based on the Solvent-Selectivity Triangle

Group Designation	Representative Solvents
I	Aliphatic ethers (close triethylamine)
II	Aliphatic alcohols
III	Pyridine, tetrahydrofuran, *N*,*N*-dimethylformamide, dimethyl sulfoxide, 2-methoxyethanol
IV	Acetic acid, ethylene glycol, formamide, benzyl alcohol
V	Dichloromethane, chloroethane, ethylene chloride
VIa	Aliphatic ketones and esters, dioxane
VIb	Acetonitrile, benzonitrile, propylene carbonate, aniline
VII	Benzene, toluene, chlorobenzene, aromatic ethers, nitrobenzene
VIII	Water, 2,2,2-trifluoroethanol, m-cresol (close chloroform)

group are selected taking their mutual solubility into account, keeping in mind that most organic solvents of intermediate polarity are miscible and that water forms the largest number of two-phase systems with low mutual solubility. A Σpider (spider) diagram provides an alternative method for visualizing the selectivity groups to the solvent-selectivity triangle [39].

A main strength of the solvent-selectivity triangle approach is its simple visual interpretation, although from empirical experience, it is likely that some solvents were incorrectly classified with respect to their neighbors and the relative position of selectivity groups with respect to the apexes of the triangle is difficult to justify if the prototypical compounds provide a suitable measure for individual intermolecular interactions [35]. The P' solvent polarity scale does not include the capability for dispersion interactions, and solvents with small P' values, for example, alkanes and cycloalkanes, produce erratic results and cannot be included in the solvent-selectivity triangle. This was justified by the assumption that solvents dominated by dispersion interactions were not important for the classification of solvent selectivity. Experimental difficulties in determining partition constants for solvents of high cohesive energy have resulted in a number of these solvents not being included in the classification or having uncertain assignments. The most significant limitation, however, is associated with the role of prototypical compounds to define specific intermolecular interactions and the incomplete correction for differences in cohesive energy for the solvents [14, 35, 38]. The partition constants of the prototypical compounds are the result of multiple intermolecular interactions and not a single dominant interaction. Ethanol as an example is dipolar and a proton donor and acceptor, and solvents interacting with ethanol need not be a proton acceptor to register a significant value for the x_e selectivity factor due to contributions from the other intermolecular interactions. Because there are no prototypical compounds that are strong hydrogen-bond acids or bases that are not simultaneously dipolar, it is impossible to characterize intermolecular interactions based on the properties of single compounds.

2.3.3 Solvatochromic parameters

Solvatochromic parameters are determined by spectral shifts in the absorption bands of reference compounds selected to represent, in general, a single intermolecular interaction. In addition, the solvatochromic parameters are typically averages for several reference compounds for each parameter and (ideally) almost independent of the identity of the reference compounds. The most comprehensive treatments of solvatochromic properties of solvents are the Taft-Kamlet-Abboud π^* (solvent dipolarity/polarizability), α (solvent hydrogen-bond acidity), and β (solvent hydrogen-bond basicity) scales [5, 40, 41]. The π^* scale is normalized to dimethyl sulfoxide = 1

and measures the capability of a solvent to stabilize a charge or dipole by virtue of its dielectric effect. The α scale measures the solvent capability to donate a proton in a solvent-solute hydrogen bond, normalized to methanol = 1, and the β scale the solvent capability to accept a proton (donate an electron pair) in a solvent-solute hydrogen bond normalized to hexamethylphosphoramide = 1. Typical values of π^*, α, and β for some common solvents are summarized in Table 2.6. Classification of solvents with the solvatochromic parameters as variables using the selectivity triangle method [35, 42, 43], hierarchical clustering [44, 45], neural network [46], and Σpider diagrams [39] has been proposed. Fig. 2.2 illustrates the selectivity triangle approach for some

TABLE 2.6 Solvatochromic Parameters for Common Solvents

	Solvatochromic Parameter		
Solvent	α	β	π^*
Acetic acid	1.12	0.45	0.64
Acetone	0.08	0.48	0.71
Acetonitrile	0.19	0.31	0.75
Benzene	0.00	0.10	0.59
n-Butyl acetate	0.00	0.45	0.46
n-Butan-1-ol	0.79	0.88	0.47
Carbon tetrachloride	0.00	0.00	0.28
Chlorobenzene	0.00	0.07	0.71
Chloroform	0.44	0.00	0.58
Cyclohexane	0.00	0.00	0.00
1,2-Dichloroethane	0.00	0.00	0.81
Dichloromethane	0.30	0.00	0.82
Diethyl ether	0.00	0.47	0.27
Dimethylacetamide	0.00	0.76	0.88
N,*N*-Dimethylformamide	0.00	0.69	0.88
Dimethyl sulfoxide	0.00	0.76	1.00
1,4-Dioxane	0.00	0.37	0.55
Ethanol	0.83	0.77	0.54
Ethyl acetate	0.00	0.45	0.55
Ethylene glycol	0.90	0.52	0.92
Formamide	0.71	0.44	0.97

TABLE 2.6 Solvatochromic Parameters for Common Solvents—cont'd

	Solvatochromic Parameter		
Solvent	α	β	π^*
Heptane	0.00	0.00	−0.02
Hexane	0.00	0.00	−0.04
Methanol	0.93	0.62	0.60
Octan-1-ol	0.77	0.81	0.40
Propan-1-ol	0.84	0.9	0.52
Propan-2-ol	0.76	0.95	0.48
Propylene carbonate	0.00	0.40	0.83
Pyridine	0.00	0.64	0.87
Tetrahydrofuran	0.00	0.55	0.58
Toluene	0.00	0.11	0.54
Triethylamine	0.00	0.71	0.14
2,2,2-Trifluoroethanol	1.51	0	0.73
2,2,4-Trimethylpentane	0.00	0.00	−0.04
Water	1.17	0.18	1.09
p-Xylene	0.00	0.12	0.51

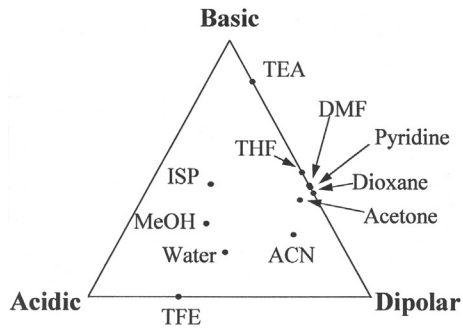

Fig. 2.2 Solvent-selectivity triangle with normalized solvatochromic parameters (solvatochromic parameter/$\Sigma(\pi^* + \alpha + \beta)$) as coordinates for some common organic solvents. *TEA*, triethylamine; *THF*, tetrahydrofuran; *DMF*, *N*,*N*-dimethylformamide; *ISP*, 2-propanol; *MeOH*, methanol; *ACN*, acetonitrile; and *TFE*, 2,2,2-trifluoroethanol.

representative organic solvents and water. The grouping of solvents is different to the solvent-selectivity triangle approach based on prototypical compounds discussed in Section 2.3.2. The most selective solvents are now found along the edges and toward the apexes of the triangle with those solvents having a blend of properties located on the face. Also indicated is that solvents, which are strong hydrogen-bond bases with weak dipole properties, are uncommon, triethylamine being the best example. The solvents acetone, acetonitrile, 2-propanol, and methanol provide a convenient range of hydrogen-bond acidity. These solvents are simultaneously strong hydrogen-bond bases and found toward the center of the triangle. 2,2,2-Trifluoroethanol is the most selective of the strong hydrogen- bond acids as it possesses no hydrogen-bond basicity and is weakly dipolar and polarizable. Acetonitrile (or dioxane), acetone (or tetrahydrofuran), and *N*,*N*-dimethylformamide (or pyridine) provide a reasonable range of hydrogen-bond basicity. Only acetonitrile is a significant hydrogen-bond acid, but all are dipolar. Results for the classification of a larger number of solvents by cluster analysis are summarized in Table 2.7. This resulted in five selectivity groups being identified with Group IV divided into two subsets. Water and 2,2,2-trifluoroethanol are identified as behaving independently. The selectivity group center coordinates provide an indication of the blend of solvent properties associated with each group.

The main problem with solvent classification methods based on the solvatochromic parameters is that only solvent polar interactions are considered ignoring the cohesive energy of the solvents [47]. For partitioning and solubility, it is necessary to consider the interactions associated with cavity formation and dispersion to obtain a complete picture of the solvation processes.

TABLE 2.7 Classification of Common Solvents by Hierarchical Cluster Analysis With the Solvatochromic Parameters as Variables

	Cluster Center Coordinates			
Cluster	π^*	α	β	Representative Solvents
I	0.265	0	0.355	Aliphatic ethers, triethylamine
II	0.735	0.030	0.424	Cyclic and aromatic ethers, aliphatic ketones and esters, propylene carbonate, acetonitrile, nitrobenzene
III	0.885	0	0.763	*N*,*N*-Dimethylformamide, dimethyl sulfoxide, pyridine
IVa	0.654	0.067	0.081	Aromatic ethers, toluene, chlorobenzene, carbon tetrachloride, 1,2-dichloroethane
IVb				Dichloromethane, chloroform
V	0.866	0.745	0.644	Alcohols, ethylene glycol
Independent				Water, 2,2,2-trifluoroethanol

2.3.4 Abraham's solvation parameter model

Abraham's solvation parameter model assumes a cavity model of solvation and uses a series of descriptors to define solute properties and a complementary series of system constants to represent solvent properties [48–51]. The transfer of a solute from an ideal-gas phase to a solvent occurs in three stages: (1) a solute-size cavity is created in the solvent requiring disruption of solvent-solvent interactions, (2) solvent molecules reorganize themselves at the cavity surface to establish favorable interactions with the solute, and (3) the solute enters the cavity and establishes appropriate solute-solvent interactions. A favorable change in free energy requires the interactions established at step (3) exceed those disrupted in step (1); the reorganization in (2) is expected to occur with little change in free energy. Solvents of different cohesive energy differ in the amount of free energy required for cavity formation. The forces contributing to solute-solvent interactions are identified as dispersion, interactions of a dipole type (orientation and induction) and hydrogen bonding. For quantitative calculations, it is necessary to parameterize the model as shown for the transfer of a solute from the gas phase to a solvent in which K is the gas-liquid partition constant:

$$\log K = c + e\mathrm{E} + s\mathrm{S} + a\mathrm{A} + b\mathrm{B} + l\mathrm{L} \tag{2.1}$$

The solute descriptors are defined in Table 2.8. The excess molar refraction, E, can be calculated for liquids but must be either estimated or determined experimentally for solids. The other descriptors S, A, B, and L are determined by experiment from chromatographic, solubility, and liquid-liquid partition measurements. The system constants for a solvent are calculated by multiple linear regression analysis with the gas-liquid partition constants for varied compounds with known descriptor values as variables [49–51]. There are chemical and statistical requirements to obtain stable values for the system constants that are then independent of solute identity for the same descriptor space. The system constants that define the solvent properties are described as e, the contribution from electron lone pair interactions (or the additional contribution of dispersion interactions that arise from loosely bound electrons in polarizable molecules); s, the contribution from interactions of a dipole type (both induction and orientation); a, the contribution from solvent hydrogen-bond basicity; b, the contribution from solvent hydrogen-bond acidity; and l, the combined and opposing contributions from cavity formation and dispersion interactions because both are strongly correlated with solute size and not easily separated out.

Visualizing the classification of solvents in a five coordinate system in three-dimensional space requires an approach that reduces the dimensionality of the coordinate system. Hierarchical cluster analysis [14, 45] and the Σpider method [39] have been used for this purpose. Hierarchical cluster analysis uses the Euclidean distance separating solvents in five-dimensional space to compute a similarity matrix.

TABLE 2.8 Identification of the Solute Descriptors and System Constants Used in the Solvation Parameter Model

Solute Property	Solvent Property	Solute Descriptor Values
E	*e*	Excess molar refraction Can be calculated for liquids from their refractive index at 20°C (η) and characteristic volume (V) $E = 10\ V\ [(\eta^2 - 1)/[(\eta^2 + 2)] - 2.832\ V + 0.526]$ For solids, it can be estimated or determined by experimental methods
S	*s*	Interactions of a dipole type (both induction and orientation) Determined by experimental methods
A	*a*	Hydrogen-bond acidity (complementary solvent property hydrogen-bond basicity) Determined by experimental methods
B	*b*	Hydrogen-bond basicity (complementary solvent property hydrogen-bond acidity) Determined by experimental methods
L	*l*	Gas-liquid partition constant at 298 K on *n*-hexadecane Determined by experimental methods
V	*v*	McGowan's characteristic volume Can be calculated from structure by summing atom constants and accounting for bond order $V = [\Sigma(\text{all atom contributions}) - 6.56\ (N - 1 + R_g)]/100$ N = total number of atoms and R_g the total number of ring structures

Solvents that are near neighbors in hyperspace and have similar properties are grouped together, and solvents further away are placed in a separate class when their separation from the nearest group (or single solvent) exceeds a threshold value until all solvents have been included in the analysis. The output is a connection dendrogram shown in Fig. 2.3 for the solvents in Table 2.9 [14, 52, 53]. The solvents are classified into eight groups with five solvents (*N*,*N*-dimethylformamide, dimethyl sulfoxide, 2,2,2-trifluoroethanol, acetic acid, and water) behaving independently. Group membership is indicated in Table 2.10. Group I contains *n*-alkane and cycloalkane solvents of low cohesion and weak or nonexistent polar interactions. Group II contains aromatic hydrocarbons and chlorobenzene solvents of low cohesion and weak polar interactions. Group III contains the haloalkane solvents, which have low cohesion and weak polar interactions but are significantly more

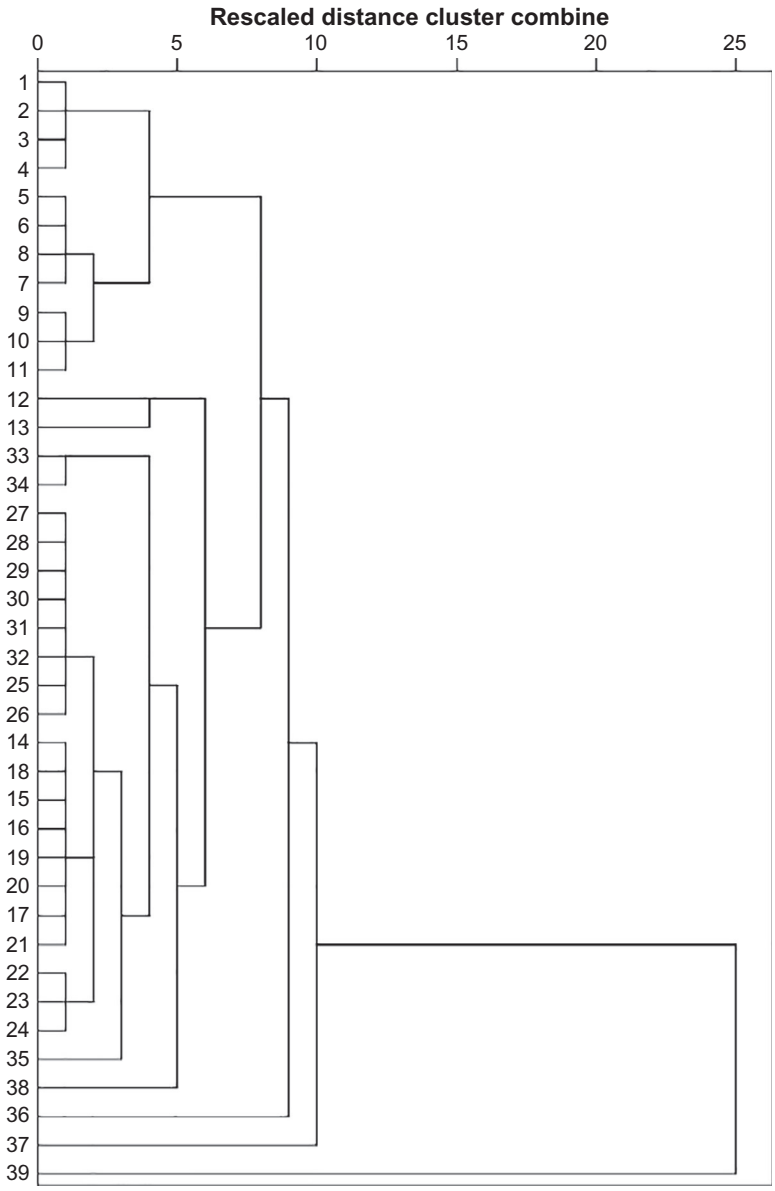

Fig. 2.3 Nearest-neighbor agglomeration cluster dendrogram for common solvents. Identification: 1 = *n*-hexane, 2 = *n*-heptane, 3 = 2,2,4-trimethylpentane, 4 = cyclohexane, 5 = benzene, 6 = toluene, 7 = *p*-xylene, 8 = chlorobenzene, 9 = dichloromethane, 10 = 1,2-dichloromethane, 11 = chloroform, 11 = 1,2-dichloroethane, 12 = ethylene glycol, 13 = formamide, 14 = ethanol, 15 = propan-1-ol, 16 = propan-2-ol, 17 = butan-1-ol, 18 = butan-2-ol, 19 = hexan-1-ol, 20 = octan-1-ol, 21 = methanol, 22 = 2-methoxyethanol, 23 = 2-ethoxyethanol, 24 = *N*-methylformamide, 25 = methyl *t*-butyl ether, 26 = diethyl ether, 27 = acetone, 28 = 1,4-dioxane, 29 = cyclohexanone, 30 = ethyl acetate, 31 = butyl acetate, 32 = tetrahydrofuran, 33 = acetonitrile, 34 = propylene carbonate, 35 = *N*,*N*-dimethylformamide, 36 = dimethyl sulfoxide, 37 = 2,2,2-trifluoroethanol, 38 = acetic acid, and 39 = water.

TABLE 2.9 Solvation Parameter Model System Constants for Some Common Solvents

	System Constants				
Solvent	***e***	***s***	***a***	***b***	***l***
Acetic acid	−0.366	1.300	2.736	2.117	0.796
Acetone	−0.387	1.733	3.060	0	0.866
Acetonitrile	−0.595	2.461	2.085	0.418	0.738
Benzene	−0.313	1.053	0.457	0.169	1.020
n-Butyl acetate	−0.414	1.212	2.623	0	0.954
n-Butan-1-ol	−0.285	0.768	3.705	0.879	0.890
Butan-2-ol	−0.387	0.719	3.726	1.088	0.905
Butan-2-one	−0.474	1.671	2.878	0	0.916
Carbon tetrachloride	−0.435	0.544	0	0	1.069
Chlorobenzene	−0.399	1.156	0.313	0.171	1.032
Chloroform	−0.560	1.259	0.374	1.333	0.976
Cyclohexane	−0.110	0	0	0	1.013
Cyclohexanone	−0.441	1.725	2.786	0	0.957
1,2-Dichloroethane	−0.337	1.600	0.774	0.637	0.921
Dichloromethane	−0.572	1.492	0.46	0.847	0.965
Diethyl ether	−0.379	0.904	2.937	0	0.963
Dimethylacetamide	−0.271	0.084	0.209	0.915	−5.003
N,*N*-Dimethylformamide	−0.869	2.107	3.774	0	1.011
Dimethyl sulfoxide	0.131	2.811	5.474	0	0.734
1,4-Dioxane	−0.354	1.674	3.021	0	0.919
Ethanol	−0.232	0.867	3.894	1.192	0.846
2-Ethoxyethanol	−0.257	1.452	3.672	0.662	0.843
Ethyl acetate	−0.352	1.316	2.891	0	0.916
Ethylene glycol	0.132	1.657	4.457	2.355	0.565
Formamide	0.310	2.292	4.130	1.933	0.442
Heptane	−0.162	0	0	0	0.983
Hexane	−0.169	0	0	0	0.979
Hexan-1-ol	−0.205	0.583	3.621	0.891	0.913
Methanol	−0.338	1.317	3.826	1.396	0.773

TABLE 2.9 Solvation Parameter Model System Constants for Some Common Solvents—cont'd

	System Constants				
Solvent	***e***	***s***	***a***	***b***	***l***
2-Methoxyethanol	−0.265	1.810	3.641	0.590	0.790
Methyl t-butyl ether	−0.536	0.890	2.632	0	0.999
N-Methylformamide	−0.142	1.661	4.147	0.817	0.739
Octan-1-ol	−0.203	0.560	3.576	0.702	0.939
Propan-1-ol	−0.246	0.749	3.888	1.076	0.874
Propan-2-ol	−0.324	0.713	4.036	1.055	0.884
Propylene carbonate	−0.413	2587	2.207	0.455	0.719
Tetrahydrofuran	−0.347	1.238	3.280	0	0.982
Toluene	−0.222	0.938	0.467	0.099	1.012
2,2,2-Trifluoroethanol	−0.547	1.339	2.213	3.807	0.645
2,2,4-Trimethylpentane	−0.230	0	0	0	0.975
Water	0.822	2.743	3.904	4.814	−0.213

TABLE 2.10 Group Membership for Solvents Classified Using the Solvation Parameter Model

	Center Coordinates					
Group	***e***	***s***	***a***	***b***	***l***	**Representative Solvents**
I	−0.13	0	0	0	1.00	*n*-Alkanes, cycloalkanes, carbon tetrachloride
II	−0.31	1.05	0.41	0.15	1.02	Benzene, *n*-alkylbenzenes, chlorobenzene
III	−0.50	1.45	0.54	0.94	0.95	Chloroform, dichloromethane, 1,2-dichloroethane
IV	0.13	1.97	4.29	2.14	0.50	Formamide, ethylene glycol
V	−0.50	2.52	2.15	0.44	0.73	Acetonitrile, propylene carbonate
VI	−0.41	1.37	2.90	0	0.94	Aliphatic ketones, ethers and esters, tetrahydrofuran, dioxane
VII	−0.28	0.78	3.79	1.03	0.88	Aliphatic alcohols
VIII	−0.22	1.64	3.82	0.69	0.79	2-Methoxyethanol, *N*-methylformamide

hydrogen-bond acidic than Group II solvents. Group IV contains the amphiprotic solvents ethylene glycol and formamide, which are significantly more cohesive, dipolar, and hydrogen-bond acidic than the aliphatic alcohol (Group VII) solvents. Group V contains acetonitrile and propylene carbonate, which are of moderate cohesion, strongly dipolar, weakly hydrogen-bond acidic and basic. They are distinguished from the other strongly dipolar solvents by their characteristic *s*/*a* ratio. Group VI contains aliphatic and cyclic esters, ethers, and ketones, which are solvents of low cohesion, moderate dipolarity, and strong hydrogen-bond basicity but no hydrogen-bond acidity. Group VII contains the aliphatic alcohols, which are cohesive amphoteric solvents (moderately dipolar and strong hydrogen-bond acids and bases). Group VIII contains 2-methoxyethanol, 2-ethoxyethanol, and *N*-methylformamide, which are more dipolar/polarizable and less hydrogen-bond basic than the simple aliphatic alcohols.

Of the five solvents behaving independently, water is the most cohesive and strongest hydrogen-bond acid of the solvents in Table 2.9. 2,2,2-Trifluoroethanol is significantly more hydrogen-bond acidic and less hydrogen-bond basic than a typical alcohol (Group VII). *N*,*N*-Dimethylformamide and dimethyl sulfoxide are more dipolar and stronger hydrogen-bond bases than the other aprotic dipolar solvents (Group V). Acetic acid is significantly less hydrogen-bond basic and cohesive than the polar protic solvents of Group IV (ethylene glycol and formamide).

The grouping of solvents by the solvation parameter model appears more sensible based on empirical information and expectations with respect to family characteristics. Within group, classifications can be scrutinized to reveal subtle differences in solvent properties. Fig. 2.4 illustrates the within-group variation for the aliphatic alcohols (Group VII). Methanol can be singled out as being more cohesive, dipolar, and hydrogen-bond acidic than the other alcohols. The effect of chain length on the system constants indicates a smooth change with chain length with longer-chain alcohols being less cohesive and weaker dipolar and hydrogen-bond acid/base solvents. Water solubility also declines with chain length, and for aqueous two-phase systems, some fine-tuning of selectivity should be feasible by varying the chain length of the n-alcohols due to changes in both the properties of the alcohol and in the saturation water concentration.

2.3.5 Conductor-like screening model for real solvents

The conductor-like screening model for real solvents (COSMO-RS) is a model combining quantum theory, dielectric continuum models, surface interactions, and statistical thermodynamics [21, 22]. It enables the calculation of dielectric screening charges and energies on a van der Waals-like molecular surface with solvent interactions described by ensembles of pairwise interacting molecular surfaces. Because all interactions are local and pairwise, the computational requirements are simplified

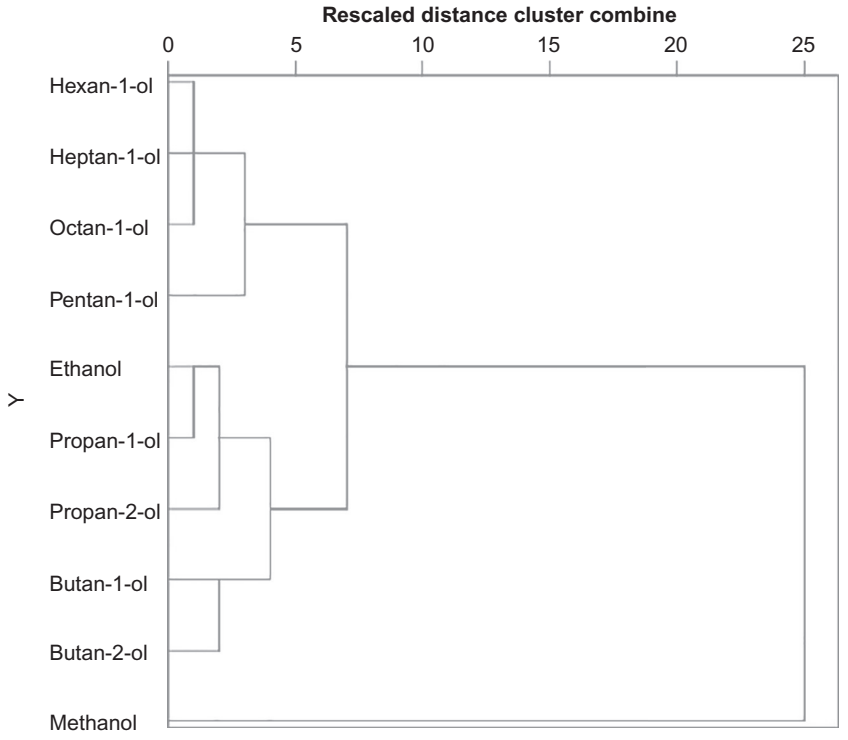

Fig. 2.4 Average linkage agglomeration dendrogram for aliphatic alcohols of Group VII with system constants of the solvation parameter model as variables.

compared with traditional approaches that treat solvents as a dielectric continuum. It allows solvent properties to be estimated without the need for experiment. The methodology is beyond the scope of this chapter, and only the application to the classification of solvents is discussed here. It has been shown that Abraham's solute descriptors and the theoretical descriptors of COSMO-RS possess a large overlap of their chemical content but are not identical [54].

Durand et al. [55] proposed a two-step statistical procedure to classify 153 liquids with 61 variables derived from the COSMO-RS model. Principal component analysis was used to reduce the dimensionality of the data set to three factors explaining 85% of the variance resulting in the classification of the liquids into 10 groups identified in Table 2.11. The classification is similar to the solvation parameter model taking into account the different number of solvents in the two data sets. The largest and most heterogeneous group of solvents is Group III, roughly 25% of the data set, containing most of the ethers, esters, ketones, and nitriles. This group is located toward the center of the principal component score plot indicating weak to moderate

TABLE 2.11 Classification of Common Solvents by the COSMO-RS Method

Group	Assignment	Representative Solvents
I	Strong electron-pair donor bases	Triethylamine
II	Weak electron-pair donor bases	*n*-Butylamine, ethylenediamine
III	Aprotic dipolar	Anisole, benzaldehyde, butan-2-one, ethyl acetate, diethyl ether, methyl *tert*-butyl ether, cyclohexanone, tetrahydrofuran
IV	Aprotic highly dipolar	Acetone, acetonitrile, dimethyl sulfoxide, propylene carbonate, nitroethane
V	Apolar	*n*-Hexane, benzene, carbon tetrachloride, chlorobenzene, diphenyl ether
VI	Asymmetrical halogenated hydrocarbons	Bromobenzene, chloroform, dichloromethane, 1,2-dichloroethane
VII	Amphiprotic	Ethanol, benzyl alcohol, aniline, octan-1-ol
VIII	Polar protic	1,2,3-Propanetriol, 2-aminoethanol, methanol, *N*-methylformamide
IX	Organic acids	Phenol, trifluoroacetic acid, 2,2,2-trifluoroethanol
X	Polar saturated	Formamide, water, acetic acid, ethylene glycol

contribution from all intermolecular interactions. These assignments are equivalent to Group VI in the solvation parameter model except for nitriles. The apolar solvents in Group V represent about 22% of the data set and contain *n*-alkanes, alkenes, cycloalkanes, benzene and methylaromatics, monohaloalkanes, mono- and dichlorobenzenes, chloroethylenes, and carbon tetrachloride. These low-polarity aliphatic and aromatic compounds are classified into two separate groups (Groups I and II) by the solvation parameter model. The classification of the polar liquids is generally the same.

Levet et al. [23] proposed a different approach to solvent classification using theoretically derived descriptors for 236 solvents. Eleven quantum descriptors were calculated using density function theory for the categories: (1) reactivity parameters (energy of the highest occupied molecular orbital (HOMO) and energy of the lowest unoccupied molecular orbital (LUMO)); (2) geometric parameter (surface area); and (3) electrostatic parameters (dipole moment, polarizability, maximum and minimum Mulliken charges, maximal and minimal electrostatic potential values computed on the isodensity surface, and two further electrostatic potentials computed on the solvent-accessible surface). Solvent classification was achieved using hierarchical

TABLE 2.12 Classification of 236 Solvents Using Theoretical Descriptors Described by Levet et al. [23]

Group	Description	Number of Solvents	Representative Solvents
I	Hydrogen-bond donors	39	Short chain alcohols and aliphatic carboxylic acids, phenols
II	Hydrogen-bond donors with high polarizability	17	Tributylamine, glycols, long-chain (>C_7) aliphatic alcohols
III	Hydrogen-bond acceptor/electron-pair donor	37	Amines, pyridines, aniline, anisole, dioxane
IV	Aprotic dipolar	28	Ethyl acetate, cyclohexanone, acetone
V	Aprotic dipolar/ polarizable	25	Ketones (<C_7), sulfolane
VI	Aprotic strongly dipolar	13	Amides, acetamide, carbonates
VII	Aprotic very strongly dipolar	9	Acetonitrile, nitroethane, nitrobenzene
VIII	Aprotic apolar	16	Linear and cyclic alkanes
IX	Aprotic apolar with π-bonds	19	Alkyl aromatic hydrocarbons, cyclohexene, 1-methylnaphthalene
X	Halogenated hydrocarbons	33	Carbon tetrachloride, chloroform, bromoethane, benzene, dichloromethane, carbon disulfide, halogenated benzenes

cluster analysis of the autoscaled descriptors resulting in 10 selectivity groups identified in Table 2.12. The solvents in Group 1 (hydrogen-bond donors) are characterized by a high maximum electrostatic potential corresponding to the presence of labile hydrogens and is distinguished from Group II (hydrogen-bond donors with high polarizability) by an above-average contribution from polarizability and hydrophobic surface area. The solvents in Group III are characterized by both high HOMO energy and maximum electrostatic potential characteristic of electron-pair donor solvents. The solvents in Group IV are characterized by low LUMO energy and a large difference between HOMO and LUMO energies distinguished from the solvents in Group V by high electrostatic potentials. The solvents in Group VI (aprotic strongly dipolar) are characterized by both high dipole moments and large hydrophobic surfaces and are distinguished from Group VII (aprotic very strongly dipolar) by strong binding of electron pairs (small difference between HUMO and LUMO energies and low HOMO energies). Group VIII and Group IX apolar solvents are differentiated by

high electrostatic potentials arising from the presence of π-bonds in the Group IX solvents. Group X contains the halogenated hydrocarbons and some other solvents of low polarizability and variable dipolarity. This group seems to be a residual group containing solvents with different properties when compared with the selectivity groups for the other methods of solvent classification. In general, there is good overall agreement for the methods described in Section 2.3 for solvent classification using either theoretical or experimental solvent properties as descriptors with some differences in the total number of selectivity groups for each method and a small fraction of solvent disagreements between methods [24, 25, 55]. The similarities are far more striking than the differences.

2.4 Distribution Model for Water-Organic Solvent Two-Phase Systems

The solvation parameter model has been used to characterize over 50 water-organic solvent two-phase systems with representative examples summarized in Table 2.13 [52, 53, 56]. For transfer between condensed phases, Eq. (2.1) is modified by replacing the L descriptor by the McGowan characteristic volume, V [48–51]:

$$\log K_p = c + eE + sS + aA + bB + vV \tag{2.2}$$

The transfer of a neutral solute between two condensed phases occurs with near-complete cancelation of dispersion interactions, unlike the case for transfer from an ideal-gas phase. The v system constant, therefore, is dominated by the difference in the cavity term for the two solvents and is correlated with the difference in the cohesive energy of the solvents. The liquid-liquid partition constants, K_p, refer to neutral compounds or ionizable compounds in the neutral form. The other solute descriptors and solvent system constants are the same as defined in Table 2.8.

For general interpretation the system constants for selected water-organic solvent systems are shown in Fig. 2.5 [57]. These are ordered to group solvents with similar system constants as neighbors. System constants with a positive sign favor distribution to the organic solvent and those with a negative sign to water. System constants with values close to zero indicate little preference for either phases and are not important for characterizing the distribution process. For the water-organic solvent systems in Fig. 2.5, the variation in the system constants is small with $v > 4$, $b > -4$, and a either ≈ -3.5 or close to 0 for the two-phase systems. This is a clear indication of the dominant properties of water, which is the most cohesive and hydrogen-bond acidic of the common organic solvents with low mutual solubility. The extraction of neutral organic compounds from water by any organic solvent of low mutual solubility depends mainly on solute size (large V favors extraction) with hydrogen-bonding interactions favoring solubility in the aqueous phase (compounds with a large B are difficult to extract). Dipole-type interactions are less important but form

TABLE 2.13 System Constants for Two-Phase Partition From Water to Organic Solvents

	System Constants				
Solvent	***e***	***s***	***a***	***b***	***v***
Benzene	0.464	−0.588	−3.099	−4.625	4.491
n-Butan-1-ol	0.434	−0.718	−0.097	−2.350	2.682
Butan-2-ol	0.480	−0.639	−0.050	−2.284	2.758
Butyl acetate	0.428	−0.094	−0.241	−4.151	4.046
Carbon tetrachloride	0.532	−1.159	−3.56	−4.594	4.618
Chlorobenzene	0.381	−0.521	−3.183	−4.700	4.614
1-Chlorobutane	0.273	−0.569	−2.918	−4.883	4.456
Chloroform	0.105	−0.403	−3.112	−3.514	4.395
Cyclohexane	0.784	−1.678	−3.740	−4.929	4.577
1,2-Dichloroethane	0.294	−0.134	−2.807	−4.291	4.180
Dichloromethane	0.102	−0.187	−3.058	−4.090	4.324
Diethyl ether	0.561	−1.016	−0.226	−4.553	4.075
Ethyl acetate	0.591	−0.669	−0.325	−4.261	3.666
Heptane	0.670	−2.061	−3.317	−4.733	4.543
Hexane	0.579	−1.723	−3.599	−4.764	4.344
Hexan-1-ol	0.460	−0.940	0.142	−3.284	3.792
Methyl *t*-butyl ether	0.307	−0.817	−0.618	−5.097	4.425
Octan-1-ol	0.562	−1.054	0.034	−3.460	3.814
Propylene carbonate	0.168	−0.504	−1.283	−4.407	3.421
Toluene	0.527	−0.720	−3.010	−4.824	4.545
2,2,4-Trimethylpentane	0.555	−1.737	−3.677	−4.864	4.417

two subgroups: polar solvents with dipolarity/polarizability similar to water (s close to −0.4) and have only a small influence on the extraction, and low-polarity solvents unable to compete with water in dipole-type interactions (s close to −1.5) favoring transfer to the aqueous phase. Solvent hydrogen-bond basicity also shows two typical behaviors for solvents of low mutual solubility with water. Polar organic solvents competitive with water as a hydrogen-bond base (a close to −0.3) exert little preference for distribution of hydrogen-bond acids to either phases, and solvents of low polarity that compete poorly with water for extraction of hydrogen-bond acids (a close to −3.5) favor distribution to the aqueous phase making extraction more

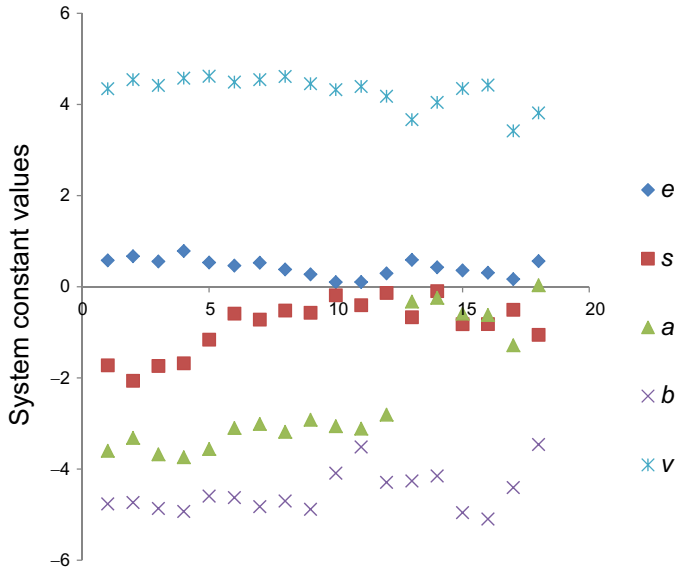

Fig. 2.5 Plot of the system constants of the solvation parameter model for representative binary two-phase water-organic solvent systems.

difficult. Solvents with small *a* system constant generally contain a relatively high volume fraction of water, which possibly contributes to the hydrogen-bond basicity of the water-saturated organic phase. Fig. 2.5 provides a striking indication of the limited possibility to vary selectivity for the extraction of neutral organic compounds with binary two-phase aqueous systems. The selectivity space covered by the aqueous biphasic systems in Table 2.13 is reasonably covered by the five solvent systems in Table 2.14 (and for other organic solvents from the same selectivity groups, if they have similar water saturation).

TABLE 2.14 Representative Liquid-Liquid Extraction Systems Covering the Selectivity Space Available With Aqueous Two-Phase Systems

	System Constants					
Organic Solvent	***e***	***s***	***a***	***b***	***v***	***c***
n-Heptane	0.678	−2.061	−3.317	−4.733	4.543	0.325
Dichloromethane	0.102	−0.187	−3.058	−4.090	4.324	0.319
Diethyl ether	0.561	−1.016	−0.226	−4.553	4.075	0.248
Ethyl acetate	0.591	−0.699	−0.325	−4.261	3.668	0.441
Octan-1-ol	0.562	−1.054	0.034	−3.460	3.814	0.088

2.5 Green Solvents

The term "green solvent" was introduced to describe sustainable solvents that minimize the environmental impact and health and safety concerns associated with their use in laboratory and manufacturing processes [58, 59]. The common solvents typically used in extractions are mainly volatile organic compounds (see Table 2.1) derived from fossil fuels and are not considered sustainable green solvents. Although these topics are not discussed specifically in this section, the reader will find useful information in solvent selection guides produced by different industrial sector groups, which address wider issues for the selection and use of green solvents for manufacturing processes [60, 61].

The greening of extraction processes is governed by the 12 principles of green analytical chemistry, which looks to eliminate or reduce the use of chemical substances (solvents, reagents, additives, etc.) and energy in extraction methods; to minimize waste, risk, and hazards associated with a method; and to promote the use of sustainable solvents [62, 63]. A practical approach to achieve these goals is the current trend toward downsizing of extraction methods (e.g., liquid-phase microextraction) and the use of solventless extraction (e.g., solid-phase microextraction), which eliminate or use minimal solvent amounts. Another approach is extraction methods based on supercritical fluid carbon dioxide and pressurized hot water as a substitute for conventional organic solvents [58, 63–65]. However, the focus of this section is the substitution of traditional volatile organic solvents by alternative solvents compatible with the principles of green analytical chemistry.

Since the established properties of volatile organic solvents cannot be changed, attention has shifted to identifying new types of solvents based on, or derived from, biomass, aqueous solutions of surfactants, ionic liquids, and deep eutectic solvents.

2.5.1 Bio-derived solvents

Bio-derived solvents are produced from sustainable biomass by low-energy conversion processes. The production of biodiesel is an example of a process that has reached large scale. Typical compounds derived from biomass considered potential solvents for extraction include alcohols (e.g., ethanol and various dialkoxypropanols and alkoxypropanediols), esters (e.g., ethyl lactate, triacylglycerols, and methyl oleate), ethers (e.g., 2-methyltetrahydrofuran, trialkoxypropanes), ketones, and terpenes (e.g., limonene) [58, 66, 67, 68]. The more polar solvents have unfavorable viscosity, but the replacement of alcohol groups with esters or ethers lowers the viscosities to between 0.5 and 5 cP. Solvatochromic parameters for some representative bio-derived solvents are summarized in Table 2.15. These solvents provide partial coverage of the selectivity space for traditional organic solvents with less favorable

TABLE 2.15 Solvatochromic Parameters for Some Representative Green Solvents

	Solvatochromic Parameters			Viscosity (cP)
Solvent	π^*	α	β	
(i) Bio-derived solvents				
1-*n*-Butoxy-3-isopropoxy-2-propanol	0.16	0.77		4.6
1,3-Di-*n*-butoxy-2-propanol	1.09	0.10		5.5
3-Butoxypropane-1,2-diol	0.68	0.91		42
Glycerol	1.04	0.93	0.67	1200
Ethyl lactate	0.69	0.64	0.63	2.4
Methyl oleate	0.43	0		5.6
1,3-Dibutoxy-2-methoxypropane	0.24	0.10		0.88
2-Methyltetrahydrofuran	0.53	0	0.58	0.6
Limonene	0.16	0	0	0.8
(ii) Room-temperature ionic liquids				
1-Ethyl-3-methylimidazolium				
Bis(trifluoromethylsulfonyl)imide	0.96	0.69	0.25	
Phosphinate	1.09	0.52	0.97	
Dicyanamide	1.08	0.53	0.35	
Perchlorate	1.11	0.56	0.41	
Acetate	1.09	0.40	0.95	
Hexafluorophosphate	0.99	0.66	0.20	
Dimethoxyphosphate	1.06	0.51	1.00	
Hexanesulfonate	0.98	0.65	0.71	
1-Butyl-3-methylimidazolium				
Bis(trifluoromethylsulfonyl)imide	0.96	0.63	0.24	
Chloride	1.09	0.39	0.70	
Hexafluorophosphate	1.03	0.65	0.22	
Tetrafluoroborate	1.04	0.63	0.39	
Trifluoromethylsulfonate	1.01	0.62	0.47	
1-Hexyl-3-methylimidazolium				
Bis(trifluoromethylsulfonyl)imide	0.97	0.63	0.25	
Chloride	1.02	0.48	0.94	

TABLE 2.15 Solvatochromic Parameters for Some Representative Green Solvents—cont'd

	Solvatochromic Parameters			Viscosity (cP)
Solvent	π^*	α	β	
Hexafluorophosphate	1.02	0.57	0.58	
Trifluoromethylsulfonate	0.98	0.67	0.52	
1-Octyl-3-methylimidazolium				
Hexafluorophosphate	0.88	0.58	0.46	
Tetrafluoroborate	0.98	0.62	0.41	
1-Butylpyridinium				
Bis(trifluoromethylsulfonyl)imide	0.90	0.52	0.18	
Tetrafluoroborate	1.08	0.53	0.21	
1-Butyl-3-methylpyridinium				
Bis(trifluoromethylsulfonyl)imide	0.97	0.54	0.28	
Ethylammonium				
Nitrate	1.24	0.85	0.46	
Formate	0.80	0.85	0.73	
Di-*n*-propylammonium				
Thiocyanate	1.16	0.97	0.39	
Tetrabutylammonium				
2-[Bis(2-hydroxyethyl)amino]ethanesulfonate	1.07	0.14	0.81	
2-(Cyclohexylamino)ethanesulfonate	1.01	0.34	0.98	
2-Hydroxy-4-morpholinopropanesulfonate	1.07	0.03	0.74	
Cholinium				
Levulinate	1.00	1.07	1.03	
Malonate	1.04	1.55	0.62	
Glycolate	1.08	1.29	0.79	
(iii) Deep eutectic solvents				
Cholinium chloride: levulinic acid	1.00	0.51	0.57	
Cholinium chloride: malonic acid	1.08	1.39	0.42	
Cholinium chloride: glycolic acid	1.08	0.49	1.08	
Cholinium chloride: urea	1.14	1.42	0.50	

Continued

TABLE 2.15 Solvatochromic Parameters for Some Representative Green Solvents—cont'd

	Solvatochromic Parameters			Viscosity (cP)
Solvent	π^*	α	β	
Cholinium chloride: ethylene glycol	1.07	1.47	0.57	
Menthol: acetic acid	0.53	1.64	0.60	
Menthol: octanoic acid	0.41	1.77	0.50	
Tetrabutylammonium chloride: octanoic acid	0.76	1.41	0.99	
Tetrabutylammonium chloride: octanoic acid (1:2)	0.80	0.84	1.19	
Tetrabutylammonium bromide: octanoic acid (1:2)	0.84	0.98	1.09	
Tetrapropylammonium chloride: octanoic acid (1:2)	0.80	0.90	0.96	

viscosity for liquid-liquid extraction. The use of bio-derived solvents for extraction in laboratory studies has received little attention so far as their novelty lies in their production and not in their solvation properties [58].

2.5.2 Surfactant-based solvents

Surfactants self-assemble into aggregates, called micelles, at concentrations exceeding a critical value, the critical micelle concentration, in both aqueous and organic solvents. Surfactants are characterized by their head groups (anionic, cationic, zwitterionic, or neutral) connected to a long alkyl chain, typically, which may contain polar substituents. Micelles are dynamic structures made up of surfactant monomers with their hydrocarbon chains packed into a central core surrounded by the polar head groups when dispersed in water. Compared with conventional solvents, they are spatially heterogeneous (the core region is hydrocarbon "like" and largely anhydrous, and the surface region is polar and solvated by water) [69, 70]. On account of their small size and shape, they have a high surface-to-volume ratio and can be considered interfacial solvents. In addition, the size, shape, and aggregation number of the micelles depend on their immediate environment (ionic strength, ion type, pH, etc.). The spatial heterogeneity of micelles has fueled speculation that solutes of different polarity are localized in different regions of the micelle, while other models suggest a more homogeneous environment for all compounds. To accommodate these different opinions, it was suggested that the solubility regime might be different for low sample concentrations, for example, extraction from dilute solutions, compared with processes such as detergency, where higher sample concentrations are common [71]. The mechanism for extraction may be unclear, but surfactant-based

solvents are in common use with several practical applications developed based on cloud point and coacervate extraction techniques [70, 72].

Surfactant-based solvent systems for extraction from water are not well characterized. The solvation parameter model was used to evaluate the transfer of neutral organic compounds from the gas phase to aqueous sodium dodecyl sulfate solutions ($e = 0.436$, $s = -0.492$, $a = -0.211$, $b = -1.747$, and $l = 2.883$) [73] and to aqueous cetyltrimethylammonium bromide solutions ($e = 0.808$, $s = -0.628$, $a = 0.808$, $b = -2.612$, and $l = 2.918$) [74] at surfactant concentration above the critical micelle concentration. Compared with traditional organic solvents, the micellar phases are quite polar and competitive with water as a hydrogen-bond base. Water is more cohesive, dipolar/polarizable, and stronger hydrogen-bond acid. The results for water-micelle partition constants were found to be predictable from retention factors obtained by micellar electrokinetic chromatography (MEKC), and the large surfactant databases available for MEKC might prove useful for selecting surfactant-based aqueous phases for extraction [75, 76].

2.5.3 Ionic liquids

Ionic liquids are low melting point-salts forming liquids composed entirely of ions. Room-temperature ionic liquids are a subset of ionic liquids with melting points below room temperature and are the most interesting for solvent extraction [59, 77–79]. Several hundred room-temperature ionic liquids are now known, and an increasing number are commercially available. The main types of room-temperature ionic liquids are alkylammonium, tetraalkylammonium, tetraalkylphosphonium, 1,3-dialkylimidazolium, guanidinium, and *N*-methylpyridinium salts formed with organic and inorganic anions such as bis(trifluoromethylsulfonyl)imide, hexafluorophosphate, tetrafluoroborate, perfluoroalkylsulfonate, and chloride. Some representative room-temperature ionic liquids and their physicochemical properties are summarized in Table 2.16 [59]. Qualitatively low ion symmetry and effective charge delocalization or shielding for one or both ions and weak hydrogen bonding between ions favor the formation of organic salts with a low melting point. The density of typical room-temperature ionic liquids is greater than water (>1.0 g/mL) and favors phase separation in two-phase systems with water or organic solvents. The viscosity of room-temperature ionic liquids is strongly correlated with the properties of the anion: lower viscosity is associated with small anions with a diffuse negative charge and a limited capability for hydrogen bonding. Low is a relative term and typically refers to values >30 cP, while individual values span the range from about 30 to $>10^3$ cP [77]. These high viscosities stabilize suspended droplets utilized in some liquid-phase microextraction techniques but otherwise contribute to poor penetration of porous solid materials and restrict mass transfer at solvent interfaces. The viscosity of room-temperature ionic liquids can be lowered into a useful range for some

TABLE 2.16 Representative Physical Properties for Typical Room-Temperature Ionic Liquids Used as Solvents for Extraction at 25°C

Ionic Liquid	Density (g/mL)	Viscosity (cP)	Surface Tension (mN/m)	Water Solubility (g/100 mL)
1-Ethyl-3-methylimidazolium				
Bis(trifluoromethylsulfonyl) imide	1.470	37	43.4	
Tetrafluoroborate	1.297 (20°C)	38 (20°C)	48.2 (20°C)	
Thiocyanate	1.116	24.5	55.3	
Trifluoroacetate	1.285	25 (30°C)		
Trifluoromethylsulfonate	1.209	42.9	41.3	
1-Butyl-3-methylimidazolium				
Bis(trifluoromethylsulfonyl) imide	1.429	52	32.5	0.80
Hexafluorophosphate	1.373	450		1.88
Tetrafluoroborate	1.208	95	43.8	Miscible
Thiocyanate	1.070	52	45.9	
Trifluoromethylsulfonate	1.290	90 (20°C)	22.9 (20°C)	
1-Butyl-2,3-dimethylimidazolium				
Bis(trifluoromethylsulfonyl) imide	1.40	67.4	23.4	0.56
Hexafluorophosphate	1.38	393	48.8	1.88
1-Hexyl-3-methylimidazolium				
Bis(trifluoromethylsulfonyl) imide	1.377	71	30.8	0.34
Chloride	1.034	18,000		Miscible
Hexafluorophosphate	1.294	483	54.3	0.75
Tetrafluoroborate	1.208	314 (20° C)		Miscible
Trifluoromethylsulfonate	1.233	116		
Tris(pentafluoroethyl) trifluorophosphate	1.557	119	33.2	
1-Hexyl-2,3-dimethylimidazolium				
Bis(trifluoromethylsulfonyl) imide	1.340	89.8	31.8	0.22

TABLE 2.16 Representative Physical Properties for Typical Room-Temperature Ionic Liquids Used as Solvents for Extraction at 25°C—cont'd

Ionic Liquid	Density (g/mL)	Viscosity (cP)	Surface Tension (mN/m)	Water Solubility (g/100 mL)
Hexafluorophosphate	1.30	560	43.4	0.75
1-Octyl-3-methylimidazolium				
Bis(trifluoromethylsulfonyl) imide	1.310	70.1	30.3	0.10
Hexafluorophosphate	1.238	682		0.20
Tetrafluoroborate	1.110	439		
Trifluoromethylsulfonate		492		
1-Butylpyridinium				
Bis(trifluoromethylsulfonyl) imide	1.462	76	35	
Tetrafluoroborate	1.217 (20°C)	75 (20°C)		
1-Butyl-3-methylpyridinium				
Bis(trifluoromethylsulfonyl) imide	1.429	82	35.8	
Trifluoromethylsulfonate	1.306	142	38.6	
Tetrafluoroborate	1.189	226	47.5	
Tris(pentafluoroethyl) trifluorophosphate	1.595	135	35.3	
Tetradecyl(trihexyl)phosphonium				
Chloride		18		
Phosphinate	0.891			
Tris(pentafluoroethyl) trifluorophosphate	1.182	588	30.4	

application by increasing the temperature or dilution by a miscible organic solvent. Ionic liquids absorb ultrasound and microwave energy efficiently and are suitable for ultrasound-assisted and microwave-assisted extraction techniques. The surface tension of room-temperature ionic liquids is typically lower than water and similar to polar organic solvents. As green chemistry solvents, their favorable properties include low or negligible vapor pressure, an ability to dissolve a wide range of inorganic and organic compounds, high thermal stability, low flammability, and low toxicity (although there are contrary reports with respect to toxicity).

Solvatochromic parameters for representative room-temperature ionic liquids are summarized in Table 2.15 and can be compared with the values for typical organic

solvents in Table 2.6 [59, 68, 77, 78, 80]. The solvatochromic parameters for the room-temperature ionic liquids fall close to the range 0–1 typical of organic solvents. They are generally strong hydrogen-bond acids occupying the top half of the α scale, which contains the aliphatic alcohols, and modest hydrogen-bond bases occupying (mostly) the lower half of the β scale, containing esters and ketones, and they are dipolar/polarizable with π^* values close to 1 and similar to dimethyl sulfoxide. Three features stand out from the comparison: (1) the room-temperature ionic liquids cover only a small portion of the selectivity space for the organic solvents, (2) the room-temperature ionic liquids have similar properties to polar organic solvents and do not exhibit exceptional solvation properties for neutral compounds, and (3) none of the ionic liquids are low-polarity solvents.

The system constants (solvation parameter model) for the transfer of neutral organic compounds from the gas phase to some representative room-temperature ionic liquids are summarized in Table 2.17 [58, 78, 81]. The number of ionic liquids is too few to make global statements covering all possible room-temperature ionic liquids, especially since the solvation properties primarily reflect the identity of the anion with the cation having a secondary role. After classification by hierarchical cluster analysis with some common organic solvents, three ionic liquids 1-ethyl-3-methylimidazolium ethanesulfonate, 1-ethyl-3-methylimidazolium trifluoroacetate, and *N*-butylpyridinium ethanesulfonate entered the selectivity group containing dimethyl sulfoxide. These solvents are characterized by high cohesive energy and are strongly dipolar/polarizable, strong hydrogen-bond bases, and weak hydrogen-bond acids. Five of the room-temperature ionic liquids, all containing a bis(trifluoromethylsulfonyl)imide anion, are grouped with the aprotic dipolar organic solvents represented by acetonitrile. These solvents are cohesive, strongly dipolar/polarizable, and moderate hydrogen-bond acids and bases. The narrow range of the system constants with variation of the cation supports the important role of the anion in establishing the solvation properties of the ionic liquids. As the anion contains no hydrogen atoms, it cannot be responsible for the hydrogen-bond acidity of the ionic liquids. Three of the room-temperature ionic liquids with a tetrafluoroborate anion (and the single example with a trifluoromethanesulfonate anion) form a separate group to the organic solvents while retaining some properties in common with the aprotic dipolar organic solvents. They can be distinguished from the latter by their a/s ratio ≈ 1.5 compared with 1.0 for the aprotic dipolar organic solvents. The system constants for the room-temperature ionic liquids in Table 2.17 fall into the range $e = -0.62$–0.86, $s = 1.6$–2.85, $a = 2.1$–7.3, $b = 0$–1.07, and $l = 0.35$–0.96. This can be compared with the range for polar organic solvents and water $e = -0.60$–0.82, $s = 0.54$–2.8, $a = 0.28$–5.50, $b = 0$–4.8, and $l = -0.21$–0.98. Overall, some selectivity differences are observed for the ionic liquids in terms of the blend of intermolecular interactions, while the selectivity space defined by the

TABLE 2.17 System Constants at 25°C (Except as Noted) for Transfer of Neutral Compounds From the Gas Phase to Some Representative Room-Temperature Ionic Liquid

	System Constants				
Ionic Liquid	***e***	***s***	***a***	***b***	***l***
1-Butyl-3-methylimidazolium					
Bis(trifluoromethylsulfonyl)imide	0.148	1.946	2.261	0.872	0.688
Trifluoromethanesulfonate	0.567	1.987	3.615	0.857	0.584
Tetrafluoroborate	0.605	2.278	3.427	0.471	0.590
Hexafluorophosphate	−0.087	2.841	2.785	0.140	0.631
1,3-Dimethylimidazolium					
Dimethyl phosphate (39°C)	0.86	2.59	7.27	0	0.35
1-Ethyl-3-methylimidazolium					
Diethyl phosphate (39°C)	0.26	1.97	6.90	0	0.54
Tetrafluoroacetate	0	2.694	5.462	0.734	0.669
Ethyl sulfate	0.137	2.544	5.262	0.042	0.592
Bis(trifluoromethylsulfonyl)imide	0.205	2.304	2.194	1.072	0.641
Trihexyl(tetradecyl)phosphonium					
Bis(trifluoromethylsulfonyl)imide	−0.619	1.666	2.262	0.03	0.957
1-Hexyl-3-methylimidazolium					
Bis(trifluoromethylsulfonyl)imide	−0.116	2.079	2.141	0.429	0.704
Trimethylbutylammonium					
Bis(trifluoromethylsulfonyl)imide	0.115	2.047	2.152	0.723	0.627
1-Octyl-3-methylimidazolium					
Tetrafluoroborate	0.100	1.800	3.224	0.453	0.722
4-Methyl-N-butylpyridinium					
Tetrafluoroborate	0.487	2.484	3.190	0.558	0.606
1,2-Dimethyl-3-ethylimidazolium					
Bis(trifluoromethylsulfonyl)imide	0.214	2.347	2.075	0.896	0.655

magnitude of the five system constants is similar for the ionic liquids and typical polar organic solvents. There is further information available for the solvation properties of ionic liquids in the form of system constants acquired by gas chromatography at a wide range of temperatures above room temperature [82–84].

2.5.4 Deep eutectic solvents

Deep eutectic solvents are mixtures of two or more compounds with a freezing point well below the melting point for any of the original mixture components. For extraction, deep eutectic solvents that are liquid at room temperature are the most interesting. A large number of compounds have been used to prepare deep eutectic solvents.

At this time the most common are based on cholinium, phosphonium, or tetraalkylammonium halide salts mixed with a carboxylic acid, urea, ethylene glycol, or carbohydrates of different types [58, 85–87]. A subclass of deep eutectic solvents are the natural deep eutectic solvents prepared from sustainable natural compounds [87–89]. The formation of room-temperature liquid eutectics is favored by the mixing of a hydrogen-bond acceptor (e.g., a quaternary ammonium salt) with a strong hydrogen-bond donor (e.g., a carboxylic acid). The room-temperature deep eutectic solvents are typically of higher density than water (1.0–1.6 g/mL) and are generally viscous solvents (>100 cP) [86, 87]. The inherent high viscosity results in slow mass transfer across phase boundaries and slow mass transport within porous matrices. Dilution with an organic solvent or an increase in temperature assists in lowering the viscosity into a more useful range. Interest in these new solvents remains high at this time, and a significant number of applications have been proposed with new eutectic mixtures continually being identified [85–89].

Solvatochromic parameters were used to characterize the solvation properties of several deep eutectic solvents [90, 91]. Representative values are collected in Table 2.15. The deep eutectic solvents are generally more dipolar/polarizable than typical organic solvents and most ionic liquids. They are typically strong hydrogen-bond acids and bases compared with conventional organic solvents and ionic liquids. The hydrogen-bond basicity of deep eutectic solvents covers a wide range and is reasonably predictable for homogeneous mixture components. Other solvatochromic parameters are typically little affected by such variations in mixture composition. The available data are too limited at present to establish a global view of general solvent properties

2.6 Conclusions

Efficient solvent extraction requires the identification of a solvent with the correct combination of physicochemical and solvation properties. Since both properties vary widely for common solvents, selection starts by defining the requirements for the extraction and then matching these requirements against the known properties of available solvents. Physicochemical properties are generally well characterized. Solvation properties, however, rely upon classification methods, some of which fail to realistically place solvent into selectivity groups where solvents within a group have similar extraction properties and those in different groups another kind of selectivity. Individual selectivity groups typically contain solvents with a blend of properties defined in terms of their capacity for fundamental intermolecular interactions. Selectivity groups with a single dominant interaction are uncommon. Therefore solvents for a particular application should be selected based on the complementary properties of the target compounds and the solvents within a given selectivity group. Within

group, solvents usually possess quite different physicochemical properties allowing for fine-tuning of the selection process. In recent years, method development has favored approaches using green techniques and solvents. Increasingly, this is becoming an additional criterion for solvent selection.

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Chapter 3

Aqueous-Organic Biphasic Systems: Extraction of Organic Compounds

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Chapter Outline

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3.1 Introduction

Liquid-liquid extraction (LLE) methods are based on the distribution of one or more compounds between two immiscible solvents. A solute that is soluble in both phases will distribute between these two phases in definite proportions that depend on the experimental conditions. The equilibrium condition is achieved when the free energy of the solute is the same in both phases.

LLE separation methods are applied to the separation of liquids (liquid samples or compounds in solution) and are practiced commonly both in industries and laboratories. Extraction procedures can be applicable to both trace amounts and also to large concentration levels. Often, one or more components can be selectively extracted from one solution into the other immiscible phase and/or reextracted from the second phase into the first one. The desired separation can be achieved by the correct selection of the solvents and the simultaneous adjustment of several parameters: solvent volumes, pH, additives, masking agents, ionic strength, method of extraction, etc. These solvent extractions offer speed, simplicity, and convenience. Many extractions can typically be performed in a few minutes employing very simple apparatus, and when the extraction is conducted under equilibrium conditions, it is possible to predict accurately the efficiency of this extraction by applying the principles of chemical equilibrium.

Although numerous combinations of two immiscible phases can be envisaged, in many practical situations, one phase is an aqueous medium and the other an organic solvent. This chapter aims to provide the background theory for LLE of organic compounds between an aqueous solution and organic solvents, including the theoretical elements for the criteria in the selection of the extracting solvent and the working conditions. At the end of this chapter, the aims of using an LLE technique in different application fields are cited.

3.2 Fundamentals

3.2.1 Partition constant and distribution constants

Liquid-liquid partition is the basic mechanism underlying many separative techniques, including LLE and several liquid chromatographic separations. The distribution of a

solute is a dynamic process that involves the movement of species across the interphase of the two immiscible liquids. Thus, for the partition of a solute A between two phases α and β, the equilibrium is represented as

$$A_\alpha \rightleftarrows A_\beta \tag{3.1}$$

The partition equilibrium constant is related to the free energy required to transport one mole of A from phase α to phase β. In this chapter, we focus on the distribution between an aqueous and an organic phase. Then, expression (3.1) will be rewritten as

$$A_w \rightleftarrows A_{org} \tag{3.2}$$

where A_w and A_{org} denote the solute in aqueous solution and in the organic solvent, respectively. The chemical potential of the solute in each phase is represented by

$$\text{Aqueous phase, w}: \mu_{A,w} = \mu^\circ_{A,w} + RT \ \ln a_{A,w} \tag{3.3}$$

$$\text{Organic phase, org}: \mu_{A,org} = \mu^\circ_{A,org} + RT \ \ln a_{A,org} \tag{3.4}$$

where $\mu_{A,i}$ represents the chemical potential of compound A in phase i when the activity of A in this phase is $a_{A,i}$ and $\mu^\circ_{A,i}$ is the corresponding chemical potential of A in the standard state in phase i. Once the equilibrium is reached, the chemical potentials of A are identical in both phases, i.e.,

$$\mu^\circ_{A,w} + RT \ \ln a_{A,w} = \mu^\circ_{A,org} + RT \ \ln a_{A,org} \tag{3.5}$$

and rearranging

$$\exp(-\Delta\mu^\circ_A)/RT = a_{A,org}/a_{A,w} = K^\circ_D \tag{3.6}$$

where $\Delta\mu^\circ_A$ ($=\mu^\circ_{A,org} - \mu^\circ_{A,w}$) denotes the difference between the chemical potentials of compound A in its standard state in each solvent. Since $\mu^\circ_{A,org}$ and $\mu^\circ_{A,w}$ values are constants at a given pressure and temperature, $\Delta\mu^\circ_A$ is likewise constant, and K°_D is the thermodynamic constant for the partition process of A between the organic and the aqueous phases.

By introducing the solute activity coefficients in the respective phases,

$$a_{A,i} = \gamma_{A,i} C_{A,i} \tag{3.7}$$

where $\gamma_{A,i}$ denotes the activity coefficient for A in the phase i and $C_{A,i}$ the solute concentration in that phase. The relationship between the partition constant, K°_D, and the ratio of concentrations at the equilibrium is.

$$K^o_D = (C_{A,org}/C_{A,w})\left(\gamma_{A,org}/\gamma_{A,w}\right) = K_D\left(\gamma_{A,org}/\gamma_{A,w}\right) \tag{3.8}$$

where K_D is the distribution constant and in parentheses the activity coefficients of the distributing species in each phase is indicated. According with Eq. (3.6) the

standard states chosen for the solute in both phases determine the value of K^{o}_{D} and, then, the solute activities. For solutes in a liquid phase, two possible standard states can be chosen. One is based on the pure solute A at a given temperature and pressure; in this convention the standard chemical potential is independent of composition, that is, $\mu^{\circ}_{A,org} = \mu^{\circ}_{A,w}$ and therefore $K^{o}_{D} = 1$. The other convention is based on the hypothetical properties of the pure solute behaving as if it were infinitely diluted in the solution. In this last case, $\gamma_{A,i} = 1$ and $K^{o}_{D} = K_{D}$.

The activity coefficient can be considered as a correction term between concentration and activity. Its value is determined by the interactions between the solute molecules and the surrounding solvent molecules. Thus, when the activity coefficient is unity, the solute behaves as if it were in the ideal state, whereas an activity coefficient less than one indicates that the solute can interact with its neighbors stronger than in the standard state and vice versa. It should be clear that K^{o}_{D} depends on the chosen standard states, whereas K_{D} does not. Indeed, in LLE, K_{D} is more important than K^{o}_{D}. Estimations of K_{D} values can be obtained from the solubility parameters or, otherwise, by using extrathermodynamic models, as discussed in the succeeding text.

In the case that more than one compound is involved in the partition process between phases, each species will distribute independently of all others.

3.2.2 Brief description of molecular interactions

The previous discussion for the partition equilibrium is based on the thermodynamics of the biphasic system, that is, on the macroscopic physicochemical properties. A different approach for estimating the distribution constant is based on the discussion of the nature of the physical forces that can exist between *uncharged* species in each phase. The phase distribution equilibrium is the result of the relative attraction or repulsion that solute molecules experience in each liquid phase compared with the forces for the solvent molecules in the pure liquid state. These physical interactions are of relatively low energy in comparison with those involving ionic species. They include van der Waals (dispersion, dipole-dipole interaction, and dipole-induced dipole interactions) and specific interactions. A range of values for the energy of each specific interaction are provided in Table 3.1.

3.2.2.1 Dispersive Interactions

Dispersion is the only interaction present between two nonpolar molecules; it is manifested by the fact that these nonpolar molecules can exist in liquid and even in solid states. These interactions are produced by the varying movement between nuclei and electrons of a molecule that induce a change in the random electronic movement of the neighboring atoms or molecules with the formation of instantaneous dipoles.

TABLE 3.1 Energy Values Involved in Different Molecular Interactions

Interactions	Energy Range (kJ mol^{-1})
Dispersive (London forces)	8–30
Dipole-induced dipoles (Debye forces)	4–8
Dipole-dipole (Keesom)	4–12
Hydrogen bonding	10–40

Source: *References [1, 2].*

These interactions, known as London forces [3], increase with the number of electrons in the molecule, that is, with the molecular volume. Due to its unstable nature, dispersion forces are relatively weak, and they decrease with the six power of the interatomic distance. These forces, however, can be additive over large numbers of atoms in a system (see Table 3.1).

As a general rule a nonpolar solute mixes with the nonpolar solvent in all proportions, since the attraction between the solvent molecules is weak and does not reject to host other nonpolar molecules.

3.2.2.2 Dipole-Dipole Interactions (Keesom Forces)

Molecules that contain centers of positive and negative charge in different locations are electric dipoles. These electric asymmetries are found in localized polar bonds between atoms of different electronegativity in a molecule. A polar solvent is one composed of these permanent dipoles. These solvent molecules will be orientated in this medium, and the energy of attraction will increase for an ordered array with positive and negative clouds close together. These electric interactions are relatively strong at room temperature, but they weaken readily as temperature is increased due to the increased thermal movement of the molecules [3].

These molecules will be strongly attracted to others of similar characteristics. The inclusion of a solute molecule will be possible if the attraction of the solute by the solvent is strong enough to overcome the interactions between solvent molecules to apart them to host the solute and mix with it. The attractive energy between these two dipoles depends on the square of the permanent dipolar moments of the solute and solvent molecules and on $1/r^6$, where r denotes the molecular distance.

3.2.2.3 Inductive Interactions

When a molecule with a permanent dipole approaches to a nonpolar molecule (or to a nonpolar moiety in another chemically equal molecule), the former induces a temporary dipole in the latter so that an attractive interaction takes place. These

dipole-induced dipole forces, known as Debye interactions, are stronger for molecules with a relatively mobile electronic structure. The induction energies increase with the dipolar moment of the permanent dipole and with the polarizability of the nonpolar molecule. The molecular dipole moment of a molecule, however, is not a representative property of the polarity of that molecule. The correlation between attractive inductive and dipole-dipole energies with dipolar moments is not exact since adjacent molecules in a liquid phase will interact through the individual dipoles in a localized zone of the molecule, so these electrostatic attractions can be larger than those expected according to the experimental dipole moment [4]. Carbon tetrachloride is an example of solvent that can exert local inductive interactions even when its net dipole moment is zero.

3.2.2.4 Hydrogen-Bond Interactions

Dipolar molecules that can interact through hydrogen bonding (HB) interactions are exceptional in their behavior. The hydrogen atom forms only one covalent bond, but when it is covalently bonded to an electronegative atom, its small size and the lack of inner closed electron shells make it possible to interact with closer electronegative atoms outside (intermolecular) or inside (intramolecular) the molecule. This may occur between a molecule with a hydrogen atom attached to fluorine, oxygen, or nitrogen or, in certain cases, carbon. A few examples are

$$HOH\cdots O(H_2),\ R_3N\cdots HOR \text{ or } Cl_3CH\cdots O=C(R_1R_2)$$

In the last example the electronegativity of the chlorine atoms attached to carbon confers an acidic character to the hydrogen in the C—H bond. The strength of a HB interaction depends on the geometry of the particular combinations. The most common orientation has an angle of 180° between the three atoms involved. The strength of this interaction also depends on the nature of neighboring atoms and their acid-base character.

The organic compounds that can participate in HB interactions can be classified as follows:

I. Hydrogen-bond donors (HBD): compounds with an active hydrogen, such as acids or $CHCl_3$.
II. Hydrogen-bond acceptors (HBA): compounds that contain an electronegative atom but no active H, such as ethers, ketones, aldehydes, esters, tertiary amines, and nitriles.
III. Compounds containing both an electronegative atom and an active hydrogen atom (alcohols, fatty acids, phenols, primary and secondary amines, and nitroalkanes with hydrogen in an alpha-position).
IV. Compounds capable of forming networks of multiple H bonds, the cage effect exemplified by water, glycols, aminoalcohols, and hydroxyacids.

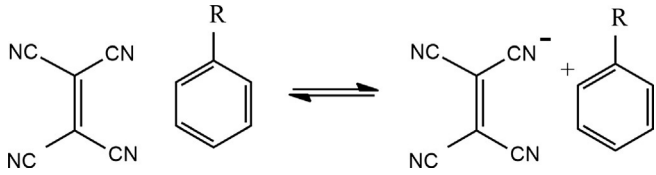

Fig. 3.1 Charge transfer complex between tetracyanoethylene and alkylbenzene.

There are no simple general physical properties that can readily estimate hydrogen-bond strength [5, 6]. Empirical parameters, such as the solvatochromic parameters, have been shown to be useful to account for a compounds HB donor or acceptor capabilities.

3.2.2.5 Charge Transfer Interactions

Hydrogen bonding is one class of specific interactions of an acid-base character or donor-acceptor transfer reactions. One type of specific interaction occurring between one electron pair donor compound (Lewis base) and one electron pair acceptor (or Lewis acid) is named a charge transfer interaction. This donor-acceptor type of bond can take place between compounds with a high polarizability, and these compounds can be n, σ, or π electron donors or acceptors. This interaction leads to the formation of a complex with ionic character. An example of this type of interaction is that between a nitrile, such as tetracyanoethylene, and alkylbenzenes (Fig. 3.1).

The total energy of interaction of the molecule A with the neighboring solvent molecules in the phase is the sum of the energies involved in each one interacting pair of atoms or molecular moieties (dispersive, inductive, dipolar, and specific, if any).

The use of mixed solvents in LLE often improves the distribution in favor of the organic phase through the additive effects of the interactions exerted by the mixture of more than one organic solvent; for example, consider an acid as a solute with HBD and HBA features: it can be extracted with higher yields using a solvent mixture of a ketone and an alcohol. Synergistic effects can often be explained by considering this effect.

Physical and chemical properties of some solvents relevant to their uses in LLE are gathered in Table 3.2. References for other solvents can be found in Refs. [7, 11–15].

3.2.3 Predictive Models of the Distribution Constants

The choice of an appropriate organic solvent for an extraction of particular interest is usually based on empirical evaluations. In general the partition of an organic compound between water and a solvent is closely related to its solubility in water, because this solubility can be considered as partition of a substance between water

TABLE 3.2 Physical and Chemical Properties of Solvents Commonly Used in Aqueous/Organic LLE

Solvent	Density (g/mL), 25°C	Solubility in Water (%w.w)	Solubility of Water	Solubility Parameter $(cal/cm^3)^{0.5}$	Dipole Moment (D)	Dielectric Constant	Solvatochromic Parameters[b]			Polarizability $(A^{\circ 3})$[c]
							α	β	π^*	
Pentane	0.621	0	0.01	7	0	1.84	0	0	−0.15	9.98
Hexane	0.655	0	0.01[a]	7.3	0.09	1.89	0	0	−0.11	11.83
Heptane	0.679	0	0.01	7.4	0	1.92	0	0	0	13.68
Cyclohexane	0.774	0.01	0.01[a]	8.2	0	2.02	0	0	0	11.04[d]
Benzene	0.869	0.18	0.06	9.2	0	2.28	0	0.1	0.55	10.4
Toluene	0.862	0.05	0.06	8.9	0.31	2.33	0	0.11	0.49	12.25
Methylene chloride	1.316	1.3	0.2	9.7	1.14	8.93	0.13	0.1	0.82	6.48
Chloroform	1.479	0.815[a]	0.07	9.3	1.15	4.81	0.2	0.1	0.58	8.23
Carbon tetrachloride	1.584	0.08	0.01	8.6	0	2.24	0	0.1	0.21	10.47
1,2-Dichloroethane	1.246	0.81[a]	0.15[a]	9.8	1.86	10.36	0	0.1	0.73	8.43[d]
Diethyl ether	0.708	6.1	1.47	7.4	1.15	4.3	0	0.46	0.27	8.79
Ethyl acetate	0.895	7.94	3.01	9.1	1.78	6.02	0	0.45	0.45	8.87[d]

MIBK	0.796	1.7	1.9	8.4	2.7	13.1	0.02	0.48	0.65	11.98[d]
1-Butanol	0.806	7.5	20.5	11.4	1.75	17.8	0.84	0.84	0.47	8.79[d]
1-Octanol	0.822	0.06	–	10.3	1.76	10.34	0.77	0.81	0.4	16.4[d]
DMSO	1.096	25.3	–	12	3.9	46.7	0	0.76	1	8.03[d]
Nitromethane	1.130	11.1	2.09	12.7	3.56	35.87	0.22	0.06	0.75	4.97[d]
Water	0.997	–	–	23.4	1.85	78.54	1.17	0.47	1.09	1.45

[a] Values at 20°C. Taken from Ref. [7].
[b] (Kamlet-Taft) hydrogen-bond donation ability (α), hydrogen-bond acceptor ability (β), and polarity/polarizability parameter (π^*). Taken from Ref. [8].
[c] Taken from Ref. [9].
[d] Taken from Ref. [10].

and its own liquid phase. Several other methods for the estimation of partition constants have been proposed. In this chapter, estimations of K_D through the regular solution theory and by the linear solvation energy relationship model are briefly discussed.

For LLE extractions in which strong interactions or secondary equilibriums are absent, an estimate of the solute distribution between two solvents, K_D, can be made from the Hildebrand's regular solution theory (RST) [16]. The combination of the equations for the chemical potential (either Eq. (3.3) or Eq. (3.4)) with Eq. (3.7) gives

$$\mu_{A,i} = \mu^{\circ}{}_{A,i} + RT \ \ln\gamma_{A,i} + RT \ \ln C_{A,i} \tag{3.9}$$

RST assumes that the entropy change and volume of mixing are negligible, and the departure from ideality is only due to the enthalpy of mixing. Thus,

$$\Delta H^{\circ}{}_{i} = RT \ \ln\gamma_{A,i} \tag{3.10}$$

where $\Delta H^{\circ}{}_{i}$ is the heat of mixing a mole of A in a large volume of phase i to form a dilute solution. This enthalpy of mixing equals the energy since the total volume is constant, and it can be expressed as the sum of the energy of vaporization of A, the energy required to form a cavity in phase i, and that of interaction of A with the neighboring solvent molecules. RST predicts that

$$RT \ \ln\gamma_{A,i} = V_A(c_{AA} + c_{ii} - 2c_{Ai}) \tag{3.11}$$

where c_{AA}, c_{ii}, and c_{Ai} are the cohesive energy densities (ratio of energy of vaporization and molar volume of pure liquids) and V_A is the solute's molar volume. It is also assumed that c_{Ai} can be estimated as the geometric mean: $c_{Ai} = (c_{AA}c_{ii})^{0.5}$.

By defining the solubility parameter, δ, as the square root of the cohesive energy density, the regular solution equation is obtained:

$$\gamma_{A,i} = \exp\frac{V_A}{RT}[\delta_A - \delta_i]^2 \tag{3.12}$$

According to this equation, deviations from ideal behavior will be positive in regular solutions and larger as the solubility parameter difference increases. By replacing Eq. (3.12) in the expression (3.8) and using the convention $K^{\circ}{}_{D} = 1$, the resulting expression is

$$K_{D,A} = \exp\frac{V_A}{RT}\left[(\delta_A - \delta_w)^2 - (\delta_A - \delta_{org})^2\right] \tag{3.13}$$

The importance of this equation is that K_D can be computed from properties of the pure components. The theory predicts that when δ_A is closer to that of the organic solvent (δ_{org}) than that of water, $K_{D,A}$ will be $\gg 1$. The solubility parameters δ_i are tabulated for many compounds [17]. Table 3.2 summarizes δ-values for commonly used solvents in LLE.

The RST expression was derived from considering only dispersive interactions. Thus, it is less accurate for polar compounds that can interact also by dipolar interactions, and the failure in predictions are more pronounced for compounds with HBD or HBA capability. Indeed, the solutions formed by such compounds in polar solvents usually have negative deviations from ideality ($\gamma_A < 1$). Other more sophisticated models for predicting solvent-solute interactions were derived from regular solution theory [5, 18]. They are based on how to divide the δ-parameter into contributions from different types of interactions based on extrathermodynamic considerations.

One of the most widely used extrathermodynamic models is the multiparametric linear solvation energy relationship (LSER) derived from the model of Kamlet and Taft [19–21] proposed to describe the solvent influence on the shifts of the lowest energy absorption bands of specific solvatochromic indicators in the ultraviolet-visible spectral region. The most widely accepted representation of the LSER partitioning model is that developed by Abraham and coworkers that is grounded on free energy considerations for solvent-solvent and solute-solvent interactions:

$$\mathrm{SP} = c + eE + sS + aA + bB + vV \tag{3.14}$$

where SP can be any free energy-related property [22]. In modeling LLE, SP corresponds to the log K_D. The solute-dependent parameter E corresponds to the solute polarizability in excess to the polarizability of an n-alkane of equal size; parameter S accounts for solute dipolarity/polarizability; parameters A and B denote solute hydrogen-bond donating (HBD) and hydrogen-bond accepting (HBA) abilities, respectively; and the parameter V reflects molecular volume. In fact the solute V-parameter accounts for both the formation of a cavity in the solvent and dispersive interactions. The equation coefficients e, s, a, b, and v, along with the constant c, are determined via multiparametric linear least-squares regression analysis of data of log K_D for a large number of solutes covering a wide range of chemical families with known E, S, A, B, and V values. These coefficients correspond to the complementary properties of the solute. For example, since A reflects the solute's HBD ability, the coefficient a is a measure of the difference between the acceptor features of both solvents. Thus a large a-coefficient implies a large difference between the basicity of water and the extraction solvent, and on the contrary, coefficients close to zero are indicative of insignificant difference in the basicity of water and the immiscible organic solvent. Finally the sign (positive or negative) of each coefficient indicates that the corresponding solute property is favorable or not for the solute transfer from one phase to the other. As a general rule the signs of the coefficients depend on how the partition is defined and point out which solvent (water or organic) interacts stronger through the specific interaction modeled by the equation. Similar reasoning can be extended to all other terms of Eq. (3.14).

A large number of solubility-related phenomena and transfer properties have been characterized using LSER equations. Two largely studied systems by the LSER

model are the partitioning between water and *n*-hexadecane and the partitioning between water and 1-octanol as representative models of numerous biological and chemical transfer processes. The LSER equations for solute transfer from water to the organic phase are [23, 24]

$$\log K_{\mathrm{D(water/C16)}} = 0.09 + 4.43V - 1.62S + 0.67E - 3.59A - 4.87B$$

$$r^2 = 0.998, \mathrm{SE} = 0.12, n = 370$$

and

$$\log K_{\mathrm{D(water/1-octanol)}} = 0.09 + 3.84V - 1.05S + 0.56E + 0.03A - 3.46B$$

$$r^2 = 0.997,\ \mathrm{SE} = 0.12,\ n = 613$$

The negative coefficients associated with the solute parameters B, S, and A (in $\log K_{\mathrm{D(water/C16)}}$ equation) indicate that the corresponding solute property is unfavorable for solute transfer to the organic solvent.

LSER equations have been used to characterize numerous separation processes due to the fact that they provide a quantitative measure of solute/solvent system interactions that are generally in close agreement with chemical knowledge. These equations can provide valuable information when applied to new systems. A comprehensive and detailed review on the history and scope of the LSER model in separation science is highly recommended [25].

3.3 Extraction Efficiency

3.3.1 Fraction extracted

The compound extracted fraction, E_{A}, is given by the ratio between the amount of solute A extracted in the organic phase (Q_{org}) and the total amount (Q_{t}). The relationship between the fraction extracted and the distribution constant is given by

$$E_{\mathrm{A}} = Q_{org}/Q_{\mathrm{t}} = C_{org}V_{org}/\left(V_{org}C_{org} + C_{\mathrm{w}}V_{\mathrm{w}}\right) = K_{\mathrm{D}}\beta/(1 + K_{\mathrm{D}}\beta) \tag{3.15}$$

where V_{org} and V_{w} are the volumes of organic and aqueous phase, respectively, and β is the phase ratio ($\beta = V_{\mathrm{org}}/V_{\mathrm{w}}$). Up to the point where the equilibrium holds, the E_{A}, often called the recovery for component A (R_{A}), is independent of its initial concentration.

The concentration of solute left in the aqueous phase, C_w, after one equilibration using volume V_{org} of organic solvent and V_{w} of aqueous phase can be easily deduced from a mass balance:

$$C_{\mathrm{w}} = C_0(1 + K_{\mathrm{D}}\beta)^{-1} \tag{3.16}$$

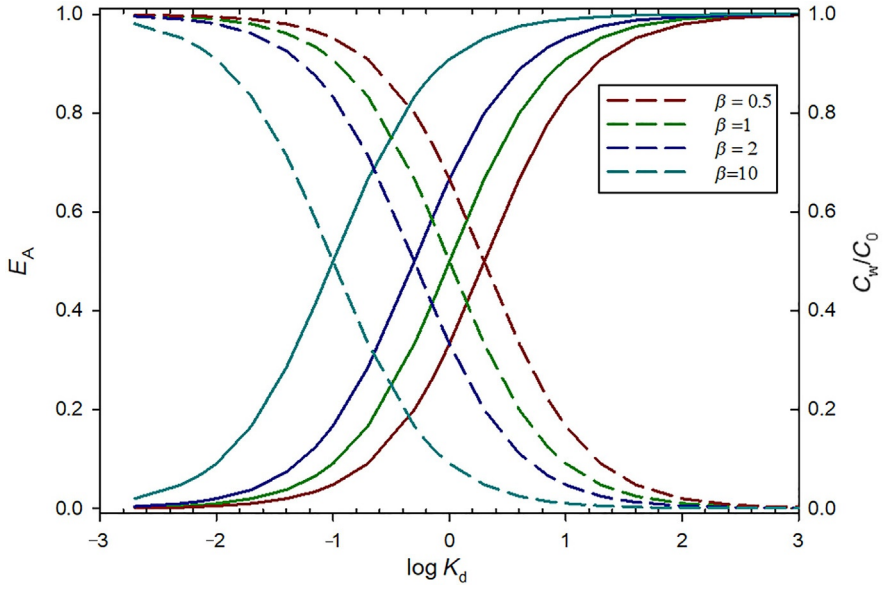

Fig. 3.2 Extracted fraction (left *y*-axis, *full lines*) and remaining concentration ratio (right *y*-axis, *dotted lines*) as a function of log K_D for four β-values.

where C_0 denotes the original concentration of analyte in the aqueous phase. For a phase volume ratio of unity, the denominator of the previous equation reduces to $(K_D + 1)$, and the expression simplifies to

$$C_w = C_0(1 + K_D)^{-1} \tag{3.17}$$

The variation of E_A as a function of K_D is shown in Fig. 3.2 for a single extraction and four different β-values (left y-axis, solid lines). On the right y-axis (doted lines), the remaining analyte concentration relative to the original concentration is also plotted. This figure clearly shows that the completeness of an extraction depends not only on the value of the distribution ratio (an increase in K_D leads to a higher extracted fraction) but also on the volumes of both phases: only when the distribution K_D is large enough an almost complete removal of compound A is possible in one extraction step, that is, E_A approaches 1. The amount of solute remaining after a single extraction tends to zero as the V_{org} approaches infinity, which is not a practical case.

The process of extraction can be improved as desired by repeated batch extractions or by using continuous extraction methods. After *n* contacts using fresh portions of organic solvent to extract the compound from a fixed volume of aqueous phase, the remaining concentration in the aqueous phase is given by

$$C_w(n) = C_0(1 + K_D\beta)^{-n} \tag{3.18}$$

and the extracted fraction $E_A(n)$ with respect to the total amount, assuming that the volume V_w remains constant after n extractions, is given by the expression

$$E_A(n) = 1 - (1 + K_D\beta)^{-n} \tag{3.19}$$

Fig. 3.3A shows the extracted fractions as a function of K_D for three successive extractions and $\beta = 1$ (solid lines) and two plots for $n = 3$ and two values of β (0.33 and 0.1). The extracted fraction increases asymptotically to unity as the number of extractions increases. The comparison of the plot for $n = 1$ and $\beta = 1$ with that for $n = 3$ and $\beta = 0.33$ clearly indicates that for a fixed volume of organic phase, higher efficiency is obtained when more steps are used, regardless of the K_D values. In practice, there is little improvement in extraction efficiency by using more than four or five portions. In Fig. 3.3B the remaining compound concentration with respect to the original is plotted on the left axis (solid lines), whereas on the right y-axis are the plots corresponding to the relative concentration of A in the organic phase with respect to the original concentration (dotted lines). This representation clearly indicates that for analytical purposes the use of many small extracting solvent amounts enhances preconcentration. Thus, for $n \times \beta = 1$, the analyte preserves the original concentration as n increases, whereas a preconcentration can be achieved for $n \times \beta < 1$.

These plots help to determine whether a given extraction is practical with a reasonable β-value or whether an extraction solvent with a more favorable K_D should be chosen.

3.3.2 Selectivity and enrichment factor

One classical application of LLE is the separation of two or more components. The degree of separation achievable depends on the differences in the distribution constants of these components with respect to the two liquid phases. Ideally, one component has high affinity for the organic phase, and the other tends to remain in the aqueous phase. A first measure of the separation of two substances A and X is the separation factor, defined as

$$\alpha = K_{D,X}/K_{D,A} \tag{3.20}$$

Another more useful parameter is the enrichment factor:

$$S_{XA} = E_X(n)/E_A(n) \tag{3.21}$$

In turn the relative concentrations remaining in the aqueous phase can be calculated by the ratio $(1 - E_X(n))/(1 - E_A(n))$.

The choice of extraction solvent and adjustment of the phase ratio determine the enrichment factors. The extraction of two compounds as a function of $K_D\beta$ is shown in Fig. 3.4.

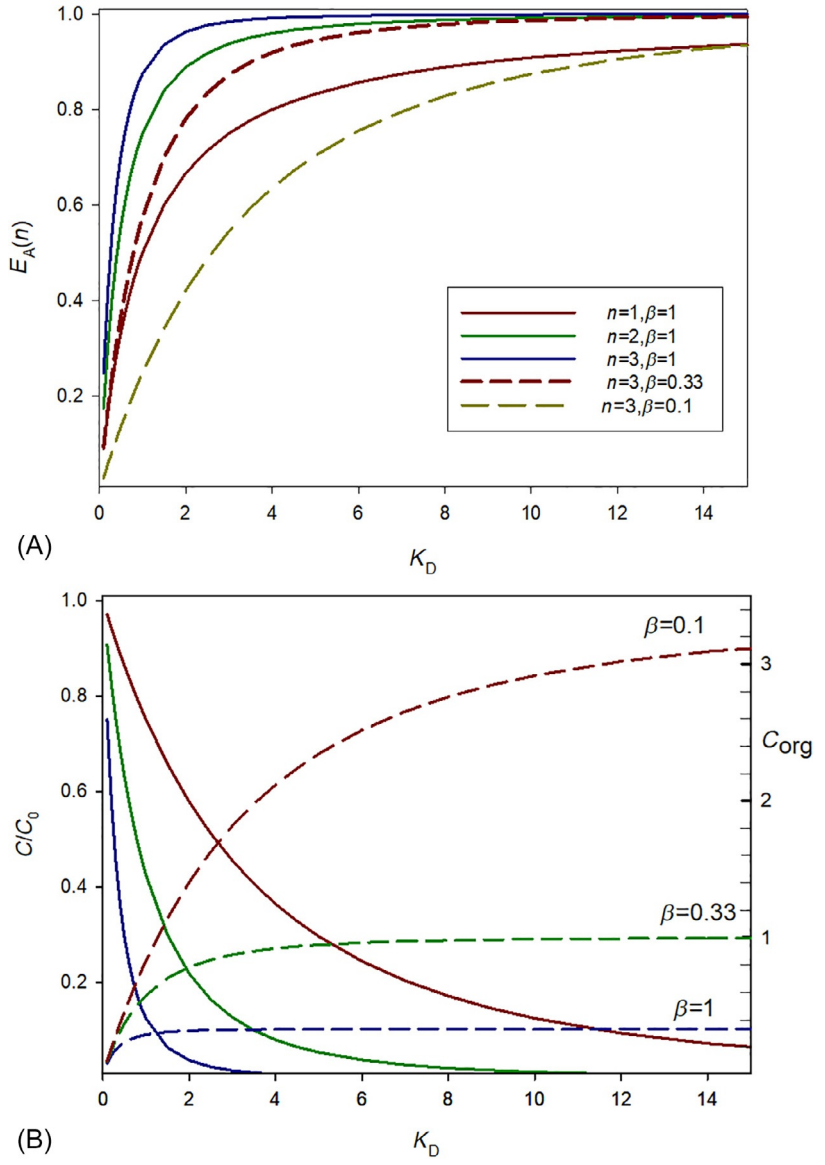

Fig. 3.3 (A) Extracted fraction as a function of K_D for one, two, and three extractions and different β-values. (B): Left *y*-axis, *solid lines*: remaining concentration relative to the original one as a function of K_D and assuming $\beta = 1$. Right axis and *dotted lines*: plots of concentration of compound in the organic phase (relative to the original one) versus K_D for $n = 3$ and three different phase ratios ($\beta = 1$, 0.33, and 0.1).

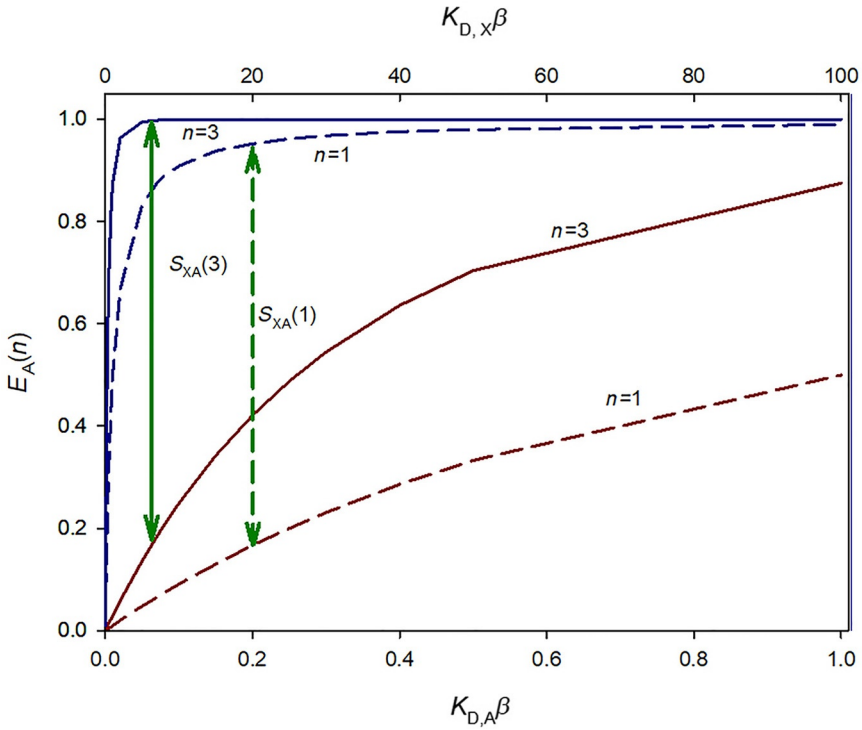

Fig. 3.4 Extracted fractions versus $K_D\beta$ for two compounds with a $K_{D,X}/K_{D,A} = 100$. Compound A: black lines, compound X: gray lines. Solid lines: $n = 3$; dotted lines: $n = 1$. The arrows indicate the enrichment factor at a given $K_D\beta$ value for one and three extractions.

In the plots, it is assumed that $\alpha = 100$. Clearly a compromise must be made between K_D and β- values. The enrichment factor S_{AX} improves as the product $K_D\beta$ decreases. However, very low values for $K_D\beta$ lead to low E_A for both components. Assuming, for instance, that $K_{D,X}\beta = 10$ and $K_{D,A}\beta = 0.001$, a single extraction will remove 90.9% of component A and only 0.1% of component X. A second extraction step of the same aqueous solution not only increases to 99.2% of component *A* in the organic phase but also increases that of component X to 0.2%. A more complete extraction of component *A* results in increased contamination by component *X*. By accepting 0.999 as a satisfactory recovery for analyte *A* ($C_w/C_0 = 0.001$), three extractions are required. The enrichment factor *S* will be 333 after three successive extractions. This means that if equal concentrations are present in the aqueous phase before LLE, the ratio between C_A and C_X in the combined three extracts will be ~1:300.

In practice, quantitative extractability and separability of a solute from others are usually mutually antagonistic, particularly in mixtures of solutes of a similar kind. Better results are achieved more readily when the chemical nature of the compounds is significantly different. Only gradual differences in the extracted fractions are usually found for compounds with similar chemical structures; then, it is necessary to resort to chemical parameters such as pH or masking agents to suppress the extraction of the unwanted component (see later). When the separation factor approaches unity, it becomes necessary to use countercurrent distribution methods in which distribution, transfer, and recombination of various fractions are performed in a sufficient number of times to achieve a separation.

3.4 Secondary Chemical Equilibria. Distribution Ratio

The distribution ratio, D, of a solute between an organic phase and an aqueous phase is defined as

$$D = C_{A,org}/C_{A,w} = \frac{\Sigma[A]_{org}}{\Sigma[A]_{w}} \tag{3.22}$$

where the numerator is the total concentration of solute in the organic phase (denoted by subscript org) and the denominator is the total concentration of A in the aqueous phase. For compounds whose distribution involves only the same chemical form in both phases at equilibrium, the distribution ratio and distribution constants are coincident, that is, $K_D = D$.

The introduction of a reversible chemical reaction for a specific compound into a separation system provides one or more additional variables for controlling the D-values of that individual component. For compounds that are weak electrolytes, for instance, the dissociation or association equilibrium can be the simplest and useful resource to facilitate the phase distribution. As a general rule, ionic species are not easily extractable with organic phases, while nonionic species are more easily extracted; the conversion of an organic acid or an amine in an ionic form by a selective management of the aqueous pH will determine its solubility in the aqueous phase. Similarly the addition of masking reagents or ion-pairing compounds to extract ions selectively or to prevent precipitation is another example of secondary chemical equilibriums to facilitate the physical distribution of components. In these cases the distribution ratio is a more realistic parameter for LLE estimations than the distribution constant. The expressions deduced for the extracted fraction (Eq. (3.15)), for the concentration remaining in the aqueous phase (Eq. (3.18)), and for the enrichment factor (Eq. (3.21)) are easily modified by substituting D for K_D.

3.4.1 Dissociation in aqueous phase. Influence of pH on LLE

Let's describe quantitatively the effect of acid-base equilibrium on the distribution process. The acid-base equilibrium of a monoprotic acid HA in water can be represented as

$$HA \rightleftarrows H^+ + A^-$$

where H^+ denotes the (hydrated) hydrogen ion. The acid dissociation constant, K_a, can be defined by the equation

$$K_a = \frac{a_H a_A}{a_{HA}} \simeq \frac{[H][A]}{[HA]} \tag{3.23}$$

where a_i refers to the activity of the species i and H, A, and HA denote hydrogen ion, deprotonated, and the acidic form of HA, respectively (charges are omitted for simplicity). The fraction α_0 is defined as the ratio between the acid form, HA, and the total concentration C_A:

$$\alpha_0 = \frac{[HA]}{C_{HA}} = \frac{1}{1 + 10^{(pH - pKa)}} \tag{3.24}$$

The distribution of a weak acid, HA, in a biphasic organic solvent/aqueous system can be expressed by Eq. (3.22). We assume that HA does not dissociate nor associate in the organic phase, that is, $C_{A,org} = [HA]_{org}$, whereas in the aqueous phase, the acid-base equilibrium will be determined by the dissociation constant. The substitution of Eq. (3.24) into Eq. (3.22) leads to

$$D = \frac{K_D}{1 + 10^{(pH - pKa)}} = \alpha_0 K_D \tag{3.25}$$

This relationship allows the distribution ratio of HA within the whole range of aqueous pH to be estimated from the corresponding values of the distribution constant and the degree of ionization, α_0. Here, it is assumed that the compound pK_a in water does not change due to the presence of solubilized solvent. In this expression, $D = K_D$ when the pH is significantly smaller than the pK_a. Thus the decrease in pH of the aqueous phase favors extraction into the organic phase. Strictly, however, always a small amount of the compound is dissociated, and in general, $D < K_D$. The same equations are also applicable to bases (pK_a refers to the acidity of the conjugate acid). For bases, extraction into the organic phase can be improved when $pH >> pK_a$.

For polyprotic acids an analogous expression for D is easily derived following the same reasoning, and the final equation for D for an acid with n replaceable hydrogens is given by

$$D = K_D \frac{[H]^n}{[H]^n + K_{a,1}[H]^{n-1} + K_{a,1}K_{a,2}[H]^{n-2} + \ldots + \prod K_{a,n}} = \alpha_0 K_D \tag{3.26}$$

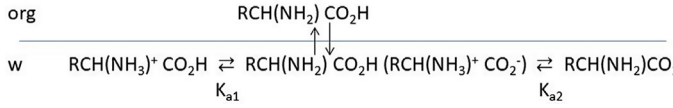

Fig. 3.5 Scheme for the acid-base dissociation of an amino acid and its transfer across the organic solvent-water interface.

Here, $K_{a,n}$ represents the successive dissociation constants for the polyprotic acid. Extraction of polyamines by organic solvents can be estimated from a symmetrical equation ($D = \alpha_n K_D$), where α_n is the ratio between the neutral base and the polyamine's total concentration.

A special case is the extraction of amphoteric compounds. Examples are 8-hydroxyquinoline, which has an acidic group (phenolic OH) and a basic (amine) group and is used to extract ions from aqueous solutions, and compounds like amino acids. The acid-base equilibriums for a neutral amino acid is represented in Fig. 3.5.

Here, $-R$ corresponds to the neutral side chains of amino acids, and K_{a1} and K_{a2} are the successive acid dissociation constants. Considering that the only species extractable in an organic phase will be $RCH(NH_2)CO_2H$, the distribution ratio is

$$D = \frac{K_D K_{a1}[H]}{[H]^2 + K_{a,1}[H] + K_{a,1}K_{a,2}} = \alpha_1 K_D \tag{3.27}$$

A graphic representation of log D as a function of pH is shown in Fig. 3.6. The plot shows the partition of tryptophan between 1-octanol and water at 25°C. Three zones are clearly noted, with the favorable extraction of this amino acid when $pK_{a1} < pH < pK_{a2}$.

Since the distribution ratio is highly dependent on the aqueous pH, compounds with small differences in their acid-base behavior can be separated by multiple contacts between an organic and aqueous phase as in countercurrent LLE or in liquid chromatography.

3.4.2 Association in the Organic Phase

Dimerization and higher degrees of association in the organic phase will lead to an increase in the distribution ratio. Consider, for instance, the association of fatty acids (HA) as dimers in the organic phase. The equilibrium reaction can be expressed as

$$2\,HA_{org} \rightleftarrows (HA)_{2,org}$$

and the dimerization constant K_{dim} is given by

$$K_{dim} = a_{(HA2),org}/a_{HA,org}{}^2 \approx \left[(HA)_2\right]_{org}/[HA]^2{}_{org} \tag{3.28}$$

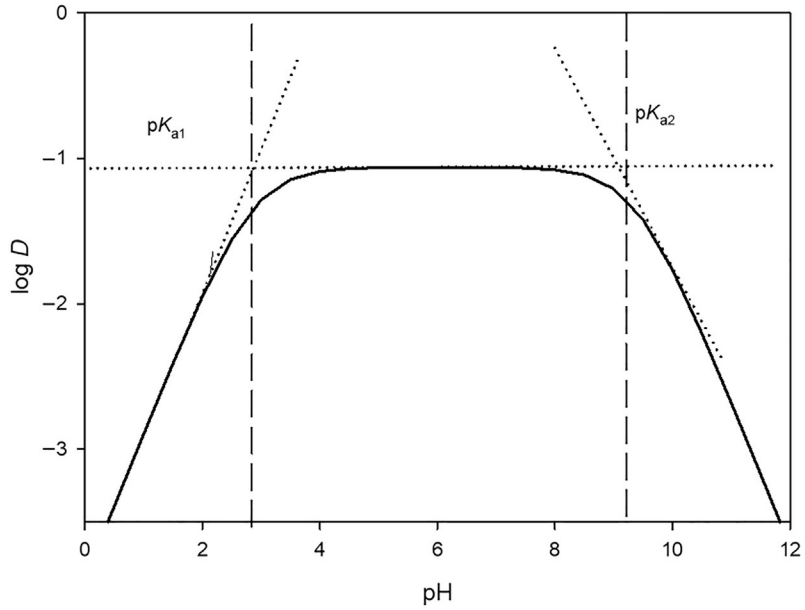

Fig. 3.6 Log D versus pH for the amino acid tryptophan ($pK_{a1} = 2.83$ and $pK_{a2} = 9.39$). Distribution constant between water and 1-octanol taken from Reference [26].

In this expression the possibility of associations in the aqueous phase is neglected. Association, however, can also take place in an aqueous solution at relatively high ionic strengths. It is difficult to describe in quantitative terms a simple equilibrium expression for these systems because a large difference would exist between concentrations and activities. In spite of the uncertainty in the values for the appropriate formation constants, however, the derived expressions are at least qualitatively useful. As a general rule the dimerization of HA favors the overall distribution of HA to the organic phase. The distribution ratio can be written as

$$D = C_{\mathrm{HA},org}/C_{\mathrm{HA,w}} = \left([\mathrm{HA}]_{org} + 2\left[(\mathrm{HA})_2\right]_{org}\right)/[\mathrm{HA}]_{\mathrm{w}} \tag{3.29}$$

The distribution ratio may be expressed in terms of the dimerization constant and the concentration of carboxylic acid in either the organic or the aqueous phase:

$$D = K_{\mathrm{D}}\left(1 + 2K_{\mathrm{dim}}\,[\mathrm{HA}]_{org}\right) = K_{\mathrm{D}} + 2K_{\mathrm{dim}}K_{\mathrm{D}}{}^{2}\,[\mathrm{HA}]_{\mathrm{w}} \tag{3.30}$$

A graph of D as a function of the aqueous concentration of HA results in a linear plot with an intercept K_{D} and slope equal to $2K_{\mathrm{dim}}K_{\mathrm{D}}^2$. The tendency of a carboxylic acid to associate to a dimer depends on the tendency of the RC=O(OH) groups to

form hydrogen bonds and on the length of the aliphatic chain R. The dimerization is, in addition, dependent on the dielectric constant of the organic solvent. Typically, very large dimerization constants are observed in nonpolar solvents such as hexane, benzene, and carbon tetrachloride and smaller ones in chloroform, which is a weak HBD. The dimerization of carboxylic acids is even smaller in HBA solvents such as oxygen-containing solvents (ethers and ketones) and is almost negligible in hydroxyl-containing solvents such as water.

A more general equilibrium describing the tendency to form molecular associations in the organic solvent from n molecules of HA is

$$n\,\mathrm{HA}_{\mathrm{org}} \rightleftarrows (\mathrm{HA})_{n,\mathrm{org}}$$

and the distribution ratio is

$$D = K_{\mathrm{D}}\left(1 + nK_{\mathrm{assoc}}[\mathrm{HA}]_{org}^{\,n-1}\right) = K_{\mathrm{D}} + nK_{\mathrm{assoc}}K_{\mathrm{D}}^{\,n}[\mathrm{HA}]_{\mathrm{w}}^{\,n-1} \tag{3.31}$$

Other representative examples of associations used in LLE include the extraction of metals by complexation with organic reagents (including chelates) and the formation of ion pairs. Both associations are discussed in other chapters.

On the other hand, extraction into the organic phase can be interfered by the formation of associations in the aqueous solution. One classical example is the extraction of iodine molecules by carbon tetrachloride or chloroform, which is decreased in the presence of I^- in the aqueous phase due to the formation, which induce the formation of a complex of I_3^-, insoluble in the organic solvent. The reaction in aqueous phase is

$$\mathrm{I}_2 + \mathrm{I}^- \rightleftarrows \mathrm{I}_3^-$$

The triiodide ion formation constant, $K_{\mathrm{f}} = [\mathrm{I}_3^-]/[\mathrm{I}_2][\mathrm{I}^-]$, and distribution ratio are related by

$$D = \frac{K_{\mathrm{D}}}{1 + K_{\mathrm{f}}[\mathrm{I}^-]_{\mathrm{w}}} \tag{3.32}$$

or

$$\frac{1}{D} = \frac{1}{K_{\mathrm{D}}} + \frac{K_{\mathrm{f}}}{K_{\mathrm{D}}}[\mathrm{I}^-]_{\mathrm{w}} \tag{3.33}$$

where K_{D} has its usual significance. A plot of $1/D$ versus $[\mathrm{I}^-]_{\mathrm{w}}$ is linear with an intercept $1/K_{\mathrm{D}}$ and slope $K_{\mathrm{f}}/K_{\mathrm{D}}$.

3.4.3 Reactive extractions

Organic acids (and also amines) can be extracted from different aqueous solutions (industrial waste stream, fermentation broth, bio oil-generated stream, etc.) by using reactive extractions. This LLE was proposed for large-scale extractions such as those

used in wastewater treatment or for recovery of an acid from a fermentation broth. In reactive (chemical) extraction of an organic acid, a basic compound, such as an amine-based additive, is diluted in the organic phase to promote the formation of acid-base complexes as the dominating factor in the extraction system. In this process the extraction is conducted at room temperature or below, whereas back extraction for recovery of acids (and/or extractants) is carried out at increased temperature. A detailed account of the extraction of carboxylic acids using lipophilic amines as additives is given in Ref. [27]. The extraction mechanism for acids with amine-based additives was divided into two categories: (i) ion-pair formation and (ii) hydrogen bonding and solvation. Ion-pair formation is the dominant mechanism when the amine additive has a basicity greater than that of the conjugate anion. The authors determined the amine's apparent basicity as the pH of half neutralization. On the other hand, in examples where the pK_a of the amine (conjugated ionic form) was lower than the pK_a of the carboxylic acid, the extraction mechanism is dominated by either hydrogen bonding or solvation interactions. In this case the extent of extraction is mainly determined by the fraction of the acid in its neutral form and, thus, is strongly dependent on the acid's pK_a value. Many reports describe the reactive extraction of carboxylic acids in numerous manufacturing processes. Some examples are the extraction of propionic acid using tri-*n*-octylamine (TOA) by methyl isobutyl ketone (MIBK) at concentrations close to those found in fermentation broth [28]. Other examples of the use of TOA included the recovery of formic acid from aqueous solutions [29] and extraction of citric acid by 2-octanol within the temperature range 303–353 K from an aqueous nonbuffered media [30]. More recently the extraction of nitrophenols and picric acid, nonbiodegradable pollutants, was studied using a secondary amine Amberlite LA in MIBK with the estimation of the equilibrium and kinetics for the extraction [31]. In all these examples, there is a need to gather information and fundamental data for the extraction equilibrium for the acid additive-solvent system close to those for real samples to design an appropriate extraction process.

3.5 Methods of Extraction

The purpose of transferring a solute from one liquid solution to another is usually the separation or purification of the specific compound. In some cases the desired component is removed from the original solution, and in other cases the desired component remains in the original solution, while the impurities are extracted. Extraction may also provide a second solution from which the component is more readily recoverable than from the original solution, changing also its concentration. The choice of the procedure is a matter of convenience but is largely dictated by the value of the distribution ratio and the particular analytical problem.

3.5.1 Batch extractions

A batch extraction is the simplest extraction procedure and the LLE technique frequently applied for separations at the laboratory scale. It is the best choice when a large D for the desired compound is readily obtainable. Through a small number of equilibration stages, the separation can be satisfactory. The operation is typically carried out using a separatory funnel, where a given volume of solution that contains the target compounds is put into contact with a given volume of an immiscible solvent. After shaking the two liquid layers are allowed to separate. The bottom layer is then set aside, and if necessary, the extraction is repeated by the addition of fresh solvent. This batch extraction process offers many advantages, especially when the extraction efficiency of the solute of interest is large after a few extraction operations. It is fast and simple, and under optimized conditions, separations are quantitative, although a complete separation is impossible. Various methods for increasing the extraction efficiency and the selectivity of an extraction can be applied.

3.5.2 Continuous LLEs

This procedure is typically used when the extraction efficiency is relatively small, and a large number of batch extractions would be necessary to achieve a quantitative separation, which is neither practical nor convenient. Thus continuous extraction with an apparatus that can be left unattended for long periods of time is advisable. The basic procedure consists of a continuous flow of volatile immiscible solvent phase passing through the solution to be extracted. The extraction solvent is continuously recycled by distillation and condensation. The extracted solutes are expected to remain in the evaporation flask. These compounds must be thermally stable and of low volatility. Although the partition equilibrium may not be achieved during the limited contact time for the two phases, the solute is being removed continuously to the extraction phase. Efficiency for the process depends on the value of K_D, the relative phase volumes, the contact area of the phases, and also the viscosity of the organic phase. One practical way to improve efficiency consists of increasing the contact area between phases. The extraction solvent can be forced to pass through the aqueous solution through fritted glass disks or small orifices, and in addition, stirrers might be used to enhance the phases contact. There are various designs for continuous extraction devices. Laboratory-size continuous LLE with lighter and heavier solvents than water are shown in Fig. 3.7.

3.5.3 Countercurrent extractions

The basic principle of countercurrent LLE systems is that after each extraction step, both phases are mixed with fresh portions of both solvents, resulting in a marked

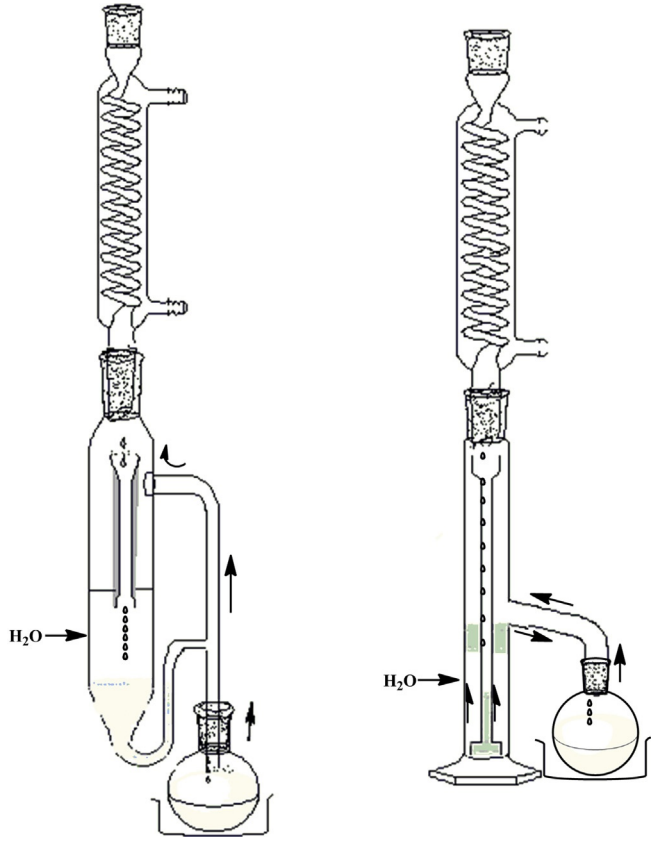

Fig. 3.7 Schematic diagram of continuous LLE with solvents heavier than water (left) and for extraction with solvents lighter than water (right).

increase in the extraction efficiency [32]. This technique is designed for the extraction of two or more compounds with a small difference in their D-values. The process resembles low-resolution column chromatography. With automated countercurrent distribution equipment, this process can be performed with several hundred transfers. It is manly useful in large-scale preparative separations. The theory of countercurrent extraction can be modeled based on a process with many discrete stages; for further details, see Chapter 10.

3.5.4 Microextractions

Microextractions refer to LLE carried out with β-values in the range 0.001–0.01. Compared with conventional LLE that uses a given volume of organic solvent,

microextraction provides less compound recovery, but the final concentration in the organic phase is significantly enhanced. In addition, the solvent volumes employed during the extraction are greatly reduced. It is typically used as a preconcentration technique before the determination step. Examples of microextraction techniques include single-drop microextraction (SDME) and dispersive liquid-liquid microextraction (DLLME). SDME uses microliters of extraction solvent that is suspended as a drop from the tip of a microsyringe needle into a liquid or vapor phase containing the analyte. This microextraction technique is discussed in Chapter 15. DLLME is a microextraction technique in which the analyte is extracted from the aqueous solution by the addition of fine droplets of extraction phase dispersed throughout the aqueous sample solution to enhance the contact between extraction solvent and sample, followed by centrifugation to recover the extraction solvent as a bulk solution. DLLME is discussed in Chapter 16.

3.5.5 Supported liquid membrane extractions

Supported liquid membrane (SLM) extraction techniques, in the form of flat sheets or hollow fibers, are another variant of LLE extraction [33]. The extraction process involves a partitioning of the analyte from the sample into the organic liquid impregnated in a membrane, followed by diffusion through the membrane into the bulk of the acceptor solution. In the three-phase mode, the analyte must be uncharged to be dissolved into the membrane, and the pH of the donor solution has to be adjusted accordingly to the pK_a of the compound. The opposite pH condition applies for the acceptor solution. Supported liquid membrane extraction is discussed in Chapter 8.

3.5.6 Automation

Automation in LLE is an important improvement over the intensive manual handling required by classical LLE. Several companies have developed analytical instrumentation that can automate all or part of the extraction and preconcentration processes. Autosamplers and workstations used for liquid and gas chromatography can perform LLE as part of an automated procedure. Similarly, robots for multipurpose tasks have options that support mixing and phase separation as part of the automated sample preparation process. The volumes used in robotic devices range from microliters to large volumes (hundreds of milliliters). Some systems mix the phases by alternatively loading the solvents into the needle and dispensing the contents back into a sample vial. Either the top or the bottom layer can be removed by adjusting the position of the needle submerged into the solution for subsequent injection or further sample preparation. Some units have vortex mixing capabilities. For further discussion, see Chapter 24.

3.5.7 Optimization of the extraction process

Optimization of LLE procedures is usually focused on improving either the extraction yield or the selectivity of the process through the selection of the extraction solvent. However, many additional experimental factors have to be considered when choosing an LLE system. First the possible chemical modification of the target compound with the aim of enhancing the distribution to the organic or aqueous phases. In addition, optimization of nonchemical factors must be considered for an effective extraction. These include the choice of the extraction technique, water and solvent volumes, extraction time, and others such as cost and environmental impact.

3.5.7.1 Selection of Solvent

When more than one solvent provides similar distribution constants and the possibility of using other solvents exists, the physical properties of these solvents must be considered. Solvents of high vapor pressure are easily removed after extraction. Also the degree of miscibility and the possible formation of emulsions with the aqueous phase must be taken into account. The safety and toxicity must also be considered. Information about physical properties of organic solvents can be found in Refs. [7, 11–15]. Sometimes a mixture of two or more solvents is used to obtain the desired extraction efficiency. A synergistic effect is obtained in some cases with solvent mixtures. Synergism is observed when a larger distribution ratio is obtained for a given compound by using a mixture of two solvents. A synergistic coefficient is defined as

$$C_S = \log \frac{K_{D,A+B}}{K_{D,A} + K_{D,B}} \tag{3.34}$$

where $K_{D,A}$ and $K_{D,B}$ are the distribution constants in solvents A and B, respectively, and $K_{D,A+B}$ is the distribution constant for the mixture. This effect was observed, for instance, in the reactive extractions of carboxylic acids using an amine and organophosphoric acids dissolved in the organic solvent [34]. The organophosphoric acid additive interacts with carboxylic acid forming an interfacial complex soluble in the organic phase, while the amine additive increases the hydrophobicity of this compound by solvation.

3.5.7.2 Phase Ratio

The use of a larger extraction solvent volume not only favors partitioning but also dilutes the concentration in the organic phase. The volume of solvent must be adjusted to obtain not only an efficient extraction but also a convenient volume for recovery after extraction. Continuous solvent extraction can be used to obtain higher recoveries. Several liters of aqueous solutions, for instance, are continuously

extracted with dichloromethane to concentrate several diverse pesticides present at concentrations of ppb from environmental water samples [35].

3.5.7.3 Salting-Out Effects

A high ionic strength in the aqueous phase markedly affects the activities of all components of the system. Addition of salts generally increases the distribution ratio for neutral organic compounds. A secondary effect due to the addition of salts is the formation of associations or ionic pairs in the aqueous phase, introducing other secondary chemical equilibrium in the system.

3.5.7.4 Extraction Time and Shaking

The extraction process is generally fast enough to achieve equilibrium in a few minutes with proper mixing. Some systems, however, can be slow, and kinetic aspects of the extraction process have to be considered. The mass transfer rate will be dominated by the slower step: the rate of formation of the extractable compound (if any) or the mass transfer across the interphase. A thorough discussion of this issue is beyond the scope of this chapter.

3.6 Conclusions

LLE has been an important separation technique in laboratory studies and manufacturing processes since the first decades of the 19th century. Thus an enormous number of applications in diverse fields have been described. In most cases the LLE process was used for one of the following objectives:

(1) Isolation and preconcentration of an analyte prior to an analytical determination. For systems with relatively large K_D, a large volume of aqueous phase containing the analyte can be mixed with a small volume of organic solvent to achieve a final concentration that tends to $1/\beta$ as K_D tends to infinity. A variety of samples are processed with methods that include a LLE pretreatment. For instance, many protocols for isolation of one (or more) analytes from biological samples (plasma, urine, animal tissues, and vegetal materials) or from environmental samples include a pretreatment by LLE.
(2) Simplification of the sample matrix and preconcentration to enhance the signal for quantitation.
(3) As a selective separation technique. For instance to isolate an analyte free from interferences. In this case a large difference in the D-values between the analyte and specific interferences is necessary.

(4) As a technique for purification of a compound. The recovery of many industrial products includes an aqueous/organic LLE step. Some examples of technological importance are product recovery from downstream fermentation broths (antibiotics, amino acids, and steroids); extraction of essential oils used in flavor and fragrances, food, and pharmaceuticals; recovery of organic acids from environmental waters; and the removal of high-boiling organics of environmental concern, such as phenols, nitrated aromatics, and anilines from wastewater.

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Chapter 4

Fundamentals of Solvent Extraction of Metal Ions

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Chapter Outline

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4.1 Introduction

Solvent extraction (or liquid-liquid extraction) of metal ions is a technique of separating metals present, usually in the form of ions, in an aqueous solution (feed) from which they are transferred to a water-immiscible organic solvent contacted with this aqueous phase [1–3]. It is also a method of physicochemical investigations, widely used in coordination chemistry.

Immiscibility or rather limited miscibility of the two liquids is consistent with an old Latin maxim *similia similibus solvuntur*. Different physicochemical properties of immiscible liquids lead to substantial differences in their behavior. Strong interactions between the polar molecules of water make water a liquid under normal conditions. The energy of these interactions overcomes the negative entropic effect of ordering the structure of liquid water. In contrast, in inert organic solvents where weak nonspecific interactions of the type of van der Waals forces prevail, the entropic effect makes their liquid structures disordered. Various solutes, in particular metal ions and/or their compounds formed in the biphasic system, differ in their affinities to water and to organic solvents; therefore they unevenly distribute between the two liquid phases. Moreover, their distribution depends not only on the properties of the solute but also on the chemical properties and compositions of both liquid phases, which can be easily adjusted in the experiment. This makes solvent extraction so flexible and versatile technique of separating metal ions.

After contacting and subsequent separation of the two liquid phases, the extracted metal is usually back extracted (stripped) from the loaded organic solvent to a fresh aqueous phase with the chemical properties different from those of the initial aqueous feed solution. Such batch extraction is mainly used in laboratories. Industrial applications of the method require a more efficient technique. Modern multistage processes of continuous countercurrent extraction, carried out in pulsed columns or in batteries of either mixer settlers or centrifugal extractors, allow for a significant increase in the scale of the process and also ensure effective separation of metals, even those with very similar chemical properties, for example, lanthanides. That is because the countercurrent streams of the aqueous feed solution and the receiving organic phase meet at each stage of the system, where the process of transferring a given solute is repeated for the increasingly depleted aqueous phase and the increasingly enriched organic phase. This leads to a better separation of the elution bands of consecutive metals in the organic phase stream. In some variants of this technique, for example,

extraction chromatography [4], in particular high-performance liquid chromatography (HPLC), and separations using supported liquid membranes (membrane solvent extraction) [5, 6], the organic phase or the liquid extractant is immobilized on a solid support, but the principle of the separation remains the same.

Solvent extraction requires the use of pure organic solvents immiscible with water. Therefore the first extractive separations, initially the separations of organic compounds, were reported only in the second half of the 19th century. The rapid development of solvent extraction aimed at separating and purifying metals began in the 1940s. That was due to the need to purify ton amounts of uranium nitrate for uranium enrichment and for plutonium production in nuclear reactors, which was done within the US Manhattan Project [1].

Nowadays, solvent extraction is used in an extremely wide range of the amounts of separated metals. Microextraction technique of sample preparation for microanalysis consists in solvent extraction of analytes (also metals) from a few milliliter samples of aqueous solution to a drop (a few microliters) of an organic solvent and is followed by an instrumental analysis of the organic phase [7]. However, the most spectacular in terms of scale of the processes is the use of solvent extraction to study the chemical properties of superheavy elements that are obtained in extremely small quantities of single atoms. The method is based on determining the extraction behavior of short-lived (a few seconds half-lives) isotopes of, for example, rutherfordium and dubnium, formed by irradiating actinide targets with heavy ion beams [8]. At the other end of this scale, huge amounts of base metals [3] are produced industrially using hydrometallurgical technologies that include leaching the metals from ores (mainly low grade), followed by solvent extraction and electrowinning (SX-EW) [9]. It is estimated that the world production of copper from ores by SX-EW in 2017 (several million tons) represented about 16% of the total production of refined copper [10]. This amount increases to >25% of the world production when considering copper that has been recovered from reprocessed scrap, for example, waste electric and electronic equipment, where hydrometallurgical technologies are widely used for the recovery of various metals [11]. Another example of the industrial use of solvent extraction is the reprocessing of spent nuclear fuels. Thousands of tons of this highly radioactive material are treated annually in several nuclear reprocessing plants around the world to extract fissile materials for recycling and to reduce the volume of nuclear waste [12] (cf. Chapter 24).

Attempts were also made to use multistage solvent extraction processes for separating isotopes of certain elements. In this way the enrichment of natural uranium into the fissile isotope U-235 was investigated as a possible alternative to the classical gaseous process using UF_6 [13]. Studies were also carried out on solvent extraction separation of lithium isotopes, aimed at obtaining material enriched in Li-6, to be used for the production of tritium as a fuel for fusion reactors. Currently, various derivatives of benzo-15-crown ethers are extensively studied as lithium extractants in ionic liquids, promising for lithium isotope separations [14].

4.2 Thermodynamic Considerations

4.2.1 Partition and distribution

Let us consider a system of two immiscible liquids: an aqueous solution containing dissolved metal compounds and/or metal ions, and an organic solvent (diluent) that contains a lipophilic ligand (extractant). Upon contacting the two liquids with each other, the extractant forms lipophilic complex compound(s) with the metal ions. The formed complex(es) and sometimes other chemical forms of the metal present in the aqueous phase move into the organic phase until dynamic equilibrium is established. The free energy change that accompanies this process may be expressed in terms of chemical potentials, μ, of the components of the two solutions. The value of μ is defined as the free energy change when 1 mol of the component is added to an infinite amount of the given solvent. In the equilibrium the chemical potentials of a metal-containing solute, S, in both liquid phases are equal to each other:

$$\mu_{S,aq} = \mu_{S,org} \tag{4.1}$$

and

$$\mu^{o}_{S,aq} + RT \ln a_{S,aq} = \mu^{o}_{S,org} + RT \ln a_{S,org} \tag{4.2}$$

where $\mu^{o}_{S,aq}$ and $\mu^{o}_{S,org}$ are the standard chemical potentials of the solute in the selected standard states; subscripts aq and org denote the aqueous and organic phase, respectively; R is the gas constant; T is the absolute temperature; and $a_{S,aq}$ and $a_{S,org}$ are the activities of S in the given solution:

$$a_{S,sol} = \gamma_{S,sol} \cdot c_{S,sol} \tag{4.3}$$

There are various means of expressing the activity of a solute in solutions [1, 2]. When the selected standard states are hypothetical solutions of S (e.g., 1 M) with the properties of infinitely dilute solutions, the activity coefficients, $\gamma_{S,sol}$, are the measures of the nonideal behavior of the solute, caused by its nonzero concentrations in the real solutions. On the other hand the energies of the solute interactions with the solvents are reflected in the values of standard chemical potentials of the solute in both liquid phases. In dilute solutions, when $c_{S,sol} \to 0$, the activity coefficients $\gamma_{S,sol} \to 1$, so

$$\lim_{c_S \to 0} \frac{c_{S,org}}{c_{S,aq}} = \exp\left(\frac{\mu^{o}_{S,aq} - \mu^{o}_{S,org}}{RT}\right) = P_S \tag{4.4}$$

where P_S is the *partition constant* of the solute, defined as the ratio of the concentrations of S in the organic and the aqueous phase at equilibrium, when these concentrations approach to zero. Also, other names, *distribution constant*, *partition*

coefficient, etc., can be found in the literature, but they are not recommended. The value of P_S, which is determined experimentally at low concentrations of S, is then the measure of the difference of the energies of interactions of the given solute with the aqueous and the organic phase. In a biphasic system of a given composition, P_S depends only on the temperature. The solvent extraction process, when only one, well-defined chemical form of a given metal exists in both liquid phases, is called *liquid-liquid partition*.

However, the cases of solvent extraction of metals in the form of only one particular solute, the same in both liquid phases, are relatively rare (see Section 4.3.1). More commonly, significant amounts of various chemical forms of the metal(s), M_j, coexist in equilibrium in the biphasic system, particularly in the aqueous phase. The name *distribution process* is then used, and the metal extraction efficiency is expressed by means of the value of its *distribution ratio*, D_M. The distribution ratio is defined as the ratio of the total concentration of a given metal (regardless of its chemical form) in the organic phase to its total concentration in the aqueous phase, when the liquid phases are in contact with each other:

$$D_M = \frac{\Sigma_j [M_j]_{org}}{\Sigma_j [M_j]_{aq}} \tag{4.5}$$

where $[M_j]_{org}$ and $[M_j]_{aq}$ denote the concentrations of a jth chemical form of the metal in the organic and in the aqueous phase, respectively. A common case is the extraction of a metal ion, M^{n+}, by an acidic chelating extractant HL. A series of consecutive ML_j^{n-j} complexes with the anion, L^-, is formed in the aqueous phase, but only one of them, the neutral ML_n chelate, is transferred to the organic phase:

$$M^{n+} + nHL_{org} \overset{K_{ex,M}}{\rightarrow} ML_{n,org} + nH^+ \tag{4.6}$$

If all the chemical forms of the metal present in the biphasic system remain in equilibrium, Eq. (4.5) may be expressed as

$$D_M = P_{ML} \cdot f\left(\beta_{j,M}, [L^-]_{aq}\right) \tag{4.7}$$

where subscript ML (also in further equations) relates to the extractable neutral ML_n chelate and $f(\beta_{j,M},[L^-]_{aq})$ is a function of the concentrations of different chemical forms of the metal in the aqueous phase. The concentrations of the consecutive complexes can be expressed as the products of their stability constants, $\beta_{j,M}$, and the $[L^-]_{aq}^j$. This makes it possible to determine experimentally the number of these complexes and their stability constants when studying the dependence of D_M on the extractant concentration or on the pH of the aqueous phase. When the extractants used are not too lipophilic, the experimental conditions can be adjusted so that the determined D_M value is equal to P_{ML} [15].

It is also possible to determine the stoichiometries and stability constants of hydrophilic metal complexes formed in the aqueous phase of solvent extraction systems containing a hydrophilic stripping ligand. The ligand competes with the extractant for the metal ion and selectively strips the metal ion from the organic phase. The method involves a study of a relationship between a certain function of D_M and the concentration of the stripping ligand. A recent example is the determination of conditional stability constants of two consecutive americium(III) complexes with a tri-*N*-dentate anionic 2,6-bis(5,6-di(sulfophenyl)-1,2,4-triazin-3-yl)pyridine ligand [16].

4.2.2 Separation Factor

Let us consider the extraction of a metal ion, M^{n+}, with an acidic chelating extractant HL, which proceeds according to Eq. (4.6). The extraction constant, $K_{ex,M}$, can be expressed as

$$K_{ex,M} = \beta_{n,M} \cdot P_{ML} \cdot K_a^n \cdot P_{HL}^{-n} \tag{4.8}$$

where K_a is the dissociation constant and P_{HL} is the partition constant of the extractant.

In the practice of extractive separations of metal ions, the distribution of a metal between the two liquid phases is described by the ratio of total concentrations of the metal in the organic and in the aqueous phases—the distribution ratio, D_M, which depends on speciation of the metal in both liquid phases. Selectivity of the separation of two different metal ions, A^{n+} and Z^{n+}, is commonly expressed by means of their separation factor, $SF_{A/Z}$, equal to the ratio of the distribution ratios of the metals:

$$SF_{A/Z} = D_{M(A)}/D_{M(Z)} \tag{4.9}$$

and after transformation using Eqs. (4.5), (4.8), and (4.9),

$$SF_{A/Z} = \frac{\beta_{n,A} \cdot P_{AL}\left(1 + \Sigma_j \beta_{j,Z}[L^-]^j\right)}{\beta_{n,Z} \cdot P_{ZL}\left(1 + \Sigma_j \beta_{j,A}[L^-]^j\right)} \tag{4.10}$$

where $\beta_{j,M}$ denotes the stability constant of ML_j, $P_{ML} = [ML_n]_{org}/[ML_n]_{aq}$ is the partition constant of the neutral ML_n complex, and $[L^-]$ is the equilibrium concentration of the anion L^- in the aqueous phase. For simplicity the charges of the ML_i species have been omitted.

Expressing *SF* as the ratio of the extraction constants of the metal ions [17] is justified only when the sum of concentrations of all the ML_j^{n-j} complexes in the aqueous phase is much lower than $[M^{n+}]_{aq}$. For weakly acidic chelating extractants, this can occur at low pH. Then, practically, the only form of the metal in the aqueous phase is the hydrated cation, $M(H_2O)_m^{n+}$, and only the neutral complex, ML_n, is

transferred into the organic phase. In this case, Eq. (4.10) simplifies to the ratio of the respective β_n and P_{ML} products:

$$SF_{A/Z} = \frac{K_{ex,A}}{K_{ex,Z}} = \frac{\beta_{n,A} \cdot P_{AL}}{\beta_{n,Z} \cdot P_{ZL}} \tag{4.11}$$

The effect of differences in the stability constants of the two complexes on the $SF_{A/Z}$ value significantly reduces with increasing pH of the aqueous phase, that is, when the concentrations of the ML_j^{n-j} complexes become comparable with that of the free metal cation, $[M^{n+}]_{aq}$. In the extreme case, when—at a low acidity—practically the only form of the metal in the aqueous phase is the neutral chelate, ML_n, the SF value becomes dependent only on the P_{ML} ratio. The negative effect of lipophilic adducts formation by coordinatively unsaturated metal chelates on the SF value, observed in synergic extraction processes, will be discussed in Section 4.3.4.

4.3 Metal-Containing Solutes in Solvent Extraction Systems

The metal species most often extracted from aqueous solutions into immiscible organic solvents are various metal complexes with lipophilic organic ligands—extractants—present in the organic phase. Depending on the physicochemical properties of the extractants, the complexes are formed either in one of the liquid phases or at the interface (see Section 4.6.1) and finally concentrate in the organic phase. However, also simpler metal species are extracted into the organic phase—uncharged metal compounds in particular oxides and salts or even charged metal ions. The following overview of the various extractable forms of metals will begin with the simplest extractable solutes.

4.3.1 Metal ions

High electric potential of metal ions present in aqueous solutions causes that the arrangement of the nearby dipole molecules of water is different from that observed in bulk water—the ions are strongly hydrated. The affinity of the metal ions to the water solvent increases with increasing charge and decreasing radius of the ions. Born equation is widely used to evaluate the free energy of hydration, ΔG, that is, the free energy of transfer of an ion of the charge q and radius r from vacuum to the liquid (water) of the dielectric constant ε:

$$\Delta G = -\frac{q^2}{2r}\left(1 - \frac{1}{\varepsilon}\right) \tag{4.12}$$

Because the respective free energy of ion solvation in a given organic solvent (of smaller ε) is much less negative, the free energy of transfer of a small single ion from water to the organic solvent is positive and resists the transfer. In contrast, relatively large monovalent ions, for example, tetramethylammonium cation, are readily transferred from water to immiscible inert organic solvents due to hydrophobic effect (see Section 4.4.1).

The transfer of ions must not interfere with the principle of electroneutrality of each liquid phase. Because of that the transfer of metal ions from the aqueous to the organic phase must be accompanied either by coextraction of an equivalent number of counterions from the aqueous phase or by the release of an equivalent number of ions of the same charge from the organic phase back to the aqueous feed solution. In the former case, small hydrophilic metal ions are pulled out of the aqueous into the organic phase by equivalent amounts of large amphiphilic counterions dissolved in the aqueous phase. The experimental partition of an ion depends also on the properties of the counterion. Direct experimental determination of partition constant of a single ion is impossible. To overcome this problem an extrathermodynamic assumption has been formulated that the partition constants of complex ions of the same structure but with the opposite charge (e.g., tetraphenylarsonium cation and tetraphenylborate anion) are equal to each other [18].

The transfer of metal ions from an aqueous to an organic phase, accompanied by the parallel transfer of equimolar amounts of large, usually monovalent counterions, sometimes followed by partial association of these ions in the organic phase with low dielectric constant [19, 20], is called solvent extraction through *ion-pair formation*. The examples include the extraction of monovalent alkali metal cations together with large monovalent anions, for example tetraphenylborate [21]. Also, anionic metal complexes are extracted this way, together with amphiphilic monovalent cations of moderately long alkyl chains, as the counterions. The examples are oxoanions of multivalent metals in the highest oxidation states: pertechnetate, TcO_4^-; hydrogen chromate, $HCrO_4^-$; or other metallate anions, for example, $AgCl_2^-$ [1, 15]. The concentration-dependent association of these oppositely charged ions in the organic phase improves their extraction by shifting the ion-transfer equilibrium.

Extraction of metal ions into the organic phase of ionic liquids (ILs) most often proceeds according to this model, both as the cation and anion exchange. However, with the increase in the lipophilicity of the cation and anion of the IL, it becomes more probable to extract the whole molecules of metal salts, formally according to the model of ion-pair extraction [22, 23].

4.3.2 Simple inert molecules (neutral metal compounds)

As stated earlier the electric charge of metal ions makes them poorly transferred from water to inert organic solvents. However, certain metals can be extracted from acidic

aqueous solutions to inert organic solvents as simple uncharged compounds, oxides or salts, by physical distribution of these solute molecules, without the formation of defined adducts (solvates) with the solvent. For example, tetroxides of ruthenium and osmium, RuO_4 and OsO_4, obtained by oxidizing the metal ions present in dilute aqueous solutions of H_2SO_4 or HNO_3, are readily transferred into organic solvents. Also, neutral salts of several metal ions of low coordination numbers (CN), in particular covalent halides of mercury, germanium, arsenic, or antimony, are extractable from aqueous solutions of high acid (or salt) concentrations to inert solvents, in particular aliphatic hydrocarbons, by physical distribution [1, 15]. It is worth to mention that also the whole molecules of various metal salts can be transferred into an organic phase by polytopic zwitterionic organic ligands that complex the metal cation and attendant anion(s) in separate binding sites [24].

4.3.3 Metal complexes with lipophilic ligands

Much more frequent cases of solvent extraction of metal ions consist in transforming the metal cations into hydrophobic compounds—complexes, preferably uncharged, with lipophilic organic ligands (extractants) present in the organic phase. The high thermodynamic activity of these metal complexes in the aqueous phase, which makes them well extractable into inert organic solvents, is due to two factors: (i) hydrophobic effect exerted on the complexes with large molecular volumes (see Section 4.4.1) and (ii) their zero charge caused by attaching anionic ligands. The extractants of metal cations are either neutral ligands, mono- or polydentate, or anionic chelating ligands formed by acid dissociation of polydentate extractants. All these ligands are lipophilic organic Lewis bases that convert multivalent metal cations into well-extractable complexes. The neutral ligands form either neutral solvated salts or cationic chelates readily extracted by ion pairing with the anions of a mineral acid present in the aqueous phase. The anionic chelating ligands form well-extractable neutral metal chelates. Some details are given in the succeeding text.

4.3.3.1 Solvated Salts

The first solvating extractants, used in early (mid-20th century) large-scale processes of uranium purification and actinide separations, were organic solvents of basic properties, the carboxy compounds: diethyl ether, methyl isobutyl ketone, dibutyl carbitol, isoamyl alcohol, etc. [25]. Later on, more efficient extractants were introduced, less water-soluble phosphoryl compounds of higher basicity: tri-*n*-butyl phosphate (TBP) and trioctylphosphine oxide (TOPO), which had to be dissolved in water-immiscible inert organic solvents (diluents) such as kerosene.

Let us consider a multivalent metal cation in an aqueous solution of a significant concentration of an inorganic acid, contacted with a diluent containing a lipophilic extractant. Water molecules that hydrate the metal cation become replaced by

inorganic anions and by the molecules of this extractant [26], which results in the formation of a large neutral molecule of the solvated salt, well extractable into inert organic solvents. An example is the extraction of uranium(VI) from HNO_3 solutions by TBP when two monodentate TBP molecules solvate the uranyl cation, forming a well-extractable hydrophobic complex $UO_2(TBP)_2(NO_3)_2$. This method is still widely used for reprocessing spent nuclear fuels in the PUREX process [12].

4.3.3.2 Neutral Metal Chelates

Well-extractable neutral molecules of metal chelates are formed when the charge of the extracted metal cation is neutralized by anions of chelating ligands of moderate or large molecular volumes, attached in the inner coordination sphere of the metal cation. The donor atoms of the chelating ligands are usually O, N, or less frequently S. The formed chelate rings consist of four to seven atoms, the five- and six-membered rings being the most stable. If the number and the kind of the donor atoms and the properties of the chelating ligands attached to a multivalent metal cation fully satisfy its coordination requirements, the neutral metal chelates formed are coordinatively saturated and thus dehydrated in the inner sphere of the cation, which makes them well extractable into inert organic solvents.

The case of extracting metals by anionic chelating extractants is well illustrated by the examples of their extraction by an amphiphilic, weakly acidic bidentate ligand, acetylacetone. Numerous multivalent metal cations in aqueous solutions form, at moderate pH, uncharged, coordinatively saturated chelates with the enol form of the $acac^-$ anion, for example, $Th(acac)_4$, $Sc(acac)_3$, and $Be(acac)_2$, fairly extractable into inert organic solvents [1, 3, 15, 25, 27–29]. The central metal cations in such chelates are effectively screened by the ligand donor atoms from surrounding solvent molecules, so only the coordinated ligands contact with the solvent water. As results from theoretical DFT calculations, the presence of the metal cation inside the chelate molecule results in some displacement of partial charges on the ligand atoms, different for various metal ions even of the same valency [30]. These differences make the energies of interactions of these chelates with the solvent water different. As a result the partition constants of coordinatively saturated β-diketonates of tri- and tetravalent metals decrease with decreasing ionic radius by more than two orders of magnitude in both (Sc—Co and Th—Zr) series studied [28, 29].

One can expect that due to geometric reasons, the coordinatively saturated neutral chelates should be formed by small multivalent metal cations and bidentate anionic ligands of relatively large bite angles [31, 32], which form rather six- than five-membered chelate rings [33]. The opposite relationships lead to the formation of coordinatively unsaturated metal chelates, for example, lanthanide(III) tris-acetylacetonates or zinc(II) bis-acetylacetonate, which are strongly inner-sphere hydrated in aqueous solutions (see Section 4.4.2), so they are poorly transferred from

the aqueous to the organic phase. The transfer can be greatly enhanced by replacing the inner-sphere water molecules with lipophilic neutral mono- or bidentate ligands, that is, by the formation of well-extractable lipophilic adducts. Such synergic effects in solvent extraction [26] will be discussed in Section 4.3.4. Also the electronic structure of the central metal cation in the chelate (see Section 4.3.4) and the size and electronegativity of the donor atoms in the coordinated bidentate anionic ligands strongly affect the extractability of the chelates. For example, the low coordination number of the central metal ion in zinc monothioacetylacetonate results in the strong improvement of its extractability and in the diminution or even disappearance of the synergism in its extraction [34]. An additional improvement in the extraction of metal chelates with S-donor ligands is due to their weaker outer-sphere hydration compared with their counterparts with O-donor ligands (see Section 4.4.3).

4.3.3.3 *Cationic Metal Chelates*

Certain polydentate neutral extractants bind the cations of multivalent metals so strongly that Gibbs free energy of attaching the third molecule of the tridentate extractant to the cation of CN = 9–10 is much more negative than the energy of attaching a mono- or bidentate anion of an acid or salt present in the aqueous solution. The examples are heterocyclic tri-/tetra-*N*-dentate bis-triazinyl ligands or tri-*O*-dentate diglycolamide ligands, extensively studied as extractants in new processes of separation of actinides(III) from lanthanide fission products [35, 36]. The large, coordinatively saturated cationic ML_3^{3+} metal chelates are readily extracted from aqueous HNO_3 solutions together with three nitrate anions, according to the ion-pairing model. The charge of the extracted metal cations is neutralized by the coextracted NO_3^- anions that do not enter, however, the inner sphere of the M^{3+} cations.

4.3.4 Synergic effects in solvent extraction

If the central metal ion in the molecule of a neutral complex is not coordinatively saturated by the donor atoms of the organic ligand (extractant) and by anions (if any), then the complex dissolved in water becomes additionally hydrated in its inner coordination sphere by one or more water molecules. Further water molecules, hydrogen bonded to them, form an outer hydration layer of the metal ion in the complex. Such strong hydration makes the coordinatively unsaturated complexes poorly extractable into inert organic solvents. However, formation by such complexes, for example, uranyl thenoyltrifluoroacetonate, of lipophilic adducts with organic Lewis bases (see Section 4.3.3.2) results in a great improvement of the efficiency of the extraction (Fig. 4.1). Solvent extraction of metal ions in the form of the adducts, that is, neutral mixed-ligand complexes, where one of the ligands is the anion of a chelating extractant and the other is a Lewis base, is called *synergic extraction* [37].

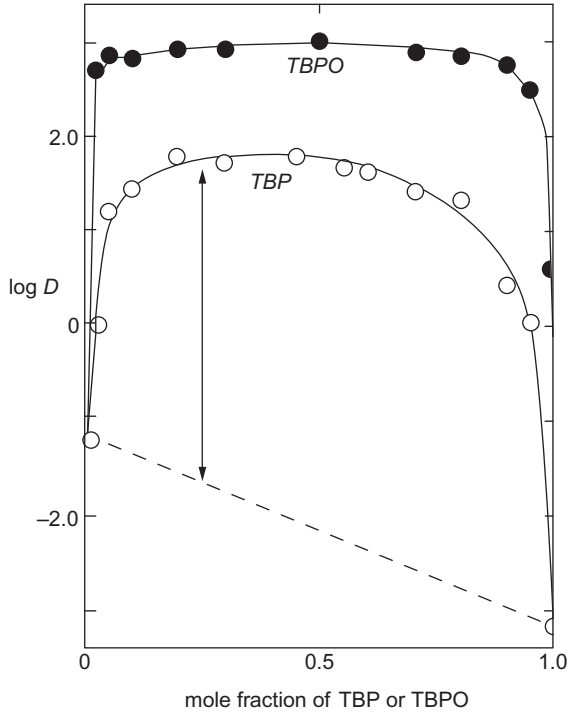

Fig. 4.1 Synergic extraction of U(VI) from 0.01 M HNO_3 by mixtures of thenoyltrifluoroacetone (TTA) with tributylphosphate (TBP) or with tributylphosphine oxide (TBPO) at a constant total molarity of $[TTA]_{org} + [TBP]_{org} = 0.02$ M or $[TTA]_{org} + [TBPO]_{org} = 0.02$ M in cyclohexane. *(From Rydberg J, Choppin GR, Musikas C, Sekine T. Chapter 4. Solvent extraction equilibria. In Solvent extraction, principles and practices, 2nd ed. Rydberg J, Musikas C, Choppin GR, Cox M (eds.). New York: Marcel Dekker; 2004. p. 116, with permission.)*

It should be mentioned here that in spite of prevalence in the chemical literature of another term, *synergistic*, the term *synergic* is recommended by IUPAC as the only linguistically correct form [38].

Early studies on the stabilities and extractabilities of mixed-ligand adducts of coordinatively unsaturated metal chelates with neutral Lewis bases as synergists have shown that the synergic effect increases with increasing basicity of the synergist and with decreasing stability of the metal chelate [37, 39–41]. The latter relationship should be limited, however, to the case of chelates of the same metal ion with the ligands of the same donor atoms and of the same number of atoms in the chelate ring. The steric hindrance against coordinating additional ligands becomes strengthened by the rigidity of the molecular structure of the chelate molecule. For example, scandium α-diketonates are more stable than $Sc(acac)_3$, but the small bite angles in the

five-membered chelate rings of the former chelates make their molecular structure open for adduct formation, while the rigid molecular structure of $Sc(acac)_3$ makes this chelate coordinatively saturated [33]. On the other hand, scandium β-diketonates with ligands less basic than Hacac, less stable than $Sc(acac)_3$, are coordinatively unsaturated, so they also easily form inner-sphere adducts, showing a synergic effect in their extraction. The metal–ligand bonds in scandium hexafluoroacetylacetonate are so weak, and its molecular structure is so flexible that the CN of the Sc^{III} ion in the chelate increases not only to 7 but also to 8, for example, in the $Sc(hfa)_3(TOPO)_2$ adduct or in the $Sc(hfa)_4^-$ anion extracted as the $Sc(hfa)_4^-$ tba^+ ion pair when tetrabutylammonium (tba^+) cations are present in the system [42]. Likewise the small bite angles of the coordinated tropolonate ligands allow the formation of a similar $Sc(trop)_4^-$ anion in the aqueous phase at a higher pH [33].

Sometimes the chelating extractant itself, at an excess concentration, plays the role of the synergist and enhances the extraction. Such autosynergic effect was described, for example, in solvent extraction of strontium by oxine [43] or later on in the extraction of lanthanides by diketones [15, 44]. The influence of the properties of metal ions on the degree of coordinative unsaturation of their chelates is more complex than expected based merely on the geometric factors and the valence of the central metal ion. The strength of the metal–ligand bond depends also on the charge density on the central metal ion; therefore, it increases with decreasing ionic radius across the lanthanide series. Because of this the stability of the $Ln(TTA)_3TBP$ mixed-ligand adducts increased across the lanthanide series, while the opposite effect found in the $Ln(TTA)_3(TBP)_2$ adducts was due to steric hindrance [45]. The result observed in the extraction of lanthanides at high acetylacetone concentrations in the diluent, benzene, seemed to be inconsistent with the previous conclusion. The stability of the self-adducts $Ln(acac)_3(Hacac)$ decreased across the lanthanide series, that is, with the decreasing radius of the Ln^{3+} ion [44]. However, this weakening of the autosynergic effect caused by the bidentate Hacac ligand is consistent with the effect observed in [45], when two monodentate TBP ligands were attached to the $Ln(TTA)_3$ chelate. In both cases the effect of the steric hindrance caused by coordination of the two donor atoms prevailed over the effect of the increase in the charge density on the central metal ions.

The electronic structure of the central metal cation in a chelate is another important factor that determines the degree of the chelate coordinative unsaturation and the possibility of the appearance of a synergic effect in the extraction. Investigations on the solvent extraction of tropolonates of trivalent metal ions with the TOPO synergist allowed to distinguish two groups of the metal ions, of d^0 and d^{10} configurations, strongly differing in their synergism. In the extraction of the d^{10} ions, either no (Ga) or weak (In and Tl) synergism was observed, increasing with the ionic radii, whereas in the extraction of the d^0 ions (Sc and Y—the counterparts of In and Tl, of similar ionic radii), strong synergistic effects appeared (Fig. 4.2) [46]. Theoretical

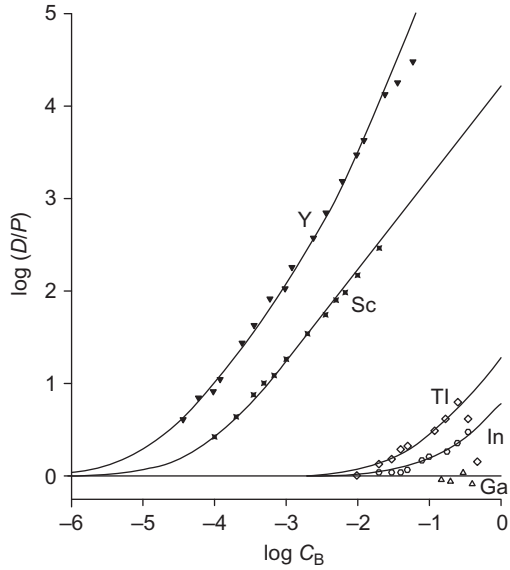

Fig. 4.2 The effect of the total molar concentration of TOPO, C_B, on the distribution of tropolonates of trivalent gallium, indium, thallium, scandium, and yttrium between inert organic solvents and various aqueous phases at 298.2 K. *(From Narbutt J, Czerwiński M, Krejzler J. Seven-coordinate d^0 and d^{10} ions. Computational and experimental studies on tris(tropolonato)metal(III)—TOPO adducts. Eur J Inorg Chem 2001;3187–98, with permission.)*

DFT calculations explain this difference as the result of much more negative energies of adduct formation for the chelates of the d^0 ions, because their unoccupied $(n-1)$d orbitals are easily available to form bonding molecular orbitals in the adducts. In contrast the $(n-1)$d orbitals of the d^{10} ions are fully occupied, while the empty virtual *n*d orbitals have too high energy to participate in the bonding. Therefore the metal chelates of the d^0 ions form weak 1:1 adducts with CN 7, which are hypervalent compounds with the bond order less than one [46]. This explains why the TOPO adducts with tropolonates of Sc and Y are much stronger than those of the In and Tl chelates, while the effects of the differences in the ionic radii of the metal ions prove to be less important.

The changes in the partition constants of the $Ln(acac)_3$ chelates, observed across the lanthanide series, reflect the opposite changes in the formation constants of their mixed-ligand adducts [15, 44]. Because the former effect is mainly due to the differences in the degree of the inner-sphere hydration of the chelates, one may conclude that both effects partly compensate each other. Therefore an increase in the concentration of a synergist decreases the selectivity of the separation of metal ions extracted in the form of coordinatively unsaturated chelates, because of reducing

their differentiation due to different hydration of the chelates. For example, in the system 10^{-2} M tropolone in toluene/water (pH 5), the separation factor $SF_{Sc/Y} = 190$ [46], while the $SF_{Sc/Y}$ values evaluated for the same system containing TOPO in the concentrations of 10^{-4}, 10^{-3}, and 10^{-2} M dropped to 50, 30, and 10.4, respectively [47]. The negative effect of lipophilic adduct formation by coordinatively unsaturated metal chelates on the separation of metal ions, observed in synergic extraction processes has a general character.

4.4 Solute-Solvent Interactions in the Aqueous Phase

Interactions of the solute with water, both with bulk solvent and with its free hydrating molecules in the aqueous phase, play the fundamental role in the solvent extraction processes. Bulk liquid water at ambient temperature contains supramolecular aggregates associated by means of hydrogen bonds and free, unbound water molecules. Enhancing the local structure of liquid water around a hydrophobic solute leads to the so-called hydrophobic effect [48–50], also known as hydrophobic hydration, which promotes the transfer of the hydrophobic solute from the aqueous to the organic phase.

Metal complexes with organic ligands—the solutes in aqueous solutions—interact with the solvent water also on other ways. Apart from the hydrophobic effect exerted by the hydrocarbon fragments of the solute, electronegative ligand atoms on the solute surface form hydrogen bonds with water molecules (outer-sphere hydration), while the solvent-accessible fragments of the central metal cation (in coordinatively unsaturated complexes) directly coordinate water molecules (inner-sphere hydration). All the interactions can be examined separately, though their contributions to the total hydration of the solute are not additive [51–53]; the outer-sphere hydration on the solute surface disturbs the hydrophobic effect in the vicinity, which decreases the partition constant of the solute [52, 53].

4.4.1 Hydrophobic effect

The hydrophobic effect increases the thermodynamic activity of large hydrophobic molecules of metal complexes formed in the aqueous phase of solvent extraction systems, which promotes their transfer from the aqueous to the organic phase. Poor solubility of liquid hydrocarbons in water, decreasing with increasing chain length, is the result of local structuring (by means of hydrogen bonds) of liquid water around the hydrophobic surface of the solute molecule. The positive contribution from one methylene group to the standard free energy of dissolution of liquid hydrocarbons in water, $\Delta G^{o}_{S}(CH_2) \approx 3.6\,kJ\,mol^{-1}$ at 25°C [19, 20], significantly decreases with increasing temperature. This value almost entirely corresponds to a negative

entropy change that results from the ordering of the water structure, while the negative enthalpy of hydrogen bonds formation is compensated to a great extent by the positive enthalpy of removing the hydrocarbon molecule from its own liquid phase. Another approach to the problem, based on the work required to form cavities able to accommodate the solute molecule of a given size and shape in both liquid phases, assumes that the free energy of cavity formation is more positive in water than in common organic solvents [54]. Both approaches predict a negative free energy change for removing hydrophobic solutes from water, which means their spontaneous transfer to organic solvents. This makes the hydrophobic effect the main driving force that causes metal complexes with organic ligands to be transferred from aqueous solutions to inert organic solvents.

The changes in the standard thermodynamic functions of partition of coordinatively saturated BeL_2 chelates (where the HL extractants are acetylacetone and its homologues), calculated per one added CH_2 group in the ligands, are very close (with the opposite signs) to those reported for dissolving liquid hydrocarbons in water, but the values of the standard free energies of partition were by several dozen kilojoule per mole less negative than minus free energies of dissolving liquid hydrocarbons of the same molar volumes in water [27]. The former observation has been interpreted in terms of the hydrophobic effect as the driving force that transferred the BeL_2 chelates into the organic phase, while the latter one turned our attention to a specific hydration of the coordinatively saturated metal chelates in the aqueous phase, which we then called the outer-sphere hydration of metal complexes (see Section 4.4.3).

However, the hydrophobic effect alone, though so important in solvent extraction, is of little interest from the point of view of separating metal ions of the same valency, because it generates similar changes in the free energy of partition of isostructural metal complexes, which does not practically differentiate the metal ions. Such differentiation is due to a specific hydration of metal complexes in the aqueous phase.

4.4.2 Inner-sphere hydration of metal complexes

In contrast to the hydrophobic effect, specific hydration of metal complexes in the aqueous phase decreases their activity in the aqueous phase, which decreases the partition constants of the complexes in the two-phase systems. Hydrophilicity of coordinatively unsaturated complexes of numerous metals, in particular trivalent lanthanides, is mainly due to coordination of water molecules to the central metal ions in their primary (inner) coordination sphere—the inner-sphere hydration. The poor extraction of such complexes results from the considerable energy required to dehydrate their molecules when they are transferred from the aqueous to the organic phase. However, the dehydration of coordinatively unsaturated metal

complexes, accompanying their transfer, is not always complete. For example, some coordinatively unsaturated lanthanide thenoyltrifluoroacetonates extracted into an organic phase retained two or three water molecules in the inner coordination sphere of the Ln(III) ion in the $M(TTA)_3$ chelate [55, 56], while no residual hydration in the organic phase was found in Eu(III) chelates extracted with di (2-ethylhexyl) phosphoric acid [55, 57]. The presence of a synergist such as benzoic acid, TBP, or TOPO in the system results in the formation of mixed-ligand adducts, for example, $Eu(TTA)_3(TOPO)_n$ ($n = 1,2$), with gradual replacing of the remaining inner-sphere water molecules in the hydrated chelates, up to complete dehydration of the extracted species [55–57]. This shows that the extraction of metal ions by weakly basic chelating ligands, which form coordinatively unsaturated chelates, is accompanied by the release of mainly the water molecules of the second sphere, H-bonded to the water molecules coordinated directly to the central metal ion.

The energy of the inner-sphere hydration depends on the properties of both the metal ion and the ligand. For example, the smaller the Ln(III) ion, the more stable is the $Ln(acac)_3$ chelate, and the higher is its partition constant [44]. This correlation is due to the weakening—across the lanthanide series—of the inner-sphere hydration of the chelates. This conclusion, seemingly surprising in view of the known effect of the hydration energy increasing across the series of Ln^{3+} ions, can be explained in terms of the progressive decrease in the coordination number of the Ln^{3+} ions, caused by lanthanide contraction, and of the competition for the Ln^{3+} ion between the acetylacetonate and water ligands. The latter results in weakening of the metal–water bonds by the metal–acac bonds that strengthen across the lanthanide series.

The influence of the strength of the metal–ligand bond on the extent of inner-sphere hydration of coordinatively unsaturated metal complexes is well illustrated by the results of studies on liquid-liquid partition of Zn(II) chelates with the homologous ligands: acetylacetone, hexanedione, and heptanedione [34]. Minute increases in the ligand basicities, caused by a small inductive effect of additional CH_2 groups, result in strengthening the Zn—O bonds, in a gradual decrease in the CN of the Zn(II) ion and in a decrease in the inner-sphere hydration of the chelates within the series. Because of that the changes in the partition constants of these coordinatively unsaturated chelates are unusually high. The average difference between the free energies of partition per one CH_2 group in the ligand, found in the series of zinc alkanedionates, $\Delta G_p^o(CH_2) \approx -4.55\ kJ\,mol^{-1}$ [34], is significantly more negative than that caused only by the hydrophobic effect alone, as observed for the analogous but coordinatively saturated beryllium chelates, $\Delta G_p^o(CH_2) \approx -3.33\ kJ\,mol^{-1}$ [27]. Accordingly, the synergic effect of trioctylphosphine oxide in the Zn(II) extraction weakens along the series of these homologous ligands [34].

4.4.3 Outer-sphere hydration of metal complexes

Another type of specific hydration of metal complexes in aqueous solutions is due to the fact that the solvent water molecules readily form hydrogen bonds with the donor oxygen atoms of the coordinated ligands. Systematic studies aimed at interpreting the observation that coordinatively saturated neutral metal acetylacetonates are much more hydrophilic than hydrocarbons of similar molar volume, already mentioned in Section 4.4.1, have led to the correct explanation of this difference. Experimental determination and the analysis of standard thermodynamic functions of partition of coordinatively saturated beryllium β-diketonates allowed to conclude, against the views of some authors, that each of the donor oxygen atoms (sp^2) in the coordinated ligands retains one lone electron pair not engaged in the bonding; therefore, these oxygen atoms behave as proton acceptors in the hydrogen bonds with solvent water molecules [27]. The hydrogen bonding of water to coordinated ligands (Fig. 4.3), called outer-sphere hydration of metal complexes [27], has been proved directly in the ^{1}H NMR and IR studies of coordinatively saturated $Be(acac)_2$ and $Co(acac)_3$ chelates in benzene solutions with variable water concentration [58]. According to theoretical DFT calculations based on the self-consistent isodensity polarized continuum model, the hydrate of a neutral chelate has a larger dipole moment than the chelate itself; thus, it more strongly interacts with bulk water. These calculations confirmed the formation of the outer-sphere hydrogen-bonded hydrates of $Sc(acac)_3$ and $Co(acac)_3$ and correctly evaluated the enthalpies of the water → heptane transfer of the chelates [30].

Based on the effect of redistributing the partial charges on the ligand atoms, caused by the presence of a metal cation inside the molecule of a coordinatively saturated

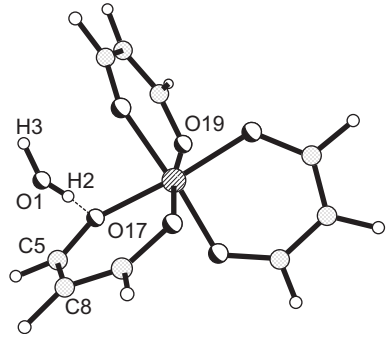

Fig. 4.3 The hydrogen bond (O1–H2–O17) between a H_2O molecule and the donor O atom in the coordinated malonaldehyde ligand in the hypothetical $Sc(mala)_3$ chelate (the model of $Sc(acac)_3$). *(From Czerwiński M, Narbutt J. Outer-sphere hydration and liquid-liquid partition of metal(III) chelates—density functional calculations. Eur J Inorg Chem 2005;555–62, with permission.)*

chelate [30] (Section 4.3.3.2), one can conclude that the outer-sphere hydration is not limited to the hydrogen bonding of water with the donor oxygen atoms in the coordinated ligands, but it extends on the whole molecule of the metal complex. This explains the unexpected (see Section 4.3.3.2) decrease of the partition constants with the decreasing radius of the metal ion, observed in four series of coordinatively saturated chelates (acetylacetonates and 2,4-hexanediones) of 3d metals(III) (Sc to Co) [28] and 4d–6f metals(IV) (Th to Zr) [29], where the partial charges on the donor oxygen atoms become less negative along the series. The use of another solvation model based on discrete solvent representation, for example, [59], for estimating the free energies of hydration of such solutes, would shed more light on the problem.

The correlation between the degree of outer-sphere hydration and the radii of the central metal ions in coordinatively saturated chelates allowed to explain the inversion of the hydrophilic properties of two pairs of Sc(III) and Lu(III) chelates, observed for two different pendant-arm macrocyclic ligands: hexadentate 1,4,7-triazacyclononane-1,4,7-triacetic acid (H_3NOTA) and octadentate 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (H_4DOTA) [60]. The neutral, coordinatively unsaturated Lu(NOTA) chelate ($CN_{Lu} \approx 8$) was hydrated in the inner sphere, in contrast to its coordinatively saturated counterpart Sc(NOTA) ($CN_{Sc} \approx 6$). The opposite order found for the coordinatively saturated anionic complexes $Sc(DOTA)^-$ and $Lu(DOTA)^-$ [60] was in line with the stronger outer-sphere hydration of the former, expected [28] due to the smaller radius of the scandium ion.

In the cases when one oxygen atom in the bidentate ligand is replaced by less electronegative sulfur, the coordinatively saturated chelate becomes much less outer-sphere hydrated and more lipophilic, as found for the pair $Co(acac)_3$–$Co(Sacac)_3$ [61]. If such replacement occurs in the ligands of coordinatively unsaturated chelates, a dramatic decrease in the coordination number of the central metal ion can be observed, for example, for the pair $Zn(acac)_2$–$Zn(Sacac)_2$. The monothioacetylacetonate chelate is almost coordinatively saturated ($CN_{Zn} \approx 4$), which can be concluded from the very poor synergism in the extraction with TOPO and from much higher partition constant, much lower enthalpy and entropy of partition, and a lower salting-out coefficient (see Section 4.4.4) of the chelate as compared with the respective values determined for its coordinatively unsaturated counterpart $Zn(acac)_2$ with $CN_{Zn} \approx 5 \div 6$ [34]. Both the very strong Zn—S bonds and the larger radius of sulfur than oxygen atom limit the access of TOPO to the Zn(II) ion in $Zn(Sacac)_2$, in contrast to the easy access in its coordinatively unsaturated counterpart $Zn(acac)_2$. Although the differences in the thermodynamic functions of partition of both chelates result also from a weaker outer-sphere hydration of $Zn(Sacac)_2$, the effect of its much weaker inner-sphere hydration is decisive. Similar differences in the solvent extraction of other zinc chelates with various O,O-, O,N-, S,S-, and S,N-donor ligands have also been reported [39].

Hydrogen bonding of water molecules to the donor oxygen atoms in coordinated ligands is the main but not the only reason of the outer-sphere hydration of metal chelates. The presence of electronegative nondonor atoms in the ligand, such as oxygen in hydroxypyranone and hydroxypyridinone ligands, reduces the partition constants of the metal complexes as a result of hydrogen bonding of water molecules to these nondonor atoms on the surface of the complex molecule [62]. The estimated Gibbs free energy contributions from such outer-sphere hydration per one hydrophilic group, —OH, —NH_2, >C═O, and —CH_2—O—CH_2—, and even for such two nearby groups, —CH_2—O—CH_2—O—CH_2—, in a molecule are between −16 and −22 kJ mol^{-1} at 298 K [20].

4.4.4 Salting out in solvent extraction systems

Hydration of electrolytes added to an aqueous solution decreases the thermodynamic activity of solvent water, which causes an increase in the activity of nonelectrolytes present in the same solution, followed by their salting out to the organic phase of the two-phase solvent extraction systems. The magnitude of the salting-out effect depends on the kind and on the concentration of an electrolyte dissolved in the aqueous phase. The salting-out phenomena were intensively studied in relation to biochemical issues already from the end of the 19th century, in particular by Franz Hofmeister and by Iwan M. Setschenow, and they are now well described, for example, in [63–65]. Inorganic salts have been empirically ordered (Hofmeister series) according to their ability in promoting the precipitation (salting-out) of a protein from an aqueous solution: $CO_3^{2-} > SO_4^{2-} > S_2O_3^{2-} > H_2PO_4^- > F^- > Cl^- > Br^- \approx NO_3^- > I^- > ClO_4^- > SCN^-$ for anions, and $(CH_3)_4N^+ > Cs^+ > Rb^+ > NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+}$ for cations [64]. The sequence for anions parallels the salting-out series for small solute molecules while the sequence for cations is rearranged. The most efficient salting-out anions (small, polyvalent, of high charge density) are strongly hydrated and most strongly decrease the thermodynamic activity of solvent water. The salting-in effects are caused by large, polarizable ions, for example, tetraalkylammonium and Cs^+ cations and tetraphenylborate and SCN^- anions, which interact with solutes by dispersion forces and/or enhance the structure of the bulk water, which reduces the hydrophobic effect of the solute removal. Also small, hard and polyvalent cations, for example, Li^+, Mg^{2+}, Al^{3+}, can exert salting-in effects by specific binding to the Lewis basic sites of certain solutes [64, 65].

Neutral metal chelates are also nonelectrolytes, and their partition constants in the solvent extraction systems increase (with some exceptions) with increasing concentration of a given electrolyte. Scarce information related is available in monographs dealing with solvent extraction of metal ions, for example, [1–3]. Based on the well-known Setschenow equation [63], one can formulate the following relationship

between the partition constant, P_{ML}, of a neutral metal chelate, ML_n, in the solvent extraction system, and the concentration, c_{el}, of the electrolyte in the aqueous phase:

$$\log \frac{P_{ML}(c_{el})}{P_{ML}(0)} = k_s \cdot c_{el} \tag{4.13}$$

where $P_{ML}(c_{el})$ and $P_{ML}(0)$ are the partition constants determined at a given concentration of the electrolyte and at the absence of the electrolyte in the aqueous phase, respectively, and k_s is the salting-out (or salting-in if $k_s < 0$) coefficient.

The stronger the specific (inner- and outer-sphere) hydration of metal chelates, the greater are their salting-out coefficients [34, 61].

4.5 Solute-Solvent Interactions in the Organic Phase

4.5.1 Inert solvents. Regular solutions

Inert organic solvents interact with solutes by various types of cohesive forces. In general the dispersion forces prevail. The energy of the dispersive interactions is different for various solvents and various solutes, but in general, these energies and their differences are much lower than the energies of specific interactions that lead to the formation of defined molecular adducts. If a liquid solute dissolves in a solvent without the heat and volumetric effects, the solution is said to be ideal [66]. However, many nonideal organic solutions have a sufficient thermal energy to entail the random distribution of the solute molecules, which results in the nearly ideal entropy of mixing. Only a small heat of mixing is the measure of the solute-solvent interactions in such solutions. The works of George Scatchard on the thermodynamics of nonelectrolyte solutions, in the first half of the 20th century, led to the expression of the cohesive energy of interactions between different molecules in such solutions as the geometric mean of the cohesive energies for the pure components. The nonelectrolyte solutions with the ideal entropy of mixing have been dubbed *regular* by Joel Hildebrand who extensively studied the solubility of nonelectrolytes. The square root of the cohesive energy density, that is, the energy of vaporization per unit volume of the nonelectrolyte, was then termed the *Hildebrand solubility parameter*, δ:

$$\delta = \sqrt{\frac{\Delta H_v - RT}{V}} \tag{4.14}$$

where ΔH_v is the molar heat of vaporization, R is the gas constant, T is temperature, and V is the molar volume of the nonelectrolyte.

The solubility parameter approach appeared useful for describing the nonspecific solute-solvent interactions in organic solutions, and in the early 1960s

the theory of regular solutions [67] was used by Siekierski to evaluate the effect of inert organic diluents on the distribution of metal complexes in solvent extraction systems [15, 66]. The early works on this topic have been reviewed by Irving [66]. The key paper described the distribution of germanium in the form of covalent GeX_4 molecules (X^- is a halide anion) between aqueous solutions of a constant high concentration of the respective acid, HCl, HBr, and HI, and a series of 18 organic solvents [68]. The authors derived the equation that expressed the ratio of the distribution ratios (in molar fractions), $D_c/D_{c,s}$ of the GeX_4 solutes (c) in these solvent extraction systems (where $D_{c,s}$ relates to the selected standard solvent) as a function of the molar volume of the solute, V_c, and of the solubility parameters of the solute, δ_C, of a solvent, δ_o, and of the standard solvent, δ_s:

$$\ln \frac{D_c}{D_{c,s}} = \frac{V_c}{RT}\left[(\delta_c - \delta_s)^2 - (\delta_c - \delta_o)^2\right] \tag{4.15}$$

Plotting the experimental $\ln(D_c/D_{c,s})$ versus the known δ_o values (Fig. 4.4) for each GeX_4 salt, the authors obtained three parabolas and evaluated three δ_C values, $\delta_{GeCl4} < \delta_{GeBr4} < \delta_{GeI4}$, corresponding to the maxima of the parabolas. This shows that the high-symmetry GeX_4 molecules form regular solutions practically in all the organic solvents studied.

Later on, other authors reported similar relationships between the distribution ratios of various metals and solubility parameters of inert diluents, observed in other solvent extraction systems [66, 69]. It should be added that conventional units, $cal^{1/2}\ cm^{-3/2}$, were used for the solubility parameter in the older papers, while the SI units, $J^{1/2}\ m^{-3/2}$ and more often $MPa^{1/2}$, are used now [69].

Among the organic solvents studied in [68], there were also aromatic and acidic ($CHCl_3$ and $CHBr_3$) solvents potentially able to interact specifically with metal-containing solutes. Nevertheless the respective experimental D_c values for the inert GeX_4 solutes did not significantly differ from the values predicted by Eq. (4.15). This indicated that the dispersive solute-solvent interactions prevailed in the systems studied. In contrast, these aromatic and acidic solvents significantly increased the experimental D_c values for similar but less inert solutes. This effect was interpreted as the result of molecular adduct formation in the organic phase by these reactive solvents with the extracted metal complexes [70, 71] (see Section 4.5.2).

Numerous attempts were undertaken later on to extend the Hildebrand-Scatchard approach on the other types of weak molecular interactions. For example, Hansen introduced a more comprehensive solubility parameter that accounted not only for dispersion forces but also for hydrogen bonding and polar interactions [72]. His approach permits the calculation of the solubility of various solutes in a broad range of solvents. This possibility has a great practical importance; however, it seems to be of little use in fundamental studies carried out by solvent extraction. The concept of

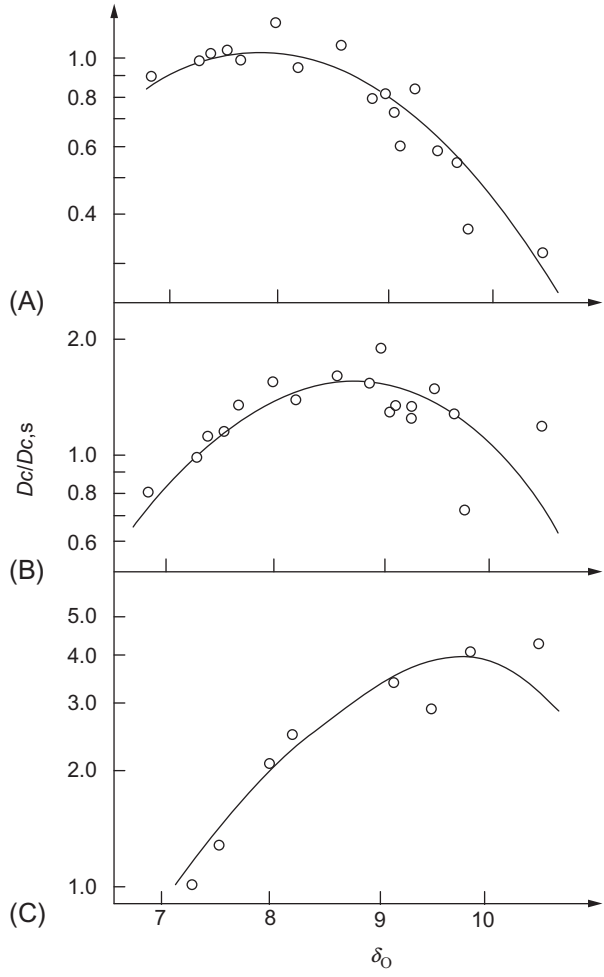

Fig. 4.4 The dependence of the $D_c/D_{c,s}$ ratio on the solubility parameter, δ_o [cal$^{1/2}$cm$^{-3/2}$], of solvents in the extraction of germanium(IV) halides into inert solvents from aqueous solutions of high concentrations of the respective acids: (A) 7.30-M HCl, (B) 6.50-M HBr, and (C) 5.20-M HI, at 20 ± 2°C. Standard solvent—*n*-hexane ($\delta_o = \delta_s = 7.3$). *(From Siekierski S, Olszer R. Relation between the partition coefficient of GeX_4 molecules (X = Cl, Br, I) and the solubility parameter of the solvent. J Inorg Nucl Chem 1963;25:1351-7, with permission.)*

solubility parameters and the examples of its application in various fields, including solvent extraction and liquid chromatography, are extensively presented in the handbook by Barton [69]. The handbook also brings a huge amount of information and an extremely broad bibliography on the subject.

Inert organic solvents, in particular aliphatic hydrocarbons, are widely used as diluents for various specific extractants. In certain cases the inertness of the diluents plays the decisive role in improving the efficiency of metal ion extraction. This is reported by Tasker et al. on the example of solvent extraction of copper(II) with salicylaldoxime ligands. The strength of the ligands as the extractants and their selectivity for copper(II) have been explained as due to the stability of the coordinated ligand dimer—the 14-membered pseudomacrocyclic hydrogen-bonded assembly, which provides a cavity of nearly ideal size for the copper(II) ion [73]. The weak interligand hydrogen bonding in the outer sphere of the copper(II) complexes would not be possible, however, for acidic diluents that competitively interact with the same hydrophilic fragments of the coordinated ligand, disturbing this way the interligand H-bond interactions.

4.5.2 Reactive solvents

Solutions of various specific extractants in inert organic diluents can be considered the solvents reactive toward metal ions. However, the solvent extraction of metal ions by complex formation with the extractants has been broadly discussed in Section 4.3.3; therefore in the present section, we limit the problem to real, one-component organic solvents and to their interactions with metal ions.

The first organic solvents used for solvent extraction of metal ions, water-immiscible carboxy compounds of basic character, ethers, ketones, etc. [25], played a diverse role in the system; apart from being the bulk organic phase, they specifically interacted with the extracted metal salts. These solvents formed lipophilic adducts (solvates) with coordinatively unsaturated metal salts by direct coordination through their donor oxygen atoms to the metal cations. However, after the introduction of more efficient extractants with higher basicities, the carboxy compounds lost their usefulness for solvent extraction (Section 4.3.3.1).

Organic solvents of acidic character, for example, chloroform [74] and chlorophenols [75], easily form hydrogen bonds with metal complexes in the organic phase, which improves the extraction [74, 75]. Chloroform forms such outer-sphere adducts not only with the complexes with ligands of the donor oxygen atoms (see Section 4.4.3) but also with those with pyridine-type ligands where the donor nitrogen atom of the coordinated ligand have no more free electron pair. In the latter case the $CHCl_3$ molecule forms the H-bond with the π-electrons of the coordinated pyridine-type ligands [71, 74]. The acetylacetonates most strongly solvated by chlorophenols are $Zr(acac)_4$ and $Hf(acac)_4$ [75], as is the case of their outer-sphere hydration [29]. This parallelism shows that the hydrogen bonding of metal chelates with the lipophilic acidic solvents in the organic phase partly compensates the similar effect of their outer-sphere hydration in the aqueous phase.

Also aromatic hydrocarbons interact specifically with certain metal salts, similar but not as inert as germanium tetrahalides. The examples are trihalides of the

metal(III) cations of the Group 15. The stereochemically active lone electron pair on the cations makes the MX_3 molecules slightly distorted trigonal pyramids with permanent dipole moments. The MX_3 molecules readily form adducts with aromatic hydrocarbons that donate electrons from their π-systems to the metal cations. In the adduct the lone electron pair of the cation, for example, Sb^{III}, in the tetrahedral MCl_3 pyramid is directed parallel to the plane of the aromatic molecule [76]. Solvent extraction of arsenic(III) and antimony(III) from aqueous solutions of high HCl concentrations was studied as a function of increasing concentration of various aromatic solvents, B, in two organic phases containing different inert diluents as the standard ones. The observed increase in the distribution ratios of the metals, greater for Sb^{III} and increasing with the increasing number of methyl groups in the aromatic ring of the solvent molecule, has been interpreted in terms of donor-acceptor interactions between the extracted metal(III) trichlorides and the aromatic solvents as the electron donors [70]. The authors calculated the stability constants of the 1:1 $MCl_3 \cdot B$ adducts for all the aromatic solvents studied, using various models of the associated organic solutions. The $SbCl_3 \cdot B$ adducts appeared significantly stronger than their $AsCl_3 \cdot B$ counterparts [70].

Also, other p-block metal ions in lower oxidation states form complexes with strongly basic ligands, in which the lone electron pair on the metal ion is stereochemically active. This conclusion was based on the low degree of coordinative unsaturation of the neutral bis(acetylacetonato)lead(II) molecule, which was revealed in the much weaker synergy in its extraction with TOPO than that observed for $Zn(acac)_2$, and it has been confirmed by the analysis of the crystal structure of $Pb(acac)_2$ (distorted square pyramid with four short, partly covalent chelating Pb—O bonds) [77]. In contrast, solvation of lead(II) ions in acetylacetone solution indicates (LAXS method) the coordination of five neutral Hacac molecules around the lead(II) ion with the formation of 10, much longer Pb—O bonds of mainly electrostatic character, which proves that the $6s^2$ lone pair on the Pb(II) ion in the solvate is stereochemically inactive [77]. Also stereochemically inactive is the $6s^2$ lone pair on the Pb(II) ion in the $[Pb(CyMe_4\text{-}BTBP)_2(NO_3)]^+$ cation with the neutral $CyMe_4$-BTBP (Fig. 4.6) ligands, in the crystal structure of which the eight long Pb—N bonds were seen [78].

4.6 Kinetics of Solvent Extraction Processes

4.6.1 Diffusional and kinetic regimes. Mechanisms of metals extraction

The kinetics of the solvent extraction processes depends on the rates of both the chemical reactions that occur in the two-phase system and the diffusion of the species participating in the chemical reactions. The slowest step, either chemical or diffusional, controls the rate of the whole process. The solvent extraction systems are

particularly difficult to be analyzed. That is because not only do the chemical reactions take place in the two different bulk liquid phases but they can also occur in the thin liquid-liquid interface [79]. Even a simple physical partition of a neutral molecule between the two liquid phases is accompanied by a chemical change in the solvation environment of the partitioned molecule. The analysis becomes much more complicated when extractants are used that complex the metal ions. Assuming that the extracted metal complex is formed at the interface, one should take into account also the effects of diffusion of the reaction substrates from both bulk liquid phases to the interface and the diffusion of the product—the metal complex—from the interface to the organic phase.

The effects of slow diffusion of the system components through the bulk liquid phases may be neglected when both solutions are vigorously stirred. The two-film model considers thus the diffusion of the solutes through two stagnant thin layers on the aqueous and organic side of the interface, called diffusion films or diffusion layers. In all solvent extraction systems, a limiting thickness of the diffusion films is 10^{-3}–10^{-4} cm, depending on the physicochemical properties of both solutions and on the specific hydrodynamic conditions. The film diffusion is often the rate-controlling step in solvent extraction processes, which then run in a *diffusional regime*. Contrary, when at least one of the chemical reactions is sufficiently slow in comparison with the rate of diffusion, the extraction rate can be described in terms of chemical reactions that take place either in the bulk phases or at the interface. In such a case the solvent extraction proceeds in a *kinetic regime*. Also a *mixed diffusional-kinetic regime* occurs, when the rates of both the chemical reactions and film diffusion processes are comparable [79].

The chemical reactions, the kinetics of which must be taken into account in the case of solvent extraction of metal ions by chelate formation, involve dehydration of the metal ion, acid dissociation of the extractant to form the chelating anion, sometimes structural preorganization of the chelating molecule, for example, slow keto-enol conversion, and the formation of a lipophilic molecule of metal chelate, its dehydration, solvation, sometimes aggregation in the organic phase, and so on. The ligand exchange with subsequent complex formation is the reaction of *nucleophilic substitution*. Breaking of a coordination bond between the metal ion and the first ligand, in particular water, and the formation of a new coordination bond with the second (organic) ligand occurs through the formation of short-lived intermediates in which the coordination number of the metal ion is either lower (dissociative mechanism, S_N1) or higher (associative mechanism, S_N2) than in the initial species. A more detailed classification of the reaction mechanisms is also used [79]. The knowledge of the reaction mechanism, that is, of the consecutive steps of the complex formation process, is essential to obtain information on the factors affecting the extraction rate and to correctly describe the whole extraction process.

The rates of the complex formation reactions depend on the activation energies of the reaction intermediates. The activation energy is the difference in the energies of the activated complex and the reaction substrates. When the extraction rate is controlled by a chemical reaction, it is generally more temperature-dependent, and the activation energy is higher than that expected for a diffusion-controlled process. The rate at which solvent molecules are exchanged between the inner coordination sphere of a metal ion and the bulk solvent is of primary importance in the kinetics of complex formation. The charge, radius, and especially the electronic structure of metal ions significantly affect the rate of this reaction, in particular water exchange in aqueous solutions (Fig. 4.5). Complexes that readily exchange the inner-sphere molecules of ligands (also water) with other ligands are termed *labile*, in contrast to *inert* complexes that exchange the ligands very slowly.

All these chemical reactions are usually going stepwise with a specific rate for every step. Formation of an extractable metal complex (chelate) can occur either at the interface or in each bulk liquid phase, depending on the hydro-/lipophilicity of the extractant, properties of the diluent, the composition of the aqueous phase, the presence of phase-transfer reagents in the system, etc. The region where the complex is formed sometimes plays the decisive role in the kinetics of the extraction.

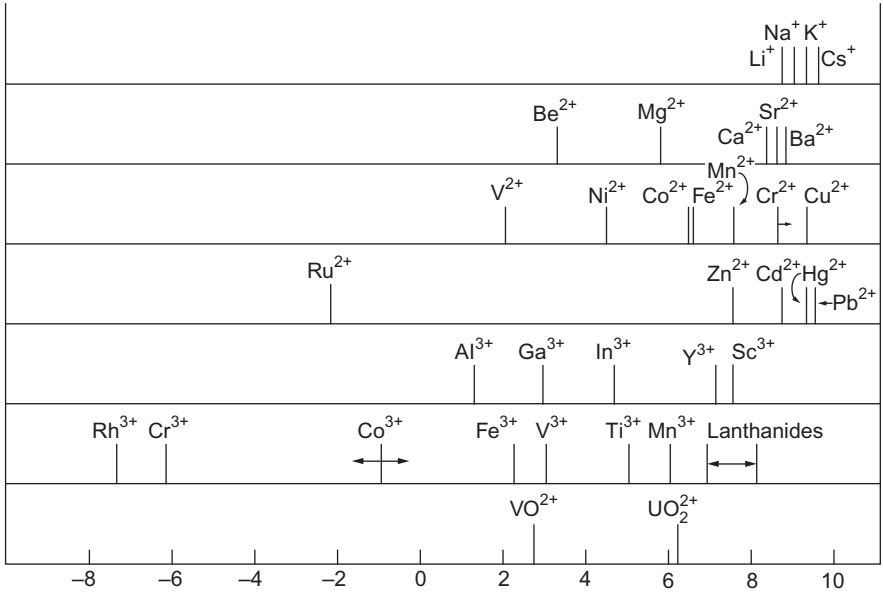

Fig. 4.5 Logarithms of rate constants for water exchange (per second) in certain M^+ to M^{3+} aqua metal ions at 25°C. The data point for UO_2^{2+} has been corrected. *(From Danesi PR. Chapter 5. Solvent extraction kinetics. In Solvent extraction, principles and practices, 2nd ed. Rydberg J, Musikas C, Choppin GR, Cox M (eds.). New York: Marcel Dekker; 2004. p. 216, with permission.)*

The rate-controlling reaction steps occur either in the bulk phase (homogenous reaction) or at the liquid-liquid interface (heterogeneous reaction). To differentiate between such chemical reactions, the interfacial tension is usually studied. For the interfacial chemical control, the effects of the interfacial area and of the interfacial activity of the extractant are of primary importance. The presence on the interface of adsorbed layers of preferentially oriented polar or ionizable extractant molecules lowers the interfacial tension, which makes it easier to disperse one phase into the other. When an extractant is a strong surfactant poorly soluble in the aqueous phase, the interfacial zone is the region where the reaction between a metal aqua ion and the lipophilic extractant most probably occurs [79]. For example, chemical reactions at the interface determine the rate of solvent extraction of copper with hydroxyoximes. Anionic surfactants accelerate the extraction, while cationic and nonionic surfactants retard it. Also the nature of the diluents used is of importance. Swelling of the interfacial layers caused by aromatic diluents decreased the concentration of the extractant molecules at the interface, which decreased the extraction rate [80]. Dramatic differences in the extraction of copper(II) from chloride solutions by three isomeric methyl 8-pyridyloctanoate extractants have been attributed to the unfavorable orientation of the nonextracting isomer at the liquid-liquid interface [81].

In contrast, when the extractant has low surface activity and relatively high solubility in the aqueous phase, the complex formation is expected to be fast and to take place in the bulk aqueous phase. The examples are the solvent extraction systems with amphiphilic extractants, β-diketones (acetylacetone, trifluoacetylacetone, thenoyltrifluoroacetone, etc.), di-ethylhexyl phosphoric acid, and so on. On the other hand, in the presence of strong complexing agents in the aqueous phase, the extraction is slow, as well as ligand substitution reactions with planar tetracoordinated complexes [3]. Rare cases when the complex formation takes place in the organic phase are mentioned in Section 4.6.2.

4.6.2 Accelerated solvent extraction of metal ions

The most obvious way to accelerate the kinetics of solvent extraction is to raise the temperature of the system. The increased fraction of high-energy solute molecules enables faster creation of the reaction intermediates with high activation energy. Various approaches based on this idea have been used in environmental applications of solvent extraction, including microwave-assisted solvent extraction [82] and extraction enhanced by sonication [83], as the preferred techniques to extract certain organic compounds from natural solid or liquid materials in many laboratories. Recently the microwave-assisted solvent extraction was successfully used to accelerate the kinetics of solvent extraction of substitution-inert platinum group metals, Ru(III) and Rh(III), from aqueous HNO_3 solutions to a thermomorphic ionic liquid without vigorous shaking, resulting in nearly quantitative yields within 10^1–10^2 s [84].

Another way to the accelerated kinetics of solvent extraction of metal ions is the use of phase-transfer reagents (phase-transfer catalysts, PTC) to facilitate the transfer of the metal ions from the aqueous to the organic phase. In this case the phase-transfer catalyst is an organic compound somewhat water soluble that forms lipophilic complex(es) with the given metal ion already in the bulk aqueous phase. This homogeneous reaction is much faster than the slow heterogeneous reaction at the interface, that takes place with a selective extractant present in the system. The formed complexes quickly migrate to the organic phase, but the distribution ratio of the metal in the given extraction system at equilibrium should be low, $D_{M,PTC} < 10^{-2}$. This is sufficient to significantly accelerate the kinetics of its extraction. On the other hand, when a PTC unselectively extracts two separated metals of similar properties, their low $D_{M,PTC}$ values do not significantly decrease the high separation factor provided by the selective extractant alone. This approach was successfully used to accelerate the rather slow extraction kinetics of americium(III) and curium(III) by $CyMe_4$-BTBP (Fig. 4.6) extractant. Another extractant, lipophilic *N*,*N*′-dimethyl-*N*,*N*′-dioctyl-2-(2-hexoxyethyl)malondiamide (DMDOHEMA), was added as the phase-transfer reagent to the organic phase (n-octanol diluent) contacted with 1 M HNO_3. The authors considered the surface active DMDOHEMA to complex the metal cations at the aqueous/organic interface and transport them into the organic phase, where DMDOHEMA could be exchanged for $CyMe_4$-BTBP in a faster ligand-exchange reaction [85]. The unexpectedly fast kinetics of actinides(III) and lanthanides(III) extraction with BTBP extractants in cyclohexanone diluent [86] has been interpreted as due to some water solubility of this ketone, which caused it to behave as a phase-transfer agent for the metal ions [87]. Also bi-*N*-dentate derivatives of 1,2,4-triazine are the phase-transfer agents for these metal ions, significantly improving poor kinetics of their extraction in similar systems with acidic aqueous phases [87].

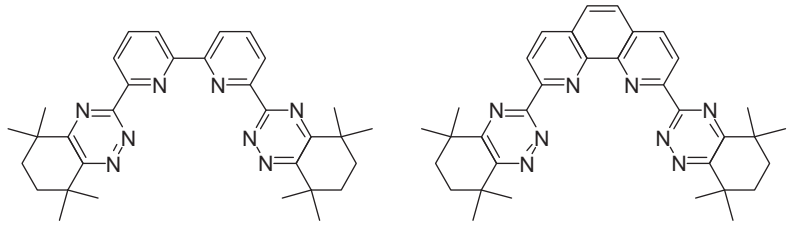

Fig. 4.6 Structures of the ligands $CyMe_4$-BTBP (1) and $CyMe_4$-BTPhen (2). *(From Lewis FW, Harwood LM, Hudson MJ, Drew MGB, Hubscher-Bruder V, Videva V, Arnaud-Neu F, Stamberg K, Vyas S. BTBPs versus BTPhens: some reasons for their differences in properties concerning the partitioning of minor actinides and the advantages of BTPhens. Inorg Chem 2013;52:4993-5005, with permission.)*

Spatial orientation of donor atoms in the free molecules of numerous polydentate extractants in solution usually differs from that observed in the same ligands coordinated to the metal ion in the extracted complex. The proper reorganization of the ligand structure not only requires an excess energy but also is a time-consuming process. This fact explains the reason for the slow kinetics of M^{3+} ions extraction with BTBP extractants [85, 87] whose donor atoms belong to four six-membered rings joined together by single C—C bonds (Fig. 4.6), thus easily rotating. To improve the slow kinetics of M^{3+} extraction, observed for the BTBPs, a novel lipophilic ligand with partly preorganized molecular structure was synthesized and studied as the extractant of actinides(III) and lanthanides(III): 2,9-bis(5,5,8,8-tetramethyl-5,6,7,8-tetrahydrobenzo-1,2,4-triazin-3-yl)-1,10-phenantroline ($CyMe_4$-BTPhen, Fig. 4.6) [88, 89].

It was expected that to achieve the cis conformation of the pyridine groups in the BTBPs that is required to form a complex, a significant energy barrier to rotation around the central biaryl C—C axis must be overcome, while this conformation is already fixed in the BTPhens. The QM calculations on the conformations of the $CyMe_4$-BTBP molecule show that just this rotation is decisive on the energy gain following the conversion from the most stable *ttt* conformer, while the rotation around the C—C bonds connecting the pyridine and triazine groups, leading to the *ccc* conformer, requires much less energy [89, 90]. Accordingly, not only the kinetics of Am^{3+} and Eu^{3+} extraction with $CyMe_4$-BTPhen was significantly faster than that with $CyMe_4$-BTBP but also the respective D_{Am}, D_{Eu}, and $SF_{Am/Eu}$ values determined under comparable conditions appeared significantly higher for $CyMe_4$-BTPhen [88] than for $CyMe_4$-BTBP [85]. The preorganization of the donor atoms with a rigid cis-locked 1,10-phenanthroline motif leads to a rapid and highly efficient separation of actinides from lanthanides. The improved extraction kinetics with the BTPhen ligand relative to its 2,2′-bipyridine counterpart was related to a higher concentration of the former at the interface [88].

4.7 Summary

Solvent extraction is currently a mature method widely used to separate metal ions present in aqueous solutions. The metals selectively extracted into the organic phase are then stripped to another aqueous solution from which they are isolated in the form of various pure metal compounds. The selective separation of metal ions can also take place at the stripping step that follows the nonselective extraction of a group of metal ions. The simplicity and effectiveness of the method and the possibility of using it in an extremely wide range of quantities of the separated metals make solvent extraction a favorable method in numerous applications. This method is used

on a laboratory scale in a variety of fundamental research in chemistry and physics. Large-scale industrial applications of solvent extraction include the production of metals from ores and their recovery from wastes, as well as the reprocessing of spent nuclear fuels from power reactors. However, many organic solvents used have adverse environmental impact. Recent studies on the alternatives to conventional solvent extraction, for example, by the use of liquid membranes or room-temperature ionic liquids, tend to minimize the environmental impact of these solvents when used on an industrial scale.

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Chapter 5

Aqueous Two-Phase Systems

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Chapter Outline

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5.1 Introduction

As shown in this book, various unit operations are used in downstream processing to extract/separate solutes from a liquid phase. Among these, aqueous two-phase systems (ATPS) were proposed as alternatives liquid-liquid extraction (LLE) processes for the separation of biological products. ATPS were discovered by Beijerinck in 1896, who observed that a mixture of gelatin, agar, and water can create a biphasic system [1]. However, there were no further developments until 1956, when Albertsson proposed ATPS composed of polyethylene glycol (PEG) and dextran for the separation of proteins, peptides, nucleic acids, viral, and LLE particles [1]. Besides polymer/polymer, Albertson also formed ATPS by mixing a polymer and an inorganic salt [1]. Subsequently, a significant number of works were reported characterizing and understanding different types of ATPS or evaluating their uses for the separation, extraction, and purification of biomolecules and particles [1–13].

ATPS are biphasic systems that can be used in LLE processes, where two water-rich phases are formed by mixing, above given concentrations, at least two different water-soluble components, such as polymers, salts, sugars, alcohols, or surfactants. Although both components are water-soluble, they separate into two phases, each richer in one of the two components. Because of the hydrophilic nature of the immiscible water-rich phases and the low interfacial tension, their use in biotechnological processes has been paramount. ATPS are frequently associated with simple, biocompatible, amenable, and easily scalable separation platforms [1–3]. Depending on the type of ATPS, a range of downstream processing scenarios can be attained, for example, achieving selective extractions, concentrating diluted solutes, or removing significant amounts of contaminants and denaturing compounds. Despite these advantages, their application to biotechnological downstream processing is predominantly confined to laboratory and academic (basic and applied) studies [2, 4–6] without significant industrial use. In recent years a large number of reviews [3, 5–26] have appeared compiling most of the advances, concepts, and applications of ATPS-based platforms.

5.2 Thermodynamic Fundamentals and Properties

The most common ATPS are generated by mixing a pair of hydrophilic polymers (polymer/polymer), a polymer with a salt (polymer/salt), or two different salts (salt/salt). Many of these combinations can generate a biphasic regime within a certain concentration range, where the phase separation is controlled by water solvation of the phase-forming agents. This section provides an overview of the thermodynamic fundamentals and key properties of ATPS.

5.2.1 Phase diagrams and tie-lines

Phase diagrams represent the potential working region for an ATPS, and any attempt at using ATPS should start with the determination of the phase diagram. As shown in Fig. 5.1, this is based on the compositions (weight or molar) of two phase-forming agents (components 1 and 2), providing a set of useful data about the system in equilibrium: (*i*) concentration of the components 1 and 2 necessary to create a biphasic system, (*ii*) the concentration of phase components in the top (light) and bottom (heavy) phases, and (*iii*) ratio of the phase volumes. Although ATPS are ternary systems, composed of two phase-forming agents and water, these are in general depicted in an orthogonal representation in which the water concentration is omitted (pure water corresponds to the origin).

The ternary phase diagram, depicted in Fig. 5.1, is constituted by two phase-forming agents (components 1 and 2) and water. A solubility curve (binodal) divides the two-phase region (above the curve) from the single-phase region (below the curve). The composition of the phases in equilibrium are related by the tie-lines (TLs) that connect two points on the binodal curve, which correspond to the

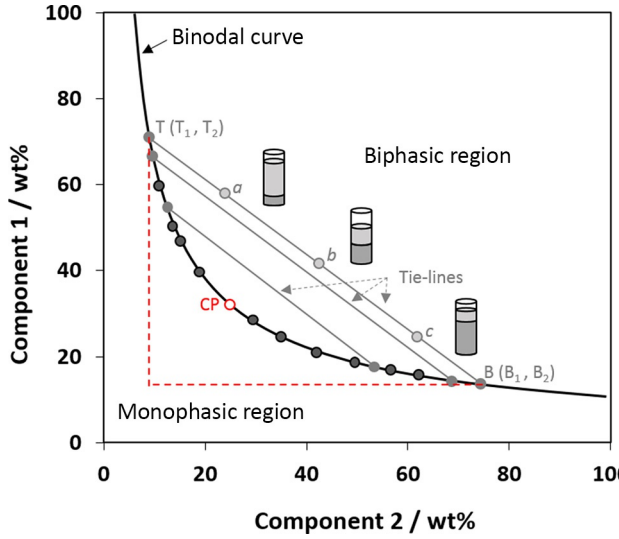

Fig. 5.1 Scheme of an orthogonal ternary phase diagram composed of component 1, component 2, and water (in weight fraction, wt%) and the respective binodal curve (—), tie-lines (—), and critical point (CP, ○). Top phase (component 1–rich phase) is plotted on the *y*-axis, and bottom phase (component 2 rich-phase) is plotted on the *x*-axis. *a*, *b*, and *c* (●) represent total compositions of three mixtures. The final composition of the top and bottom phase is represented by the nodes (●) *T* and *B*, respectively.

concentration of components 1 and 2 in the top and bottom phases. For example, *a*, *b*, and *c* correspond to three ATPS that have the same composition in equilibrium (T_1, T_2 and B_1, B_2, respectively, for the top and bottom phases). Thus, moving along the same tie-line (TL), the concentration of the phases remains the same, differing only in the total compositions and phase volume ratios—an interesting feature when ATPS are used for concentration of analytes since partition is maintained, while the volumes are reduced.

TLs are approximately parallel, and parameters such as tie-line length (TLL) and slope of the tie-line length (SLT) can be calculated, contributing to the understanding of the phase diagram and helping to draw other TLs. TLL has the same units as the concentration, and it is used to express the influence of the system composition on the partition of solutes. TLL and SLT can be related to the equilibrium phase composition according to Eqs. (5.1) and (5.2):

$$TLL = \sqrt{[T_2 - B_2]^2 + [T_1 - B_1]^2} \tag{5.1}$$

$$STL = \frac{[T_1 - B_1]}{[T_2 - B_2]} = \frac{\Delta\text{component 1}}{\Delta\text{component 2}} \tag{5.2}$$

For example, the TL of the system *a* is the segment TB, and the ratio of the length of the segments *aB* and *aT* corresponds to the top and bottom phase ratio (in wt%), as shown in Eq. (5.3):

$$\frac{m_T}{m_B} = \frac{V_T \rho_T}{V_B \rho_B} = \frac{aB}{aT} \tag{5.3}$$

where *m*, *V*, and ρ are the masses, volumes, and densities of the top (*T*) and bottom (*B*) phases. If the phase densities are known, the volume ratio of the phases can be easily determined.

As detailed by Hatti-Kaul [27], the binodal curves are mainly determined by three different methods, (*i*) turbidimetric titration, (*ii*) the cloud-point titration, and (*iii*) the analytic determination of the nodes (end points) of the systems. All these methods can be used for the determination of the binodal curve, but the most common are titration (turbidimetric and cloud-point) methods. The choice of a specific method should consider the type and nature of the phase-forming agents. For example, the determination of the solubility curves for a salt/salt ATPS can be easily carried out by cloud-point titration [3], but if some polydisperse polymers are used as phase-forming agents, in polymer/polymer or polymer/salt ATPS, a gradual decrease/increase in turbidity may occur, affecting the accuracy of the titration-based methods [27]. Although these methods are still the most applied, they are tedious and lengthy, and consume large amounts of reagents [13]. High-throughput screening alternatives have been proposed, using microfluidic devices [28] or 96-well microplate titration-based approaches [29].

The most common approach used for the description of the binodal curves is to fit them using empirical equations, particularly, the three-parameter equation (Eq. 5.4) proposed by Merchuk et al. [30]:

$$Y = A\exp\left(BX^{0.5} - CX^3\right) \tag{5.4}$$

where Y and X are the weight fractions (wt%) of components 1 and 2, respectively, and A, B, and C are adjusted parameters obtained by least-squares regression.

Merchuk's equation, initially proposed to describe polymer/salt ATPS, was successfully applied to polymer/polymer [31], salt/salt [32], and ionic liquid/carbohydrate-based ATPS [33]. Other alternative empirical equations have been proposed [23, 24], but as recently assessed by Alvarez et al. [34], Merchuck's equation still remains the best equation to fit the binodal curve.

Regarding the determination of TLs, the gravimetric method (also proposed by Merchuk et al. [30]) coupling the fitted binodal data by Eq. (5.4) and a mass balance relationship is typically used. The compositions of the coexisting phases (top and bottom) are determined mathematically by the solution of the following system of Eqs. (5.5)–(5.8):

$$Y_T = A\exp\left(BX^{0.5} - CX_T{}^3\right) \tag{5.5}$$

$$Y_B = A\exp\left(BX^{0.5} - CX_B{}^3\right) \tag{5.6}$$

$$Y_T = \frac{Y_M}{\alpha} - \frac{1-\alpha}{\alpha}Y_B \tag{5.7}$$

$$X_T = \frac{X_M}{\alpha} - \frac{1-\alpha}{\alpha}X_B \tag{5.8}$$

where Y and X are the weight fractions (wt%) of components 1 and 2, respectively, and A, B, and C are adjusted parameters obtained by least-squares regression; subscripts T, B, and M correspond to top phase, bottom phase, and the mixture, respectively; α is the ratio between the mass of the top phase and the total mass of the mixture. Despite the popularity of this approach to determine the TLs of different types of ATPS, the analytic method is still the most accurate to determine the exact composition of each component of the coexisting phases.

5.2.2 Physicochemical Properties of the Phases and Kinetics of Separation

The molecular mechanisms behind the phase separation and partition of solutes are governed by the thermodynamic equilibrium of the system, according to the type and chemical nature of the phase-forming components and characteristics of the target solute. An overview of these mechanisms is provided in the succeeding text. Nevertheless, before considering thermodynamics, it is important to review the kinetics of the phase separation and their relationship to the physicochemical properties of the

coexisting phases. Asenjo and coworkers [18, 35] stated that three main forces (gravitational, flotation, and frictional) are acting on a drop during coalescence, with the drop movement a result of their balance. Interestingly, each force depends on specific properties of the phases in equilibrium, namely, (*i*) gravitational force, dependent on the density of the drops, and (*ii*) flotation or frictional forces—dependent on the rheological properties of the phases. Therefore, the balance of these forces, along with the interfacial tension, will control the drop coalescence. Considering that in most ATPS the phase densities are similar, the behavior of the drops is mainly controlled by the rheological differences of the coexisting phases [35]. The viscosity plays a key role in the phase separation process because it determines the settling time of the phases after the mixing [35], as well as the fluid dynamics in continuous processing [18, 35] or the formation of micropatterned droplets [36]. For a fast separation, salt/salt ATPS should be used instead of polymer-based systems. However, by decreasing the polymer molecular weight or by increasing the temperature, the settling time and phase dynamics can be adjusted. Similarly, density differences can also affect the rate of sedimentation and, consequently, the kinetics of phase separation [35]. It is important to note that by changing the phase densities, it is possible to reverse the top (light) and bottom (heavy) layers. Therefore, the modulation of the individual phases, by changing the concentration of polymers or surfactants or simply by adding additives (such as salts, ionic liquids, and cosolutes) [13], can be a good approach to optimize the phase separation kinetics and consequently to tailor an ATPS to a specific application. ATPS have a very low interfacial tension, when compared with traditional LLE systems, with the smallest interfacial tension values obtained for formulations close to the CP. Like the other two properties, interfacial tension can also be adjusted [13]. The choice of ATPS should thus consider the fluid viscosity, density, and interfacial tension. Depending on the application, other properties such as osmolarity and hydrophobicity should also be considered prior to implementation [13].

5.3 Types of Aqueous Two-Phase Systems

The most common ATPS are generated by mixing a pair of hydrophilic polymers (polymer/polymer) or a polymer and a salt (polymer/salt). They have been explored since the 1980s [1, 8, 11]. A significant advance occurred in 2003, when Rogers and collaborators [37] generated an ATPS by the codissolution of two salts in water (salt/salt-based ATPS), one of the salts having one or both ions of high charge density (water-ion interactions stronger than water-water interactions), while the other salts are based on low-charge density ions (water-ion interactions weaker than water-water interactions). The delocalization of charge in the salt ions induces a lower melting temperature of the salt itself, these being categorized as ionic liquids (ILs) [37]. Therefore, in the last two decades, many studies have focused on the

development of new ILs-based ATPS, combining ILs with inorganic and organic salts, sugars, carbohydrates, polymers, alcohol, or other compounds. In this section, considering the large number of possible ATPS, only three types of ATPS are discussed, namely, *5.3.1 Polymer/Polymer*, *5.3.2 Polymer/Salt*, and *5.3.3 Salt/Salt*. Other ATPS types are briefly highlighted in the Section 5.3.4. In the Section 5.3.5, the influences of temperature and pH on the phase behavior of traditional ATPS are compared.

5.3.1 Polymer/polymer ATPS

Polymer/polymer ATPS [1] are formed when pairs of water-soluble polymers are mixed above a critical concentration inducing the formation of two phases. Since the late 1950s, several polymer/polymer systems have been characterized for different purposes [1, 8, 21]. During approximately 60 years, several combinations of hydrophilic polymers were successfully employed for the formation of two-phase systems, obtained from the mixing of: (*a*) two nonionic polymers, such as the well-known PEG/dextran-based ATPS; (*b*) one nonionic and an ionic polymer, for example, PEG/poly(acrylic acid) (PAA) and PEG/dextran-based ATPS; and (*c*) two charged polyelectrolytes, namely, sodium dextran sulfate/polystyrene sulfonate (PSS). Fig. 5.2 summarizes representative examples of polymer/polymer ATPS reported to date [1, 26, 27, 38–43].

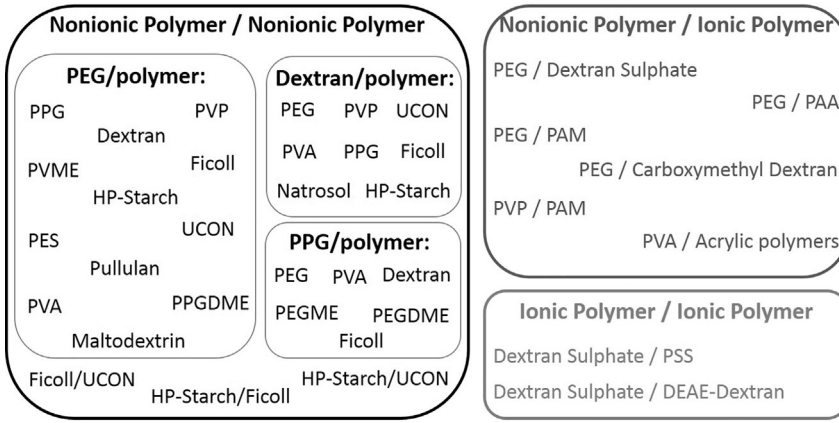

Fig. 5.2 Representative examples of polymer/polymer ATPS. (*Acronyms*: PEG, polyethylene glycol; PEGME, polyethylene glycol methyl ether; PEGDME, polyethylene glycol dimethyl ether; PPG, polypropylene glycol; PPGDME, polypropylene glycol dimethyl ether; PVP, polyvinyl pyrrolidone; PVA, polyvinyl alcohol; PES, polyether sulfones; HP, starch, hydroxypropyl starch; PAA, polyacrylic acid; PAM, polyacrylamide; PSS, polystyrene sulfonate).

Phase separation in solutions containing polymer mixtures is a common phenomenon, that is, most hydrophilic polymer pairs are "incompatible" in aqueous solutions [8]. Thermodynamically, polymer/polymer ATPS phase separation is described according to two points of view: the theory of polymer mixtures described by Flory-Huggins [44] on the basis of the energetically unfavorable segment interactions of polymers overcoming the entropy increase involved in phase separation [45, 46]; or the structure of water as a key factor of phase separation, that is, ordered polymeric water structures, supported by Zaslavsky [47]. To this day the phase formation in polymer/polymer ATPS is incompletely understood [40], and as recently highlighted by Sadeghi and Maali [43], only a few experimental and theoretical attempts have been made to fully understand the polymer/polymer phase separation mechanism.

These phase-forming mechanisms apply to nonionic-based ATPS, which, as shown in Table 5.1, are the most representative class of polymer/polymer ATPS. However, other systems can also be formed by mixing one polyelectrolyte and a nonionic polymer [39, 41, 59], two polyelectrolytes [60], or even using electrolytes as adjuvants [41, 46, 61], but their phase-forming mechanisms are far more complex. Herein, we do not detail these systems, but a comprehensive view of their phase separation mechanisms can be found in Picullel [45], Pfennig [61], Gupta [39], and Johansson [41, 62]. It is important to note that the phase behavior of polymer/polymer ATPS is strongly influenced by the presence of salts (or electrolytes). The addition of small concentrations of salt can significantly enhance the biphasic region or may allow the partitioning mechanisms of target solutes to be adjusted.

The next point to discuss is the effects that can influence the phase diagram, such as polymer concentration, molecular weight, the presence of additives, temperature, and pH. Since the last two factors also influence other types of ATPS, these will be discussed together in Section 5.3.5. Regarding the effect of polymer concentration, as highlighted in the description of the phase diagram, at low concentrations, both polymers are fully miscible in water, and no phase separation occurs. With increasing polymer concentration, a phase demixing will occur [1, 11]. It should be highlighted that depending on the nature of the polymer or its molecular weight, the effect on phase separation can be intensified or reduced affecting the "phase diagram symmetry." For example, in PEG/dextran ATPS, the increase of polymer molecular weight enhances their ATPS phase-forming ability, that is, low polymer concentrations are required for the formation of a two-phase region, as well as increasing the asymmetry between the phases [1, 11].

5.3.2 Polymer/salt ATPS

Polymer/salt ATPS, the second type of ATPS discovered in the mid-1950s [1], are formed by the dissolution of a water-soluble polymer and inorganic (or organic) salt

above critical concentrations [1, 27], promoting a salt-rich, polymer-poor bottom phase and a polymer-rich, salt-poor top phase [1, 27]. As reviewed by Hatti-Kaul [8], Grilo et al. [7], and Ruiz-Ruiz et al. [10], in the last decades, polymer/salt systems have been widely studied, carefully characterized, and applied to many separations. A wide array of polymers and salts can be combined for the formation of ATPS. As shown in Fig. 5.3, PEG and PPG polymers are the most used polymeric phase-forming agents [1, 38, 63, 64], while the inorganic phosphate, sulfate-based salts are the most common ionic components [1, 38, 63, 64]. However, ATPS composed of polymer and hydroxide [63, 64], nitrate [65], and carbonate salts [63, 64] can be produced; chloride salts [66] can also form ATPS if combined with a more hydrophobic PPG polymer. Noteworthy, more recently, a large number of ATPS used organic salts as phase-forming components, for example, citrate, tartrate, acetate, and formate salts [67–69], as more eco-friendly alternatives. Alternatively, it is possible to find less common polymer/salt systems, using polymers such polyethylene glycol dimethyl ether (PEGDME) [70], polyalkylene glycols (UCON) [71], or polyoxyethylene (20) cetyl ether (POELE) [72].

Regarding the demixing mechanisms of polymer/salt ATPS, in general, the effectiveness of different salts in promoting phase separation follows the Hofmeister series [73], where salt ions (preferentially the anion contribution) are ranked according to their salting-out ability [74, 75]. This sequence is the empirical ordering of salts according to the minimum concentration required for protein precipitation from an aqueous solution, which is, in general, the following:

$$\textit{Anions} — {CO_3}^{2-} > {SO_4}^{2-} > {S_2O_3}^{2-} > {H_2PO_4}^{-} > F^{-} > Cl^{-} > Br^{-} \approx {NO_3}^{-} > I^{-} > {ClO_4}^{-} > SCN^{-}.$$

$$\textit{Cations} — {(CH_3)_4N}^{+} > Cs^{+} > Rb^{+} > {NH_4}^{+} > K^{+} > Na^{+} > Li^{+} > Mg^{2+} > Ca^{2+}.$$

Multivalent anions and highly charged ions (i.e., with large negative Gibbs free energies of hydration), such as HPO_4^{2-} and SO_4^{2-}, are highly effective at forming

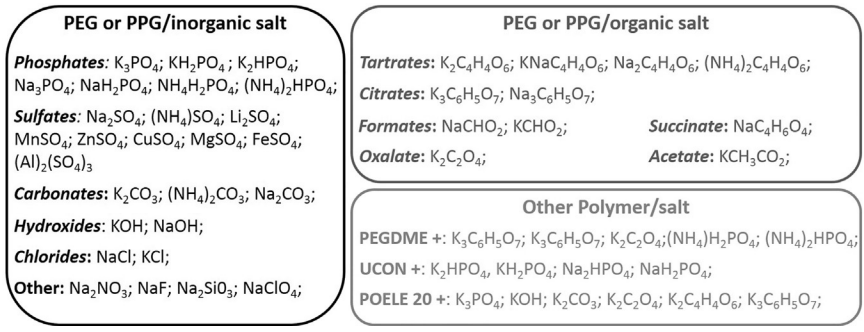

Fig. 5.3 Representative examples of polymer/salt ATPS.

polymer/salt ATPS, while, for example, chloride and bromide univalent salts are not as effective [73].

Although some details of the mechanisms are not fully understood, it seems that the phase separation results from the competition for hydration between the polymer and the salt [40]. For example, triply charged anions are strongly hydrated, being more effective in polymer salting out than the doubly charged sulfate and the monovalent hydroxide anions, that is, they are better ATPS-forming agents [73]. In the case of cations, the effect is more complex and, in some cases, results from a competition between two opposite effects, hydration and cation-polymer specific interaction (e.g., interaction between lithium and EO groups of PEO polymers) [73]. Anyway, Ananthapadmanabhan and Goddard [73] demonstrated that in most cases the fundamental forces behind the formation of polymer/salt ATPS are the salting-out aptitude of each salt and the partial dehydration of the polymer [73]. Further studies validated these earlier speculations for a wide range of salts and polymers [63, 65, 68, 69, 71, 72], even correlating the ions' salting-out aptitude with their Gibbs free energies of hydration (ΔG_{hyd}) [69, 71, 72], that is, the more negative ions' ΔG_{hyd} is, the stronger is its salting-out aptitude [76].

As discussed in the previous paragraphs, the phase separation of polymer/salt is both dependent on the type and molecular weight of the polymer and salting-out ability of the salt (i.e., position in the Hofmeister series ranking), but as for other types of ATPS, they are strongly influenced by temperature, pH (as a result of ion speciation), or even the presence of additives (as discussed later in Section 5.3.5).

5.3.3 Salt/salt ATPS

As highlighted in the previous sections, polymer/polymer and polymer/salt ATPS were for long the most studied and applied. However, due to the limited ability to manipulate the difference in polarities between the coexisting phases, their application was limited, often exhibiting narrow extraction and purification capabilities. ATPS composed of two polymers have similar phase polarities, while polymer/salt ATPS display distinctly different characteristics between the coexisting phases [3, 4]. To overcome these limitations, several alternatives were proposed, such as polymer derivatization or the use of additives [9, 10, 61].

A disruptive innovation on the ATPS field occurred in 2003, when Rogers and coworkers [37] demonstrated that a hydrophilic ionic liquid can be salted-out and concentrated from aqueous solution by inorganic salts forming an ATPS. The formation of this type of salt/salt ATPS, generally known as ionic liquid/salt ATPS, results from the codissolution of two salts, one with one or two highly charged ions (water-ion interactions stronger than water-water interactions) and other salt with low-charge density ions (water-ion interactions stronger than water-water interactions)

[3, 77]. The first salt can be any organic or inorganic salt (with mainly high-charge density ions dominated by coulomb interactions) with "salting-out" nature, while the second, due to low-symmetry and charge-delocalized ions, fits within a "particular" category defined as ionic liquids (ILs). By definition, ILs are "salts with melting temperatures below 100°C" [78]. However, the definition of ILs based on their melting temperature restricts the window for salts that can fit within ILs category [79]. Therefore, considering that salts with melting temperatures above 100°C also form salt/salt ATPS [80] and to avoid a distinction between IL/salt and salt/salt ATPS, this categorization was adopted in this chapter.

Due to the tunability of ILs (covering the whole hydrophilicity-hydrophobicity range [3]), the study of salt/salt ATPS has grown exponentially. In particular, after 2009 a large number of phase diagrams were characterized and extensively applied as potential separation platforms for a wide range of compounds [3, 4]. A detailed description of ILs-based ATPS can be found in the book by Freire [4]. In the next paragraphs, the major concepts behind the formation of the salt/salt ATPS with representative examples (Fig. 5.4), are discussed.

Imidazolium-based ILs (with halogens, sulfates, sulfonates, alkanoates, tetrafluoroborate, and triflate anions) are the most studied as the first phase-forming component, while high-charge density inorganic salts, such as phosphates, sulfates, and carbonates, are used as the second component of polymer/polymer ATPS [3, 4]. However, due to environmental concerns, some ILs-based ATPS have been proposed, in which inorganic salts have been replaced by more benign organic salts (citrates, tartrates, etc.) or even by other species such as amino acids, carbohydrates, and polymers (alternative ATPS discussed in the next subsection). Independently of the salt/salt ATPS, the use of two salts as phase-forming agents with low phase viscosities allows a quick phase splitting, overcoming an important drawback of the polymer-based ATPS [3].

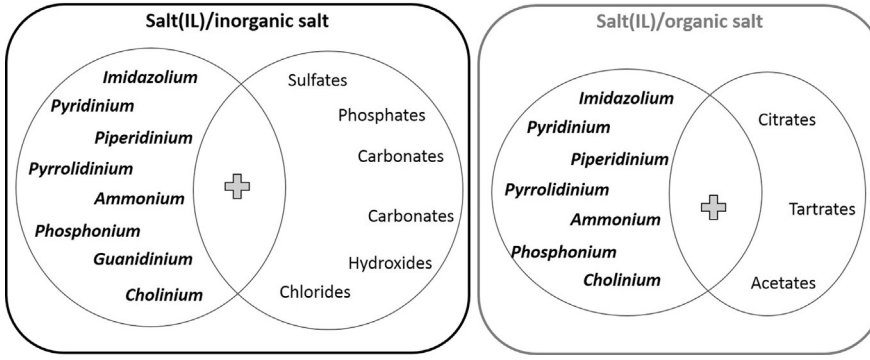

Fig. 5.4 Representative examples of salt/salt ATPS.

In these salt/salt systems, since both phase-forming components are ionic, a distribution of the constituent ions between the coexisting phases occurs but is always restricted by the electroneutrality of the overall system and individual phases [81]. Bridges et al. [81] have shown that, besides maintaining electroneutrality, the most chaotropic ion is preferentially concentrated into the top phase, while the most salting-out ion is partitioned to the bottom phase, with negligible deviation in the speciation of the ions along any given tie-line; thus each salt/salt phase diagram can be determined and interpreted on whole salt concentrations rather than ion concentration yields [81].

Regarding the phase separation mechanisms, the formation of salt/salt ATPS and its efficiency of separation are associated with the Gibbs' free energy of hydration (ΔG_{hyd}) of the most "hydrophobic" salt, namely, K_3PO_4, which due to its very negative ΔG_{hyd} is preferentially solvated [37], "salting out" the ILs from aqueous solution. The ΔG_{hyd} trend was demonstrated by the efficiency of separation in ATPS composed of 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl) with a series of inorganic salts (KOH, K_2CO_3, Na_2HPO_4, and $Na_2S_2O_3$) [37]. Similarly to the previous polymer/salt ATPS, the ability of the low ΔG_{hyd} salts to salt out different hydrophilic salts ([C_4mim]Cl, [C_4mmim]Cl, [C_4py]Cl, [N_{4444}]Cl, and [P_{4444}]Cl) can also be ranked according the Hofmeister series [81]. These first insights were further confirmed by Coutinho [32, 82–84] and Zafarani-Moattar [53, 85] research groups, which demonstrated that the cornerstone of the salt/salt ATPS formation is the salting-out effect [3], due to the creation of water-ion complexes, in opposition to the dominant ion-ion interactions in the salting-in inducing ions [77]. Therefore, as stated by Freire et al. [3], the addition of high-charge density salts (salting-out inducing salts) to aqueous solutions of salts containing low-symmetry charge-delocalized ions (ILs) leads to the preferential hydration of the first over the second and consequently to the salting out of the IL to the opposite (upper) phase. Shariari et al. [32] performed an extensive study of the molecular mechanisms for the effect of salt ions in the formation of salt/salt ATPS. Different ATPS containing 1-butyl-3-methylimidazolium triflate ([C_4mim][CF_3SO_3]) and aqueous solutions of conventional salts were evaluated, illustrating the influence of both the cation and anion of the salt on phase demixing [32]. The large set of data showed that both the cations' and anions' ability to induce the salting-out phenomenon follows the well-known Hofmeister series. Furthermore, they also found a close correlation between the IL molality required for ATPS formation and the ions' molar entropy of hydration, concluding that the creation of ion-water complexes plays a key role in the formation of salt/salt ATPS [32].

In addition to the nature of the salts, both temperature and pH significantly influence phase demixing for salt/salt ATPS. In Section 5.3.5 the general effects of both parameters are summarized. A more detailed discussion can be found in Refs. [3, 4].

5.3.4 Other types of ATPS

In addition to the well-studied polymer/polymer, polymer/salt, and salt/salt ATPS, several other ATPS formed through the mixture of a wide range of compounds have been studied, for example: (*i*) aqueous micellar two-phase systems (AMTPS), micellar and reverse micellar, using surfactants (anionic, cationic, zwitterionic, or nonionic) [14, 16, 20, 86–88]; (*ii*) IL-based ATPS with polymers [89–92], carbohydrates [33, 93, 94], amino acids [95], and others [3, 4]; (*iii*) carbohydrate-based ATPS [33, 93, 94, 96, 97]; (*iv*) copolymer-based ATPS [98–100]; (*v*) ATPS composed of deep eutectic solvents [101–103]; and (*vi*) ATPS composed of hydrophilic organic solvents, mainly short-chain alcohols [104–106]. While interesting, these systems are also more complex.

Among these "alternative" systems the most studied are AMTPS, which were introduced by Watanabe and Tanaka in 1978 for the extraction of zinc (II) [88]. These systems are formed by mixing surfactants (anionic, cationic, zwitterionic, or nonionic), that is, amphiphilic molecules with a hydrophilic and a hydrophobic core, and water, which can form aggregates known as micelles above a certain concentration (critical micellar concentration, CMC) [87]. A homogeneous micellar isotropic surfactant aqueous solution, when subjected to certain conditions, particularly temperature, can form spontaneously a micelle-rich (concentrated) phase and a micelle-poor (diluted) phase. Micelles start to interact preferentially with each other, resulting in a phase separation at a specific temperature (known as cloud-point temperature) [86]. The formation of AMTPS depends mainly on surfactant structure, charge, and concentration. In the presence of additives (e.g., inorganic and organic salts, biopolymers, alcohols, and ionic liquids), the phase demixing aptitude can be enhanced or reduced [86, 87].

A new type of ATPS was created by mixing polymers with ILs [89, 90]. While initially it was presumed that this polymer/IL systems would be simply another series of polymer/salt ATPS [90], it was soon realized that the replacement of high-charge density salts by more amenable ILs increased the complexity and changed the nature of interactions at the molecular level [89, 91, 92], enlarging the phases' polarity range, increasing the solute specific interactions, and reducing the crystallization problem [4]. Freire et al. [90] formed an ATPS composed of PEG polymers (different molecular weight) and a series of ILs (from imidazolium, piperidinium, pyridinium, pyrrolidinium, and phosphonium families). Thence, many ATPS combining ILs and different PEG- and PPG-based polymers have been used for several purposes [3, 4, 90, 92]. The formation of these polymer/IL ATPS not only is dependent on the IL ions' ability to form hydration complexes or the formation of polymer-water structure complexes but also results from a delicate balance between competing interactions that occur in IL-polymer solute pairs, where the larger the immiscibility between IL and the polymer, the greater is the phase separation [92]. Therefore, depending on the polymer type and molecular weight, nature of the ILs (e.g., the

ion chain length), and temperature, opposite phase demixing trends can occur as a result of the balance between complex interactions occurring between all the phase-forming agents [91, 92].

5.3.5 Effect of temperature and pH

ATPS phase diagrams can be influenced by many parameters, including the type and concentration of phase-forming components, temperature, pH, and the presence of additives or contaminants. In previous sections, the influence of phase-forming components on the formation of ATPS was discussed. However, both pH and temperature exhibit a strong influence on the phase separation mechanisms. A comparison of the main ATPS and their response to temperature and pH changes is presented in Table 5.1.

As the temperature increases, the binodal curves of the polymer/polymer [11, 48, 49] and salt/salt ATPS [3, 53, 54] shift to higher concentrations of phase-forming agents (away from the origin) corresponding to a decrease in the biphasic region. This shift with temperature is characteristic of an upper critical solution temperature (UCST) behavior. On the other hand, polymer/salt ATPS resemble a lower critical solution temperature (LCST) behavior in which an increase in temperature leads to an enlargement of the biphasic region, that is, less polymer and/or salt is required for the phase separation [49, 50, 107].

TABLE 5.1 Influence of pH and Temperature in the Phase Behavior of the Main ATPS Types

Temperature effect			
Increase (↑) of the temperature	Polymer-polymer ATPS [11, 48, 49]	Polymer-salt ATPS [50–52]	Salt-salt ATPS [3, 53, 54]
Biphasic region (binodal curve)	Decrease (↓) (shift to higher polymer concentrations)	Increase (↑) (shift to smaller polymer/salt concentrations)	Decrease (↓) (shift to higher salt concentrations)
Type of critical solution temperature behavior	UCST	LCST	UCST
pH effect			
Increase (↑) of the pH	Polymer-polymer ATPS [55, 56]	Polymer-salt ATPS [50–52]	Salt-salt ATPS [4, 57, 58]
Biphasic region (binodal curve)	Decrease (↓) (shift to higher polymer concentrations)	Increase (↑) (shift to lower polymer/salt concentrations)	Increase (↑) (shift to lower salt concentrations)

Several reports detail the effect of pH on phase separation of polymer/salt [101–103] and salt/salt [4, 57, 58] ATPS, but the effect of pH on the phase diagrams for polymer/polymer ATPS has been rarely studied. An exception is the study of Planas et al. [55], where different phase diagrams for systems containing EOPO-PEI titrated with lactic acid and DEX at different pH values (from 2.0 to 6.0) are described. The biphasic region increases with a decrease in pH, but the polymer concentrations in the two coexisting phases are not affected (constant slope of the TLs over the whole pH range) [55]. Yan and Cao also demonstrated that the binodal curve for two pH-responsive polymers move close to the origin (a high biphasic region) with a decrease of pH [56]. On the other hand, for both polymer/salt [50–52] and salt/salt [4, 57, 58], phase diagrams at increasing pH resulted in enlargement of the two-phase region, that is, less phase-forming components (polymers or salts) are required for the formation of the ATPS.

5.4 Applications of Aqueous Two-Phase Systems

ATPS have been utilized for the extraction, separation, and purification of a plethora of solutes. While some studies are focused on the understanding of the partition of molecules and particulates in these systems, most address their application to the extraction, concentration, and purification of products, both as downstream processing platforms or as an analytic tool. Over the last decade, approximately 100 ATPS application-based articles were published each year [7]. These were mostly focused on the separation/purification of enzymes, followed by purification of DNA, nucleic acids, monoclonal antibodies, and antibiotics, while several also addressed the extraction of metals [108]. The number of articles using ATPS as an analytic/characterization technique is also growing. In this section, some of these applications are summarized.

5.4.1 Partition of Molecules and Particulates

ATPS are regarded as useful tools for the extraction and purification of biocompounds [1, 11, 21]. Extraction by ATPS involves the transfer of a target solute from one aqueous phase to another with a partition coefficient (K), defined as the distribution ratio of the solute between the coexisting phases ($K = C_T/C_B$). Albertsson [1] proposed a model to correlate the K for a specific biomolecule, based on the different driving forces: (*i*) size-dependent (solutes are separated according to their size and surface area); (*ii*) electrochemical (solutes are separated according to their charge and the electric potential of the coexisting phases); (*iii*) hydrophobicity (solutes are separated according to hydrophobic interactions between molecules and the relative hydrophobicity of the phases); (*iv*) biospecific affinity (one of the phase-forming polymers has

specific binding sites for the target molecule); and (*v*) conformation-dependent (the conformation of the solute is the key factor for partitioning). This model, initially proposed for protein partitioning [1], can be expressed as Eq. (5.5):

$$\ln(K) = \ln(K_0) + \ln(K_{size}) + \ln(K_{elec}) + \ln(K_{hfob}) + \ln(K_{biosp}) + \ln(K_{conf})$$

where the subscripts *size*, *elec*, *hfob*, *biosp*, and *conf* refer to the electrochemical, hydrophobic, size, biospecific, and conformational contributions to K from both protein structural properties and the surrounding environmental conditions of the system. K_0 includes all other factors. As discussed in Refs. [1, 7, 11, 21], the K of a biomolecule is the result of the protein structure-related factors (such as charge, hydrophobicity, and/or surface properties) and the surrounding environmental conditions, as: (1) salt type and concentration; (2) pH; (3) phase-forming polymer type, molecular weight, and concentration; (4) the presence of polymer derivatives (charged, hydrophobic, or affinity types); (5) temperature; and (6) salt additives.

Although several theoretical and experimental studies provide some support for the key factors for biomolecule partition [109–111], these are still too complex and poorly understood. As discussed by Grilo et al. [7], depending of the type of biomolecule and ATPS, different theories are used to explain the experimental results. While some authors suggest that hydrophobicity controls the partition of biomolecules [109, 110], others suggest that electrostatic interactions play the major role [111]. Grilo et al. [7] suggested that the partitioning "should be regarded as a synergetic effect of all these different mechanisms." Thus, depending on the characteristics of the biomolecule and properties of ATPS, each mechanism will have a higher or lower influence on the partition. Since most ATPS partition mechanisms are quite complex and unpredictable, most experimental work optimized the partition according to the product properties and operation conditions, among others. In fact, the factors influencing the partition behavior of solutes in ATPS are (*i*) the polymer molecular weight and concentration, (*ii*) salt (or ionic liquid) type and concentration, (*iii*) relative hydrophobicity, (*iv*) pH and charges, (*v*) temperature, (*vi*) density and viscosity, (*vii*) interfacial tension, (*viii*) settling time, and (*iv*) solute size and concentration. The influence of each factor on partitioning for different ATPS is discussed in earlier reviews [3, 5–22, 26] and books [1, 2, 4, 27, 38].

5.4.2 Extraction and purification of products

In spite of the ATPS partition mechanisms not being fully understood, these systems have been widely used as effective downstream platforms for the recovery and purification of products ranging from biocompounds to metals. They are mainly used to replace traditional organic solvent liquid-liquid extraction procedures [5, 7, 21, 22],

but due to their low interfacial tension, they can be applied as well to stabilize fragile biological structures [1, 13]. A number of reviews provide additional details [3, 5–22, 25]. Some representative applications are presented in the succeeding text.

As expected, most ATPS are used for the extraction of bioproducts, ranging from small and simple biomolecules, such as amino acids, antibiotics, peptides, alkaloids, carotenoids, or biocolorants, to complex compounds such as proteins, enzymes, monoclonal antibodies, virus, virus-like particles (VLPs), cells and organelles, and DNA and nucleic acids [3, 5–22]. Among these, protein-related applications are the most widely studied, with several reviews focused on this topic [9, 16, 18, 21]. In particular, ATPS have been used extensively for the recovery of protein-based biopharmaceuticals (e.g., monoclonal antibodies, growth factors, and therapeutic enzymes) from complex cell cultures (like microbial, animal, and vegetal) [5, 7, 8, 12, 22, 25]. However, as suggested by Gonzalez-Valdez et al. [12], ATPS have a great potential for the selective fractionation, recovery, and purification of low-molecular weight solutes, other types of macromolecules, organelles, and even whole cells. In certain cases, due to the target-bioproduct degradation or cell culture inhibition, in situ approaches are used, where the extraction and production steps are integrated in a single unit, recovering the product during the bioconversion in a concept known as *extractive fermentation* or *extractive bioconversion* [5, 8, 11]. Alternatively, ATPS have been realized as a powerful tool for the selective extraction of metal ions [108].

5.4.3 Analytical applications

ATPS are becoming more widely used for analytic applications [4, 5, 7, 10, 13], for example, the use of ATPS for the concentration of residual drugs and pollutants in water [112–114] and food [113, 114] in which the ATPS allowed high concentration factors enhancing detection limits. The analytic aptitude of polymer/salt ATPS was also extended to label-free cell technologies, being used to differentiate promyelocytic cell line HL-60 through a high-throughput cell partitioning analysis [115]. Benavides et al. [10] highlighted the possibility of using ATPS for the molecular characterization of proteins. Since the protein partition is a surface-dependent phenomenon, where the exposed residues interact with the phase-forming agents, it can be used to discriminate between similar proteins based on their molecular properties, such as molecular size or relative hydrophobicity. Zaslavsky et al. [26] reviewed advances in analytic application of solute partitioning in ATPS, emphasizing systems that explore protein structural changes and protein-partner interaction (in vitro and in vivo). This solvent interaction analysis method allows the analysis and characterization of individual proteins in solution, detecting small changes, such as single-point mutations, chemical modifications, posttranslational modifications, aggregation, and protein misfolding, among other conformational and interactions

changes. Considering that clinical proteomics and disease biomarkers are often protein-related, ATPS seems to be a convenient technique for automated discovery and monitoring of structure-based protein biomarkers in biological fluids and subsequent use in clinical disease diagnostics.

5.4.4 Emerging and non-conventional applications

Recent advances in the biotechnology and materials science fields have enlarged their use to alternative applications, such as micropatterning and bioprinting, high-throughput 3-D tissue assembly, microcapsule production, synthetic biology, and microscale biomolecular assay development. Teixeira et al. [13] provide an overview of emerging and nonconventional biotechnology applications of ATPS, anticipating five major areas for future growth, namely, (1) application-focused ATPS polymer design, (2) *stimuli*-responsive systems, (3) scaling-industrial separation reactions, (4) therapeutic microencapsulation and drug delivery, and (5) artificial cells and synthetic biology. We also believe that these new applications will change the focus of ATPS and its communities, spreading their use beyond current applications.

5.5 Scaling-Up and Continuous Processing

The use of ATPS as a large-scale separation process or as a bioengineering tool is yet to become a widespread "reality." Albeit new emerging biotechnological uses can be performed on small scale using microdevices, the downstream processing of biological products at industrial scale is fully dependent on a proper scale-up or integration in continuous processing platforms. From the beginning the attractiveness of ATPS-based separations to industry was associated with the simplicity of scaling, equipment, and facility demands [11, 25]. Despite these advantages, the large-scale applications of ATPS are limited in number [6, 8]. Considering the use of ATPS for large-scale downstream processing, it may be divided into two categories: (*i*) batch or continuous mode by using single-stage or multistage mixing/settling units; and (*ii*) continuous mode by using countercurrent distribution, liquid-liquid partition chromatography, or continuous countercurrent chromatography (CCC). The first category has been more extensively studied, mainly in the early stages of purification (low resolution); the second category, originally designed for aqueous-organic- and organic-organic two-phase systems (as discussed in another chapter in this book), is less common for ATPS-based processes but exhibits a strong potential for use as a high-resolution bioproducts' purification platform. Rosa et al. [25] have reviewed some case studies of batch scale-up of ATPS for the manufacture of

biopharmaceuticals (viz., interleukin, human growth hormones, and monoclonal antibodies), highlighting their advantages and drawbacks. Particularly, the authors concluded that three main questions still need to be addressed to introduce these platforms in biopharmaceutical production processes: their maximum capacity, limited predictive design, and economic and environmental sustainability (compared with established chromatographic platforms) [25]. It is important to note that recycling, back extraction, and multistage procedures can reduce some of the process costs and increase the sustainable character of ATPS [6].

Albeit that the number of studies focused on continuous ATPS are scarce, these appear as the most promising for industrial purification processes. Depending on the target product, continuous processing has clear advantages in comparison with the batch mode, namely, low processing times, high productivity and purification yields, and lower cost. Espitia-Saloma et al. [19] presented a comparative analysis of different ATPS-based techniques in continuous processing, from the most common approaches using conventional column contactors to novel mixer-settler processing units. In summary, although column contactors are more studied, due to their lack of versatility and limitations related to mass transfer and separation performance, the interest in mixer-settler devices is increasing [19]. Anyway, similar to batch-mode ATPS, an effective industrial implementation of continuous systems is yet dependent on the definition of parameters such as phase recycling, feasible predictive models, practical guidelines for design and scale-up, control, and automation [19].

5.6 Final Remarks and Future Perspectives

This chapter addresses the theoretical and practical aspects of the use of ATPS. These systems were proposed by the middle of the 20th century and have since been regarded as powerful alternatives to conventional liquid-liquid extraction systems for the isolation of bioproducts. They share a series of key advantages, such as versatility, biocompatibility, low cost, and outstanding purification performances for a range of molecules and products. However, as herein summarized, they still lack a significant application at an industrial scale.

The most studied classes of ATPS are formed by mixing a large range of polymers and salts, under different process conditions (pH, temperature, and additives). Nevertheless, other types of systems were also proposed and characterized, by using ILs, carbohydrates, amino acids, or surfactants. These combinations, using benign, renewable, and biodegradable phase-forming components (such as sugars, short-alkyl chain alcohols, ionic liquids, and amino acids), have been raising the "greenness" of ATPS, but regarding a future and sustainable application at industrial scale, it is crucial to develop novel and economical approaches for the full recovery and reuse of these components.

Several theoretical and experimental studies have tried to reveal and develop models and thermodynamic relationships to characterize the phase separation mechanisms and solute partitioning. However, their widespread use has yet been limited because of their complexity and the number of possible combinations. Furthermore, additional efforts are required to fully reveal the nature of these systems and thus to allow the development of phase separation predictive models. Similarly, considering the plethora of solutes that can be partitioned/separated with these platforms and envisaging the implementation at large scale, establishing reliable models to predict solute partitioning is of upmost importance.

Finally, considering the excellent extractive performances, integrability, biocompatibility, and sustainable characteristics of many ATPS, we believe that these can be commercially applied for the purification of several products, particularly, complex bio-based materials (such as VLPs, membrane proteins, and DNA fragments) and metal-based products (recycling and urban-mining procedures). Nevertheless, their alternative uses as (bio)analytic and nonconventional biotechnological approaches are nowadays very promising and may lead to alternative industrial applications.

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Chapter 6

Octanol-Water Partition Constant

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Chapter Outline

6.1 Introduction

The partition constant of a compound between *n*-octanol and water (or aqueous solution) is the most common way of expressing the lipophilicity of a molecule of industrial, pharmaceutical, or environmental interest. Octanol/water presents several relevant advantages compared wtih other systems with organic phases such as alkanes, cycloalkanes, haloalkanes, aromatic solvents, or even other alkanols of lower or higher number of carbon atoms [1]. First of all, due to its relatively long alkyl chain and the polar hydroxyl group, it is a potential model of the lipid constituents of biological membranes. This synthetic reference model is in fact constituted

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by two binary phases, a water-saturated *n*-octanol phase and a *n*-octanol-saturated aqueous phase. In the former the concentration of water is high, about 2 mol L^{-1}. In other words, there is about one water molecule per four alcohol molecules. Under these conditions the partition of polar compounds into the octanol phase does not significantly change the structure or properties of the organic solvent, and in addition, these polar groups do not need to be completely dehydrated in the transfer process from the aqueous to the octanol phase. Concerning the *n*-octanol-saturated aqueous phase, the content of organic solvent is in the mmol L^{-1} range and does not play a significant role. Additionally, the hydroxyl moiety of *n*-octanol has hydrogen-bond donor (acidity) and acceptor (basicity) capacities, allowing the stabilization of interactions with a large variety of polar groups favoring their solubility in the organic phase. Finally, from an experimental point of view, *n*-octanol shows an acceptable viscosity and low vapor pressure and is compatible with UV detection for quantification purposes.

The measure of lipophilicity in octanol/water systems is normally provided as the ratio of the concentration of a solute in a single definite form (S) in the water-saturated octanol phase (O) to its concentration in the same form in the aqueous phase (W) at equilibrium, expressed in its decimal logarithmic form:

$$\log P_{O/W} = \log \frac{[S]_O}{[S]_W} \tag{6.1}$$

According to IUPAC recommendations, $\log P_{O/W}$ should be named as *partition ratio*. However, in scientific discussions and literature the term *partition coefficient* or better *partition constant* is widely used. Anyhow, it must be pointed out that $\log P_{O/W}$ refers only to a single species, which is a relevant consideration when dealing with ionizable acidic or basic compounds. Normally, $\log P_{O/W}$ is defined for the neutral (unionized) form of a compound, and therefore it is necessary to measure the lipophilicity at the right pH to ensure the compound is in its neutral form, commonly at a pH value at least two units below the solute pK_a for acids or two units above for bases. The higher the $\log P_{O/W}$ value, the more lipophilic the solute. When the effect of ionization should be taken into account in the measurement of lipophilicity, which is very common for drug molecules, then a *distribution ratio* or constant ($\log D_{O/W}$) is defined taking into account the different species that might be present in either phase at equilibrium. For a monoprotic acid (HA/A^-) or base (BH^+/B), $\log D_{O/W}$ can be defined as

$$\log D_{O/W(acid)} = \log \frac{[HA]_O + [A^-]_O}{[HA]_W + [A^-]_W} \tag{6.2}$$

$$\log D_{O/W(base)} = \log \frac{[BH^+]_O + [B]_O}{[BH^+]_W + [B]_W} \tag{6.3}$$

Assuming the oversimplification that ionic species do not partition into the octanol phase, the following expressions can be derived from Eqs. (6.1) and (6.2) and the acidity constant of the solute:

$$\log D_{O/W(acid)} = \log \frac{P_{O/W(HA)}}{1 + 10^{pH-pK_a}} \tag{6.4}$$

$$\log D_{O/W(base)} = \log \frac{P_{O/W(B)}}{1 + 10^{pK_a-pH}} \tag{6.5}$$

where $P_{O/W(HA)}$ and $P_{O/W(B)}$ are referred to acidic and basic species, respectively. These equations allow the definition of a lipophilicity-pH profile for the neutral species and in the pH range close to the pK_a value. The other regions (pH$\gg$pK_a for acids and pH$\ll$pK_a for bases) show an infinite decrease in log $D_{O/W}$. Experimentally, it is shown that, in fact, ionic compounds partition into the organic phase but usually as ion pairs. Therefore, the partition ratio for the ionic species depends not only on its lipophilicity but also on the nature and concentration of the counterion. As a rule of thumb, in aqueous solutions containing 0.15 mol L^{-1} of KCl or NaCl, corresponding to physiological ionic strength, log $P_{O/W}$ of ionized compounds is about 3–4 units lower [2]. Thus, more realistic equations can be used:

$$\log D_{O/W(acid)} = \log \frac{P_{O/W(HA)} + P_{O/W(A^-)} \cdot 10^{pH-pK_a}}{1 + 10^{pH-pK_a}} \tag{6.6}$$

$$\log D_{O/W(base)} = \log \frac{P_{O/W(B)} + P_{O/W(BH^+)} \cdot 10^{pK_a-pH}}{1 + 10^{pK_a-pH}} \tag{6.7}$$

Eqs. (6.6) and (6.7) have been used to calculate the log $D_{O/W}$ profiles of an acid (ibuprofen) and a base (propranolol) from log $P_{O/W}$ and pK_a values obtained from the literature (Fig. 6.1) [2]. Similar equations can be derived for polyprotic compounds, such as nitrazepam, also shown in Fig. 6.1.

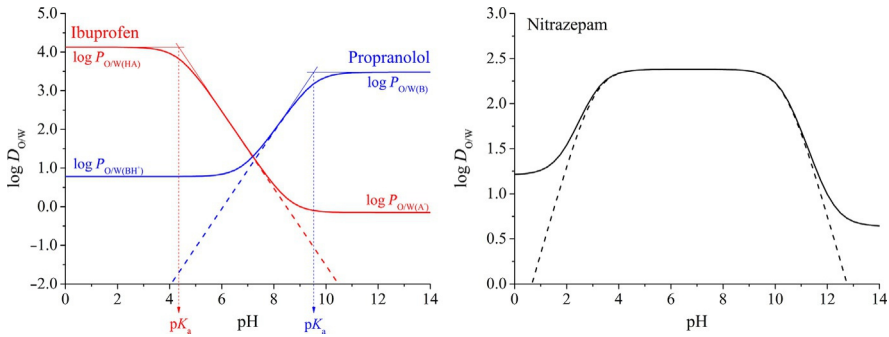

Fig. 6.1 Lipophilicity-pH profiles of ibuprofen (acid), propranolol (base), and nitrazepam (ampholyte). Dashed lines represent the oversimplified cases where only neutral species partition into the octanolic phase (Eqs. 6.4 or 6.5).

6.2 Methods Used to Determine the Octanol-Water Partition Constant

Many computational and experimental methods can be used to determine the lipophilicity log $P_{O/W}$ parameter. For computational methods, there are several software packages (e.g., ClogP, AlogPs, and ACDLabs) to estimate log $P_{O/W}$, but the results generally show a lack of consistency because different algorithms are applied in the calculation. In this sense, extensive studies of the accuracy of calculated log $P_{O/W}$ values by different software approaches have been reported [3–5].

The experimental log $P_{O/W}$ values can be obtained by direct or indirect methods. The direct methods include the classical shake-flask method, which is recommended as a standard method by the Organization for Economic Co-operation and Development (OECD) [6], and also potentiometric methods. Reversed-phase liquid chromatography (RPLC) is the most common indirect method for estimating octanol-water partition constants, also recommended by OECD as a standard procedure due to its high throughput, insensitivity to impurities or degradation products, broad dynamic range, online detection, and sample size requirements.

6.2.1 Shake-flask method

The most common method used to measure the octanol-water partition constant is the traditional shake-flask method [6, 7]. In this method, a compound is added to the partitioning solvents (water-saturated with *n*-octanol and *n*-octanol-saturated with water), both placed in a flask. Then the flask is shaken to accelerate the partition equilibrium, the phases separated, and the amount of compound in each phase determined to obtain the log $P_{O/W}$ value (Fig. 6.2). A similar method can be used to measure the log $D_{O/W}$ for ionizable compounds, provided the pH is strictly controlled.

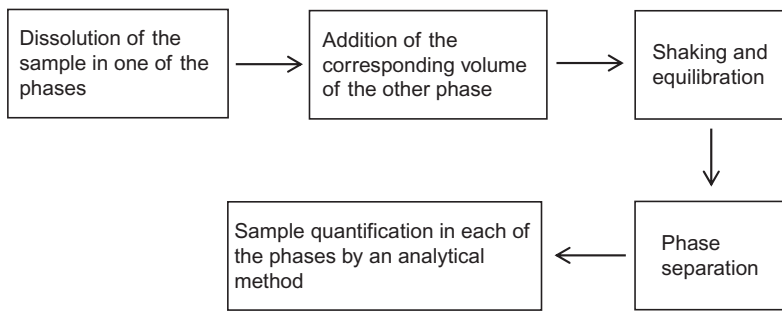

Fig. 6.2 Flowchart of the shake-flask method steps.

This is a relatively simple method, although an important number of parameters must be controlled to obtain reliable results:

i. *Temperature*: This is one of the most important parameters to control. The saturation of both solvents must be done already at the desired temperature, and then temperature must be controlled throughout the process.
ii. *Volume ratio between phases*: To optimize the ratio an estimate of the log $P_{O/W}$ or log $D_{O/W}$ value of the compound is required, since most lipophilic compounds will require larger aqueous volumes and vice versa [8]. Guidelines [6, 7] recommend that at least three different volume ratios are tested, that is, the one that fits better according to the expected log P, a ratio just above, and a ratio just below. Furthermore, each ratio should be tested in duplicate.
iii. *pH of the aqueous phase*: For ionizable compounds, the pH of the aqueous phase must be controlled in order to obtain the correct log $D_{O/W}$ or log $P_{O/W}$ values. When buffer solutions are used to control the pH of the aqueous phase, the ionized form of the compounds can form ion pairs with some of the buffer components. In that case the log $D_{O/W}$ value will be modified by this secondary chemical equilibrium, and the buffer concentration has a direct effect on the final log $D_{O/W}$ value.
iv. *Length of the shaking and equilibration steps*: In general, equilibrium between the two phases is reached quite rapidly, although it depends on the type of vials employed (glass tubes, chromatography vials, 96-well plates, etc.) and on how vigorous the agitation is. In general terms, 1 h of shaking is sufficient to achieve equilibrium. Vigorous agitation is not recommended in order to minimize the formation of emulsions, which complicate the posterior phase separation. When agitation using rotatory rollers or orbital baths is used, centrifugation can often be avoided. However, it is a mandatory step when phases are not clearly separated.
v. *Separation of phases*: The method of phase separation and how each phase is sampled for analysis are also important. Cross contamination is one of the drawbacks in this step, and it is especially important when the volume of aqueous phase is sampled, since to reach the aqueous phase the syringe needle has to pass through the octanol phase.
vi. *Quantification*: The determination of the amount of compound in each phase can be done by several analytic techniques, the most common being UV spectroscopy and liquid chromatography with either UV or MS detection.

The main advantage of the shake-flask method is that it does not require specific instrumentation. However, its main drawback is its low level of automation. In this sense, several modifications of the method have been proposed in recent years, with the aim of transforming it into a high-throughput method for routine analysis. Important modifications were proposed by Hill et al. [9] that affect almost all steps of the method:

i. The first improvement was to use chromatographic sample vials as containers to perform both equilibration of the phases and analysis of the sample in a single reservoir.

ii. The second improvement was to measure only the amount of compound in the water phase. To this end, a stock solution of the compound in aqueous phase was prepared. This stock solution was used for the preparation of the different partition vials and also for the measurement of the initial compound concentration. Then, four vials were prepared for analysis: one contained the standard stock solution and the other three different ratios of stock solution/n-octanol. The concentration in the aqueous phase was calculated according to Eq. (6.8), comparing the area of the compound in each partition with the area of the compound in the stock solution:

$$\log D_{O/W}\left(P_{O/W}\right) = \log\left(\left(\frac{A_{st}}{A_w} - 1\right)\frac{V_w}{V_o}\right) \tag{6.8}$$

In this equation, A_{st} and A_w are, respectively, the peak areas of the compound for the standard stock solution and in the aqueous phase of the partition, and V_w and V_o are the volumes of water and octanol in the partition vials.

iii. The third improvement was to use a vial roller for 90 min in the equilibration step, ensuring adequate mixing and preventing emulsion formation.
iv. The fourth improvement was to inject directly from the vial into the HPLC system without a separation step.
v. The last improvement was to use a fast generic gradient method for the analysis of the sample. The method had a duration of 7.5 min, including the reequilibration step.

The method proposed was applied to log $D_{O/W}$ determinations covering a range from -1.5 to 3.5. However, one limitation of the method is that the compound must have a reasonable aqueous solubility.

Other authors have also worked on the automation of the shake-flask method, focusing on different steps of the process. For example, Hitzel et al. [10] presented a completely automated method using an automated pipetting station to add the organic and aqueous phases into a 96-well plate. Then samples were equilibrated for 30 min, and each phase was directly injected into an HPLC system, where a fast gradient was used for analysis. This method works well for log $D_{O/W}$ values between -2 and 4 and is only limited by the solubility issue. The use of 96-well plates instead of chromatographic vials has been widely implemented in routine analysis [10–13].

On the basis of the method developed by Hill et al., Andrés et al. [8] proposed three different procedures to expand the scope of the method to compounds with larger lipophilicity. The compounds were divided into low, regular, and high lipophilicity, and the different procedures were applied according to the compounds' expected lipophilicity. These procedures optimize parameters such as sample solvent, the partition ratios, and the phase to be measured for quantification. The procedures were designed to require a minimum sample amount and were validated by compounds in the log $D_{O/W}$ range from -2 to 4.5.

Other authors evaluated the possibility of using compounds initially dissolved in DMSO, since this is the solvent used for most compound libraries. Furthermore, DMSO usually improves the solubility of compounds. Low et al. [11] demonstrated that changes in log $D_{O/W}$ were significant for DMSO content as low as 0.5%. In addition, it was shown that an increase in DMSO percentage in the samples implied a decrease in the log $D_{O/W}$ value. To overcome this problem, other authors proposed a drying step in which DMOS was removed at 40°C under vacuum [12]. Afterwards the dried sample is dissolved in either the octanol or aqueous phase.

The separation step has also been subject to improvement, especially to avoid cross contamination, since octanol may enter the sampling needle as it passes through it to reach the aqueous phase. To this end, Dohta et al. [13] developed the water-plug aspiration/injection method (Fig. 6.3). In this method a few microliters of water was taken up into the needle as a plug before sampling of the water phase. In this way, the water in the needle repelled octanol as the needle passed through it. Once the sample was taken, the needle was externally cleaned with ethanol and water, respectively, before injection into an HPLC for quantification.

Innovations have also been suggested for the quantification step. HPLC is the technique of choice in most instances due to its simplicity and the small amount of sample required. Furthermore, impurities do not interfere as they are separated

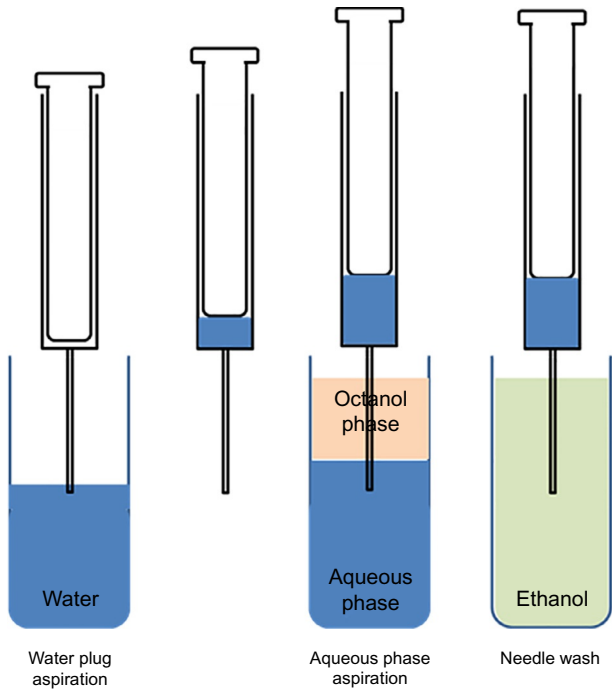

Fig. 6.3 The water-plug aspiration/injection method.

from the major component during analysis. A UV detector in most cases is sufficient for detection, although several methods based on MS detection have been developed, since LC-MS is generally the method of choice for the quantification of small molecules in industry [11–13]. In case MS with electrospray ionization (ESI) source is used, the different characteristics of the two phases (water and *n*-octanol) may create matrix effects, which can lead to inaccurate results. This drawback can be overcome in different ways. One possibility is to use the matrix matched calibration method [11], which is generally feasible due to the simplicity of both matrices. Another option is to use ionization sources other than ESI, like atmospheric pressure photoionization (APPI) [12]. In recent years, other techniques based on miniaturization have also been used for log $D_{O/W}$ determination. One example is microchip capillary electrophoresis with contactless conductivity detection (MCE-CCD), which was used to determine log $D_{O/W}$ for several drugs with an analysis time <40 s [14]. Other examples are based on microfluidic liquid-liquid extraction systems that allow high automation and perform the determination of log $D_{O/W}$ and log $P_{O/W}$ in short time frames [15, 16].

6.2.2 Potentiometric Method

The potentiometric methodology applies to ionizable compounds, especially for compounds with inadequate chromophores. This technique allows the simultaneous determination of pK_a and log $P_{O/W}$, which can be used to calculate the log $D_{O/W}$ value at any desired pH.

The methodology involves determining the shift in the pK_a when a titration is performed in a two-phase octanol-water system, compared with water. Two titrations of the compound are performed over a pH range that includes the pK_a of the compound. The first titration is carried out in the aqueous phase, and from it, the acidity constant (pK_a) is obtained. The second titration is performed in an octanol-water mixture and allows the apparent acidity constant (pK_a') determination, which is shifted from the aqueous pK_a value. Fig. 6.4 shows the pK_a shift for a monoprotic

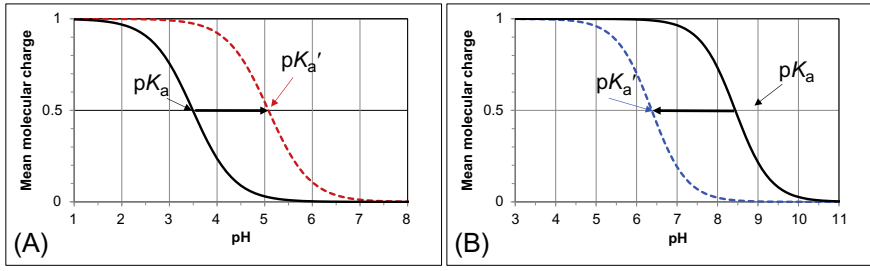

Fig. 6.4 Bjerrum plot curves for (A) a monoprotic weak acid (HA) and (B) a monoprotic weak base (B). Solid lines correspond to a titration in water and dashed lines to a titration in the *n*-octanol-water mixture.

weak acid (HA) and a monoprotic weak base (B) when the neutral form dissolves in *n*-octanol. For a monoprotic weak acid the pK_a' is greater than the value in aqueous solution. This is because the concentration of the neutral form of the acid decreases in the aqueous phase because of its partition to *n*-octanol, so the equilibrium shifts in the opposite direction of hydrogen ion generation, making the substance appear more basic ($pK_a^{'} > pK_a$). Conversely, for a monoprotic weak base (B), the decrease of neutral form in the aqueous phase causes a pH drop, making the compound appear more acidic and shifting the pK_a to lower values ($pK_a^{'} < pK_a$) [17–19].

In both cases, the extent of the shift depends on the partition of the species. A large difference between pK_a and $pK_a^{'}$ indicates a large value of log $P_{O/W}$. Eqs. (6.9) and (6.10) allow estimation of the partition constant for HA and B, respectively, if only the neutral form partitions:

$$P_{O/W(HA)} = \frac{10^{(pK_a' - pK_a)} - 1}{r} \tag{6.9}$$

$$P_{O/W(B)} = \frac{10^{(pK_a - pK_a')} - 1}{r} \tag{6.10}$$

where r is the phase ratio (V_o/V_w), V_o the volume of octanol, and V_w the volume of aqueous phase. The volume ratio selected depends on the hydrophobicity of the compound.

When the ionic species partitions into the octanol phase, the shift in $pK_a^{'}$ with respect to pK_a depends on both species (ionic and neutral), and the relationship between the partition constant and ionization constant is expressed by Eq. (6.11):

$$\left|pK_a' - pK_a\right| = \log \frac{1 + rP_n}{1 + r\,P_i} \tag{6.11}$$

where P_n and P_i are the partition constants of the neutral and ionic forms of the compound, respectively. The $|pK_a^{'} - pK_a|$ indicates the absolute magnitude of the differences between the apparent and aqueous pK_a, since acids are shifted to higher values and bases are shifted to lower values. Because there is a third parameter to estimate (P_i), three titrations have to be performed: one without *n*-octanol and two in octanol-water mixtures with different phase ratios. Usually, for monoprotic weak acids and bases, the partition of the ionized species into the octanol phase is from 3 to 4 orders of magnitude lower than for the neutral form. The *n*-octanol volume in the third titration, therefore, must be greater than for the second titration. From the two pK_a shifts and the volume ratios r_1 and r_2, the three constants pK_a, log P_n and log P_i can be calculated [18, 19].

When polyprotic compounds are analyzed, equations are more complex. The Bjerrum curves can help identify which species partition into octanol. As an example, Fig. 6.5 shows the possible curves for a diprotic acid. Thus drawing the Bjerrum curves for polyprotic compounds allows the identification of the species that partition

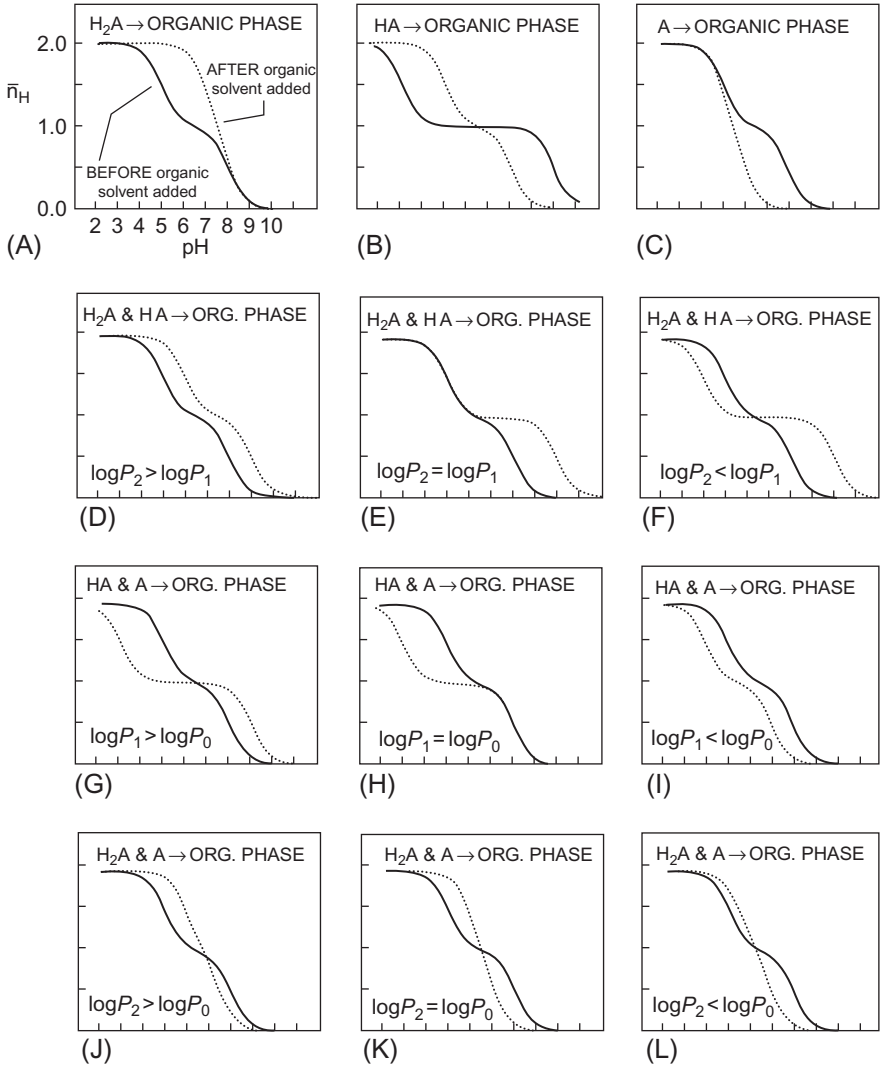

Fig. 6.5 Simulated difference curves for a diprotic substance possessing pK_as 8 and 5 and various combinations of log $P_{O/W}$. The label A in the drawings represents a weak acid; these drawings are equally valid for weak bases. *From Avdeef A. Quantitative structure-activity relationships, vol. 11. John Wiley and Sons; 1992. p. 510, with permission.*

in *n*-octanol and, consequently, choosing the appropriate equation to estimate the partition constants involved [18].

The potentiometric log $P_{O/W}$ method can be used for substances that undergo ion-pair partitioning and/or dimerization in the organic phase, provided that the sample

can be dissolved in water or *n*-octanol. The method can be used over a wide range of octanol-water volume ratios as the pH is measured in the dual-phase system, without any need for complete phase separation. However, this method cannot be used for hydrophobic weak acids and bases with high or low pK_a values, respectively, if values shift to pK_a' outside the measurable range $(2-12)$. The measurement range is ultimately defined by the volume ratio and approximately extends from $\log P_{o/w}$ of -2 to 6 in 0.15 M KCl [20].

6.2.3 Liquid chromatographic methods

The determination of $\log P_{O/W}$ by the traditional shake-flask method is long and tedious, and the titrimetric method can be applied only to ionizable compounds. The pressure in the drug discovery process in the pharmaceutical industry to measure the lipophilicity of a high number of potential drugs in a short time resulted in the investigation of fast and high-throughput techniques for the determination of octanol-water partition and distribution constants. Liquid chromatography, especially reversed-phase liquid chromatography (RPLC), is fast and adequate for this purpose.

In RPLC the main mechanism responsible for retention is the partition of the compound between an aqueous mobile phase and an organic stationary phase. Many studies have reported the selection of appropriate mobile and stationary phases that can act as a surrogate octanol-water partition system [21]. Ideally, the stationary phase should be *n*-octanol and the mobile-phase water. However, there are few available *n*-octanol stationary phases that can be used for this purpose. Several attempts have been made to immobilize *n*-octanol on the stationary phase surface with *n*-octanol-saturated water as mobile phase. A commercially available instrument was also developed [22]. The chromatographic system is calibrated by measuring the retention factor ($\log k$) of a set of standard compounds of known $\log P_{O/W}$ values. The main problem of this approach is to maintain a constant concentration of *n*-octanol immobilized on the stationary phase and to change the octanol/water volume ratio to be able to cover a wide range of partition constants (i.e., $-1 < \log P_{O/W} < 5$).

The problems with coated or immobilized *n*-octanol columns have led many authors to study the feasibility of using commercially available columns (mostly C18 and more recently IAM columns) with an aqueous-organic solvent mobile phase (acetonitrile-water and methanol-water). In these systems, $\log P_{O/W}$ values of standards are correlated with the retention factor of the measured standards ($\log k$) to obtain the following relationship [23]:

$$\log P_{O/W} = n + m \log k \tag{6.12}$$

In order to cover a wide range of $\log P_{O/W}$ values, various concentrations of the organic solvent in the mobile phase must be used for the correlation. Thus, many

authors use the retention factor linearly extrapolated to pure water (k_W) from log k measurements at several mobile-phase compositions (φ):

$$\log k = S\varphi + \log k_W \tag{6.13}$$

It is commonly believed that extrapolation to pure water provides retention/partition parameters in an environment closer to octanol/water partition, but some authors have found better relationships between log $P_{O/W}$ and log k for particular mobile-phase compositions [24]. In addition, it should be considered that the relationship between log k and φ is not usually linear, although it can be a reasonable approximation for log k values around 0.5 ± 1.0. However, different log k_W values can be obtained for the same compound, column and instrument, when they are derived from different sets of mobile-phase compositions or organic modifiers (acetonitrile or methanol, usually) [21].

The isocratic method for determination of log $P_{O/W}$ has been validated in several interlaboratory comparison tests and the results used by the OECD to prepare guidelines that should be followed to obtain reliable log $P_{O/W}$ values by RPLC [25]. The main recommendations of the guidelines are as follows:

i. The method is performed with columns packed with commercially available solid phases containing long hydrocarbon chains (e.g., C8 and C18) chemically bonded to silica.
ii. Methanol/water should be used as mobile phase with a minimum water content of 25% (v/v).
iii. The pH of the eluent is critical for ionizable compounds. The use of an appropriate buffer with a pH sufficiently below the pK_a for a neutral acid or above the pK_a for a neutral base is recommended. The pH of the eluent should be within the operating pH range of the column (2–8 usually).
iv. At least six reference substances should be used in the correlation line.
v. The reference substances should have log $P_{O/W}$ values covering the log $P_{O/W}$ range of the target compounds. Extrapolation beyond the calibration range should only be carried out for very lipophilic substances (log $P_{O/W} > 6$).
vi. The log $P_{O/W}$ values of the reference substances used for calibration should be based on reliable experimental data (Table 6.1). They should be structurally related to the target compounds.
vii. Duplicate determinations must be made to increase the confidence in the measurements.

If the earlier conditions are fulfilled, the method can determine log $P_{O/W}$ values in the range 0 to 6 with a repeatability and reproducibility of ±0.1 and ± 0.5 log units, respectively. The agreement with shake-flask values is also ±0.5 log units. Exceptionally the method can be expanded to cover log $P_{O/W}$ values between 6 and 10.

Although faster than the shake-flask method, the common RPLC isocratic methods are somewhat time-consuming, because they require measurements at

TABLE 6.1 Reference Substances for log $P_{O/W}$ Determination by HPLC [25]

Reference Substance	log $P_{O/W}$	pK_a
2-Butanone (Methylethylketone)	0.3	
4-Acetylpyridine	0.5	
Aniline	0.9	
Acetanilide	1.0	
Benzyl alcohol	1.1	
4-Methoxyphenol	1.3	10.26
Phenoxyacetic acid	1.4	3.12
Phenol	1.5	9.92
2,4-Dinitrophenol	1.5	3.96
Benzonitrile	1.6	
Phenylacetonitrile	1.6	
4-Methylbenzyl alcohol	1.6	
Acetophenone	1.7	
2-Nitrophenol	1.8	7.17
3-Nitrobenzoic acid	1.8	3.47
4-Chloroaniline	1.8	4.15
Nitrobenzene	1.9	
Cinnamyl alcohol (Cinnamic alcohol)	1.9	
Benzoic acid	1.9	4.19
p-Cresol	1.9	10.17
Cinnamic acid	2.1	3.89 (*cis*) 4.44 (*trans*)
Anisole	2.1	
Methyl benzoate	2.1	
Benzene	2.1	
3-Methylbenzoic acid	2.4	4.27
4-Chlorophenol	2.4	9.10
Trichloroethylene	2.4	
Atrazine	2.6	
Ethyl benzoate	2.6	
2,6-Dichlorobenzonitrile	2.6	

Continued

TABLE 6.1 Reference Substances for log $P_{O/W}$ Determination by HPLC [25]—cont'd

Reference Substance	log $P_{O/W}$	pK_a
3-Chlorobenzoic acid	2.7	3.82
Toluene	2.7	
1-Naphthol	2.7	9.34
2,3-Dichloroaniline	2.8	
Chlorobenzene	2.8	
Allyl phenyl ether	2.9	
Bromobenzene	3.0	
Naphthalene	3.6	
Phenyl benzoate	3.6	
Isopropylbenzene	3.7	
2,4,6-Trichlorophenol	3.7	6.0
Biphenyl	4.0	
Benzyl benzoate	4.0	
2,4-Dinitro-6-s-butylphenol	4.1	
1,2,4-Trichlorobenzene	4.2	
Dodecanoic acid	4.2	5.3
Diphenyl ether	4.2	
Phenanthrene	4.5	
n-Butylbenzene	4.6	
Dibenzyl	4.8	
2,6-Diphenylpyridine	4.9	
Fluoranthene	5.1	
Triphenylamine	5.7	
DDT	6.5	

several mobile-phase compositions, especially for sets containing compounds of widely different lipophilicities. Thus gradient methods have been developed to increase the speed of log $P_{O/W}$ determinations [26–28]. In these methods, log $P_{O/W}$ is correlated to gradient retention time, sometimes corrected by internal standards to avoid run-to-run and laboratory-to-laboratory variations [26], through an intermediate hydrophobicity index [27] or by k_w [28].

A significant limitation of RPLC method for the determination of log $P_{O/W}$ is the congeneric effect, that is, the correlation line is good and the measured log $P_{O/W}$ values accurate when the target compounds and calibration substances belong to the same chemical family. Alternatively, the correlation line for substances with different functionalities tends to exhibit poor statistical properties and have a poor predictive capability. The OECD recommends to use reference substances structurally similar to the target compounds to avoid this effect.

The origin of the congeneric effect seems to be due to the different hydrogen-bond capabilities of *n*-octanol and C18 phases. While the water-saturated *n*-octanol phase has a good ability to accept hydrogen bonds, C18 and similar bonded phases do not. Therefore, hydrogen-bond donor groups in the solute decrease chromatographic retention and partition in reference to octanol/water partition. To correct the congeneric effect, the addition of a hydrogen-bond acidity solute descriptor to the correlation model between log $P_{O/W}$ and log k has been proposed [21]. With the addition of this descriptor, the correlation can encompass neutral compounds of all functionalities giving a standard deviation of 0.2–0.4 log units.

The RPLC method is a reliable method for determination of log $P_{O/W}$ for neutral compounds or the neutral forms of ionizable compounds, but not for the determination of log D values of partly ionized compounds, that is, at specific pH values (e.g., pH 7.4) [26]. The reason is that it is practically impossible to reproduce the dissociation of ionizable compounds in the water-organic solvent mobile phase used in RPLC. The pH of an aqueous buffer changes with the addition of organic cosolvent (e.g., methanol) when preparing the mobile phase that depends on the specific buffering agent and cosolvent concentration [29]. The pK_a of ionizable compounds also changes with the addition of cosolvent but to a different extent than the pH of the buffer. Thus the degree of dissociation at a specific aqueous pH cannot be reproduced in the mobile phase unless one knows the exact pK_a of the target compound in the RPLC mobile phase, measures the pH of the buffer in the mobile phase and tunes it to the needed pH (i.e., the same pH − pK_a difference as in water). In addition, it is very doubtful that the log $P_{O/W}$ versus log k correlation stablished for neutral compounds is valid for ionized or partly ionized compounds. A method has been proposed for calculation of log $D_{O/W}$ values of ionizable compounds from the log $P_{O/W}$ of the neutral form obtained by RPLC, the pK_a in water, and log $P_{O/W}$ of the ionized form of the compound, assumed to be 3.15 log $P_{O/W}$ units less than the log $P_{O/W}$ of the neutral form [26].

Although widely extended, RPLC is not the only chromatographic method used to determine log $P_{O/W}$. Micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) were proposed as suitable alternatives to direct measurement of log $P_{O/W}$ [30, 31]. Both techniques use capillary electrophoresis instrumentation to determine the partition between an aqueous mobile phase and a cationic or anionic surfactant-based pseudoestationary phase or

microemulsion. In particular, a microemulsion of sodium dodecyl sulfate (0.05 M or 1.44%, w/w), heptane (0.82%, w/w), and 1-butanol (6.5%, w/w) in water was identified as a suitable surrogate model for octanol/water partition [32] for log $P_{O/W}$ values in the range −0.5 to 4.5 with an uncertainty <0.2 log units [33] and avoiding the congeneric effect.

Centrifugal partition chromatography and high-speed countercurrent chromatography have been used to determine log $P_{O/W}$ and log $D_{O/W}$ [34, 35]. The same principle but different instrumentation is used for both techniques. Both techniques employ a liquid mobile phase with an immiscible liquid stationary phase with no solid support. A centrifugal field maintains the liquid stationary phase in a set of cartridges, and the mobile phase is pumped through the stationary phase when centrifugal partition chromatography is used. The retention mechanism is governed solely by the partition process. The two immiscible phases can be *n*-octanol and water, and the log $P_{O/W}$ or the log D at the specific pH of the buffered water phase is obtained directly from the measured retention parameter (retention volume usually). The main handicap is that it requires specific instrumentation and is rarely used in industry.

6.2.4 Comparision between experimental methods

Some comparisons between the traditional shake-flask method and potentiometric and/or chromatographic methods have been reported [36, 37]. Takács-Novák et al. [36] carried out a validation study comparing the log $P_{O/W}$ values for 23 structurally diverse compounds determined by potentiometric and shake-flask methods. The authors concluded that the log $P_{O/W}$ values obtained by both methods were in good concordance. Port et al. [38] made a critical comparison of the shake-flask, potentiometric, and chromatographic methods to select the preferred method according to the chemical features of a compound. Table 6.2 summarizes the log $P_{O/W}$ values determined by the three methods for 66 compounds representative of a larger collection of diverse pharmaceutical compounds.

Due to the experimental variability in log $P_{O/W}$ measurements, a tolerance limit of 0.6 log units is accepted for comparison between methods [38, 39]. By this criterion, equivalent results were obtained for 60% of the compounds by the three methods, including many of the acids (Table 6.3). The results obtained by shake-flask method matched well with those obtained by potentiometry, with poorer agreement with the chromatographic method. However, it should be pointed out that several compounds, whose values were considered as nonequivalent, were in fact only slightly beyond the 0.6 limit.

From the comparison of the three methods, some remarks to assist in identifying the most suitable method are presented later [38]:

TABLE 6.2 pK_a and log $P_{O/W}$ Values for a Representative Set of 66 Compounds

		log $P_{o/w}$		
Compound	**pK_a Values**[a]	**Shake-Flask**[b]	**Potent.**[a]	**Chrom.**
Acidic				
Acetaminophen	9.39(A)	0.40 (1.0)	0.49	0.57
Atorvastatin	4.04(A)	4.00 (2.0)	4.08	4.50
Celecoxib	9.55(A)	3.90 (2.0)	3.91	4.20
Flufenamic	4.16(A)	4.64 (2.0)	5.19	4.83
Glimepiride	5.38(A)	4.02 (2.0)	3.97	4.30
Hydrochlorothiazide	8.72(A), 9.96(A)	0.00 (2.0)	−0.04	0.74
Indomethacin	3.98(A)	3.89 (2.0)	4.10	3.83
Ketorolac	3.50(A)	2.71 (2.0)	2.62	2.60
Naproxen	4.28(A)	3.12 (2.0)	3.24	2.93
R-flurbiprofen	4.35(A)	3.97 (2.0)	3.84	3.73
Rosuvastatin	4.44(A)	2.46 (2.0)	2.52	2.58
Topiramate	8.55(A)	0.47 (1.0)	0.58	–
Valsartan	3.84(A), 4.69(A)	3.37 (2.0)	3.52	3.20
Warfarin	5.01(A)	3.19 (2.0)	3.28	3.41
Zonisamide	9.49(A)	0.50 (2.0)	0.77	1.01
Basic				
Amantadine	10.62(B)	2.32 (12.5)	2.52	–
Atenolol	9.40(B)	0.13 (12.0)	0.06	0.22
Chlorpromazine	9.25(B)	5.40 (12.0)	5.27	5.44
Clofazimine	8.38(B)	6.30 (12.0)	–	5.93
Clopidogrel	4.99(B)	–	4.52	4.84
Diltiazem	7.79(B)	–	2.84	3.02
Duloxetine	9.81(B)	4.07 (12.0)	4.54	4.04
Famotidine	6.67(B)	−0.75 (11.5)	−0.36	0.65
Fluoxetine	9.89(B)	4.21 (12.0)	4.42	4.26
Loratadine	4.86(B)	4.45 (12.0)	4.88	4.30
Miconazole	5.99(B)	5.58 (12.0)	5.38	5.68
Milnacipran	9.55(B)	1.37 (12.0)	1.72	2.57

Continued

TABLE 6.2 pK_a and log $P_{O/W}$ Values for a Representative Set of 66 Compounds—cont'd

		log $P_{o/w}$		
Compound	**pK_a Values**	**Shake-Flask**	**Potent.**	**Chrom.**
Mirtazapine	3.77(B), 7.65(B)	3.06 (12.0)	3.28	2.78
Oxybutynin	7.72(B)	5.29 (10.5)	4.59	5.34
Prenylamine	9.31(B)	3.82 (12.0)	5.07	5.94
Quetiapine	3.57(B), 6.97(B)	2.91 (12.0)	3.13	2.50
Ranitidine	2.18(B), 8.38(B)	−0.24 (11.5)	0.26	0.06
Rimonabant	2.80 (B)	5.57 (12.0)	–	6.00
Sertraline	9.31(B)	4.73 (12.0)	5.17	5.38
Terfenadine	9.27(B)	4.96 (12.0)	4.47	6.08
Tramadol	9.50(B)	2.64 (11.5)	2.70	3.28
Trimipramine	9.21(B)	4.55 (12.0)	4.77	5.87
Venlafaxine	9.59(B)	2.81 (12.0)	3.05	3.74
Verapamil	8.81(B)	3.63 (11.5)	4.07	4.43
Vildagliptin	7.52(B)	−0.57 (11.5)	−0.16	−0.08
Neutral				
Carbamazepine	–	1.40 (7.0)	–	1.90
Lacosamide	–	0.21 (7.0)	–	−0.14
Levetiracetam	–	−0.14 (2.0)	–	0.48
Oxcarbazepine	–	1.17 (7.0)	–	1.08
Sulfinpyrazone	–	1.35 (7.0)	–	2.26
Taranabant	–	4.94 (2.0)	–	5.69
Amphoteric				
Clopamide	2.72(B), 8.95(A)	1.00 (5.2)	–	1.33
Folic acid	2.30(B), 3.79(A), 4.67(A), 7.97 (B)	–	0.10	–
Haloperidol	8.54(B), 10.98(A)	3.52	3.61	3.66
Isoniazid	3.53(B), 11.14(A)	−0.65	−0.85	−0.95
Isoproterenol	8.66(B), 9.95(A)	–	−0.62	–
Mebendazole	3.53(B), 9.88(A)	3.09	2.92	1.82
Nalidixic acid	6.00(A)	1.36	1.48	1.98
Omeprazole	4.25(B), 8.64(A)	2.23	2.14	1.40

TABLE 6.2 pK_a and log $P_{O/W}$ Values for a Representative Set of 66 Compounds—cont'd

		log $P_{o/w}$		
Compound	**pK_a Values**	**Shake-Flask**	**Potent.**	**Chrom.**
Pantoprazole	3.84(B), 8.22(A)	2.07	1.84	1.34
Pioglitazone	5.56(B), 6.52(A)	–	4.03	3.22
Rosiglitazone	6.26(B), 6.67(A)	–	3.10	3.29
Sulfamethoxazole	1.67(B), 5.65(A)	0.86	0.90	1.44
Tapentadol	9.44(B), 10.47(A)	–	2.88	3.37
Zwitterionic				
Benazepril	3.35(A), 5.43(B)	1.24	1.38	2.05
Ciprofloxacin	6.20(A), 8.56(B)	−1.13	−1.15	−1.20
Enalapril	3.03(A), 5.35(B)	−0.04 (4.2)	−0.09	0.14
Labetalol	7.41(A), 9.37(B)	1.45	1.37	1.74
Levodopa	2.77(A), 8.49(B), 10.29(A)	1.58 (5.6)	0.50	1.57
Ramipril	3.53(A), 5.79(B)	1.06 (4.7)	0.72	0.77
Telmisartan	3.01(B), 4.39(A), 6.02(B)	4.18	3.54	4.03

log $P_{O/W}$ are determined by three different methods: shake-flask, potentiometric, and chromatographic method.
[a] Potentiometrically determined at an ionic strength of 0.15 M.
[b] pH of the aqueous phase in brackets; determined at an ionic strength of 0.10 M.
Adapted from Port A, Bordas M, Enrech R, Pascual R, Rosés M, Ràfols C, et al. Critical comparison of shake-flask, potentiometric and chromatographic methods for lipophilicity evaluation (log Po/w) of neutral, acidic, basic, amphoteric, and zwitterionic drugs. Eur J Pharm Sci 2018;122:331–40. doi:10.1016/J.EJPS.2018.07.010. With Permission of Elsevier.

TABLE 6.3 Percentage of Compounds With Equivalent log $P_{o/w}$ Obtained by Different Techniques (Total Number of Compounds in Each Category in Brackets)

	SF-P-C	**SF-P**	**SF-C**	**P-C**
Overall	60% (48)	92% (50)	68% (57)	74% (53)
Acidic	93% (14)	100% (15)	93% (14)	93% (14)
Basic	45% (20)	90% (21)	59% (22)	64% (22)
Neutral	–	–	50% (6)	–
Amphoteric	43% (7)	100% (7)	50% (8)	70% (10)
Zwitterionic	57% (7)	71% (7)	86% (7)	71% (7)

SF, shake-flask; *P*, potentiometry; *C*, chromatography.
From Port A, Bordas M, Enrech R, Pascual R, Rosés M, Ràfols C, et al. Critical comparison of shake-flask, potentiometric and chromatographic methods for lipophilicity evaluation (log Po/w) of neutral, acidic, basic, amphoteric, and zwitterionic drugs. Eur J Pharm Sci 2018;122:331–40. doi:10.1016/J.EJPS.2018.07.010. With Permission of Elsevier.

i. The shake-flask method requires long times in solution, and is not suitable for easily degradable compounds. The potentiometric method can only be applied to ionizable compounds and is unsuitable for compounds with log $P_{O/W}$ higher than 5.5 or with solubility issues. The chromatographic method cannot be used for compounds that are insufficiently retained on the column.
ii. The shake-flask method with LC-UV, LC-MS, or NMR detection is the most universal method and is relatively simple and adequate for both neutral and ionizable compounds. Selection of a suitable aqueous phase where the compound is entirely in its neutral form provides reliable log $P_{O/W}$ values for ionizable compounds. Furthermore, the selection of a buffer where the compound is partially ionized allows measurement of log D_{pH} at the selected pH. The applicability range of this method extends from log $P_{O/W}$ of -2 to 4 (occasionally up to 5). Its major drawbacks are that it is time-consuming and for highly lipophilic or sparingly soluble compounds quantification techniques of appropriate sensitivity are required [6, 8, 37].
iii. The potentiometric method is suitable for ionizable compounds with log $P_{O/W}$ values between -1.8 and 6. Low sample purity and poor solubility of the acidic and basic species are the limiting features for reliable results [36].
iv. Chromatographic methods are fully automated and fast and provide reliable log $P_{O/W}$ values for ionizable compounds. However, to ensure the compound is in its unionized form, some caution is needed because the mobile phase contains a significant fraction of organic modifier that changes the compound pK_a value, modifying the molar fraction of the neutral species [38].
v. log $P_{O/W}$ estimated through the chromatographic retention on C_{18} columns strongly depends on the hydrogen-bond acidity of the solute. Then, solely the measurement of retention is unable to correctly describe drug lipophilicity. The combination of chromatographic retention and a hydrogen-bond donor descriptor is able to properly estimate log $P_{O/W}$ values from -1 to 7. This method is particularly convenient for highly lipophilic compounds outside the limits for the shake-flask and potentiometric methods and for solutes with stability issues in time-consuming determinations [25, 40].

6.3 Lipophilicity and Biological Activity

When describing the connection between lipophilicity and biological activity, the correlation of lipid solubility with the anesthetic effect found by Hans Meyer [41] and Ernest Overton [42] at the turn of 19th to 20th century is often mentioned. They demonstrated a systematic relationship between the solubility of chemicals in oil and their anesthetic activity, suggesting that the effect of a compound on a living entity depends on its facility to diffuse across a cell membrane and that this capability must

be proportional to a lipid/water partition ratio. In these first studies, vegetable oils were used as the organic phase. However, they were difficult to obtain in a pure form (the oil composition might vary from lot to lot), and the solubility of relatively polar compounds in these oils was poor. This led Corwin Hansch to propose *n*-octanol as the benchmark solvent for the measurement of lipophilicity [1]. This author, together with his many collaborators, is one of the main actors in the development of relationships between biological activity and log $P_{O/W}$ in linear [43] and higher-order [44] models. He also discovered that for some biological systems, it was not possible to establish a direct correlation between activity and log $P_{O/W}$, and it was necessary to introduce further terms into the model besides lipophilicity [45]. This was the birth of quantitative structure-activity relationships (QSAR) [46, 47].

Hansch's group at Pomona College (Claremont, CA, the United States) compiled a database with thousands of equations relating biological activity with different molecular descriptors. About 85% of roughly 3000 equations included a term related to lipophilicity [48]. Nowadays the database contains about 14,000 biological systems, and over half of them contain a log $P_{O/W}$ term (Table 6.4). Examples of reported linear correlations between biological activity and log $P_{O/W}$ are the hemolytic action of alcohols and esters on bovine erythrocytes; toxicity studies of alcohols, ketones, and aromatic hydrocarbons on tadpoles or invertebrates like *Daphnia magna*; or the inhibition of the synthesis of the hydrolase ATPase under the effect of alcohols, acetone, chloroform, and ether. Examples of higher-order equations containing only log $P_{O/W}$ terms are the effect of alcohols on the growth inhibition of the bacteria *Bacillus subtilis*, the concentration of phenols necessary to kill the yeasts *Candida albicans*, and the toxicity of bis-quaternary ammonium alkanes in mice.

The C-QSAR database reports a direct correlation between several properties of biomedical and environmental interest and log $P_{O/W}$ [49]. For instance, lipophilicity and molecular size are key parameters in the prediction models for skin permeation [50, 51]. Moss et al. [52] established the following relationship between permeation (K_p, in cm s^{-1}) and log $P_{O/W}$ and molecular weight (MW):

$$\log K_p = 0.74 \log P_{O/W} - 0.0091\text{MW} - 2.39$$
$$n = 116; s = 0.42; r^2 = 0.82$$

This model suggests that skin permeability increases with the lipophilicity of the tissue layer and that molecular size reduces diffusion into the skin. This model was developed using compounds with MWs from 30 to 390 and log $P_{O/W}$ from 0 to 7.5. Alternative models for different log $P_{O/W}$ and molecular size ranges have been described (e.g., Potts et al. [53], $18 \leq \text{MW} \leq 750$ and $-3 \leq \log P_{O/W} \leq 6$). Lipophilicity was also reported to be a key factor in the prediction of passage through other biological membranes, such as corneal permeability and transfer from blood to placenta or into breast milk [54]. Stępnik et al. proposed a surrogate model for

TABLE 6.4 Number and Percentage of Equations Describing Biological Activity Including log *P* as Independent Variable (Figures Calculated From C-QSAR Database [49])

Class	*N*	%	Class	*N*	%
Macromolecules	238	61%	Single-celled org. (cont.)		
Enzymes			Leukocytes	4	57%
Oxidoreductases	505	49%	Protozoa	116	65%
Transferases	201	45%	Viruses	176	50%
Hydrolases	660	45%	Yeasts	90	73%
Lyases	24	56%	Organs/tissues		
Isomerases	24	44%	Cancer	276	51%
Ligases	12	63%	Gastrointestinal tract	54	54%
Receptors	1060	41%	Heart	47	48%
Organelles			Internal/soft organs	51	71%
Mitochondria	66	70%	Liver	23	66%
Microsomes	72	63%	Nerves, brain, muscles	204	51%
Chloroplasts	71	83%	Skin	58	92%
Membranes	99	60%	Multicellular organisms		
Synaptosomes	17	59%	Animal (vertebrates)	471	67%
Single-celled organisms			Insect (bugs)	171	67%
Algae	42	82%	Fish	183	88%
Bacteria	684	70%	Human	48	79%
Cells in culture	1107	52%	Invertebrates	94	86%
Erythrocytes	68	86%	Plant	80	63%
Fungi, molds	215	68%	*Total*	*7311*	*54%*

human oral absorption of fatty acids and polyphenols based on two lipophilicity parameters (log $P_{O/W}$ and AC log $P_{O/W}$) and a steric descriptor (the molar volume) (r^2=0.983) [55].

An example of environmental applications is the uptake of organic compounds in phytoremediation research. Chang et al. [56] evaluated the plant-water partition coefficients (K_{pl}) of three compounds (4-chlorophenol, toluene, and *p*-xylene) in four different vegetables (Chinese cabbage, lettuce, scallions, and peanut) and concluded that there is a strong linear correlation between log K_{pl} and log $P_{O/W}$ ($r^2 \geq 0.805$).

Given that organic compounds can be present in different degrees of ionization, biological properties are sometimes estimated from log $D_{O/W}$. For instance, Austin et al. [57] correlated the binding to rat liver microsomes, a parameter associated with the intrinsic clearance of drugs, with log $D_{O/W}$ (for neutral and acid forms) and log $P_{O/W}$ (for basic forms) using 25 different drugs to establish the model (r^2=0.82).

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Chapter 7

Surfactant-Based Extraction Systems

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Chapter Outline

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Sample preparation is necessary for the preconcentration of target compounds and sample cleanup. For the determination of low concentrations of target compounds, the interferences initially existing in the samples must be reduced or even eliminated [1]. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are the most widely used sample preparation techniques for this purpose. Although LLE techniques are simple and undemanding, they have a several disadvantages: (1) They typically employ a relatively high number of environmentally hazardous volatile organic solvents; (2) they are not effortlessly integrated with the analytical equipment; and (3) they are limited in terms of selectivity, that is, many matrix components are coextracted from the sample and may interfere in the determination of the target compounds. To reduce the cost of high-purity solvents, the amount of solvent used in an analytical procedure should be minimized or even eliminated. Therefore it is unnecessary to organize a solvent collection system. To protect the operator and the environment from harmful effects of highly toxic reagents, they should be eliminated from analytical procedures. Over the past few years, developing more effective, miniaturized, and eco-friendly extraction techniques to minimize organic solvent consumption has attracted much attention [2]. Accordingly, to design new sustainable extraction methods, toxic reagents were substituted, and analytical techniques were miniaturized and automated. During the toxic reagent substitution process, newly designed nonconventional solvents, that is, deep eutectic solvents (DESs) [3], ionic liquids (ILs) together with their derivatives [4], and surfactant-based solvents have gradually supplanted traditional organic solvents employed in extraction procedures.

7.1 Types and Properties of Surfactants

Due to their unique properties like aggregation and remarkable reduction in surface tension, surfactants have garnered a lot of attention over the last few decades [5]. Surfactant aggregation has been studied for numerous research and industrial purposes [6]. Upon reaching a certain concentration in the solution, surfactant monomers tend to form bilayers, vesicles, micelles, and a wide range of novel nanostructures in different media. The concentration of surfactant at which micelle formation occurs is called the critical micelle concentration (CMC) [6–9]. There are a number of technological applications for micellar solutions, including analytical extraction, catalysis, detergency, drug delivery, and tertiary (or enhanced) recovery. Among these applications, analytical extraction is particularly important because of its low impact on the environment [10]. Extraction includes analyte dissolution and adsorption on the hydrophobic core of micelle aggregates. Surfactant-based sample preparation was developed as a simple and multipurpose sample handling technique for metal ions and common organic compounds from biological, clinical, and

environmental samples. In 1978, Tanaka and Watanabe [11] introduced cloud-point extraction (CPE) for metal ion preconcentration from aqueous samples. The combination of surfactants and modern extraction techniques leads to synergistic effects. These include low vapor pressure of aggregates, the capability to solubilize a wide range of inorganic and organic compounds, and high thermal stability. There are four classes of surfactants:

(i) Nonionic: With neutral hydrophilic head groups that cannot be ionized in aqueous solutions, such as glycerol monolaurate, Triton X-100, and Tween 80.
(ii) Anionic: With anionic hydrophilic head groups and usually low-polarity tail groups, these types of surfactants are commonly used as foaming agents and detergents, such as alkyl or aryl ether sulfates, sodium dodecyl sulfate (SDS), sodium lauryl, and sodium stearate.
(iii) Cationic: With cationic hydrophilic head groups and low-polarity tail groups. Typical examples include quaternary ammonium salts and fatty amine salts, such as dimethyl-benzylammonium chloride and cetyltrimethylammonium bromide (CTAB).
(iv) Zwitterionic or amphoteric: Surfactants with both cationic and anionic head groups, such as sulfobetaines and natural substances like phospholipids and amino acids

7.2 Surfactant Aggregation

Aggregates are formed by surfactants, such as micelles, in the bulk aqueous phase. The aggregate core is formed by interactions involving the hydrophobic tails, while the hydrophilic head groups are drenched in the neighboring liquid. Surfactant molecules initially organize on the surface at low concentrations. With increasing concentration, more surfactant molecules enter the surface inducing a swift decline in the surface tension. Micelle formation is the result of surface saturation and the subsequent addition of surfactant molecules. A new colloidal phase as extractant can be produced by coacervation of the micellar phase from the aqueous phase by an external force. Coacervation is a process during which a colloidal system (also called colloidal dispersion and colloidal suspension) divides into two immiscible liquid phases in the same solvent environment. The coacervate, that is, a colloid-rich dense phase (i.e., high-density colloidal phase), counterbalances a proportionately dilute liquid phase. Coacervation includes a delicate balance among hydrophobic associations, electrostatic interactions, hydrogen bonds, van der Waals, and other weak interactions. Suppression of coacervation may occur due to the reduction of weak interactions; on the other hand the enhancement of weak interactions may lead to precipitation. Based on its formation mechanisms, coacervation can be classified into two groups, that is, simple and complex. Simple coacervation consists of an

individual type of colloid-like surfactants or macromolecules. It can be produced by the addition of a dehydrating agent such as alcohols or salts. It can also be generated by raising the temperature, which favors intercolloid interactions over colloid-solvent interactions. Complex coacervation is composed of at least two biomacromolecules, polyelectrolytes, surfactants, and/or other colloid species of opposite charge, which is mainly guided by the electrostatic force of attraction in a neighborhood that is electrically neutral. The formation of complex coacervates may be affected by pH, concentration, ionic strength, mixing ratio, temperature, and molecular structures. Different solvent extraction techniques have been developed based on an external agent, including micelle-mediated extraction, supramolecular solvent-based microextraction, and CPE.

7.3 Surfactant Coacervation as Extraction Solvent

7.3.1 Cloud-point extraction

The cloud point is the temperature at which dissolved components (solids or liquids) are no longer completely soluble, precipitating as a second phase giving the fluid a cloudy appearance. In the aqueous phase the nonionic surfactant is still soluble below the cloud-point temperature. The solution divides into two phases following an increase in the temperature above the cloud point: (1) the lower or dense phase, which contains the majority of surfactants, and (2) the aqueous phase. This type of colloidal phase can play an important role in the CPE of biomolecules, organic compounds, and metal ions.

7.3.1.1 CPE of Metal Ions

The application of CPE to the extraction of metal ions is reviewed [12]. To form molecular aggregates, namely, micelle, the surfactant molecules are added to the aqueous solution to give the required concentration. The micelle dispersion is formed homogeneously above the cloud-point temperature with favorable transfer of analyte compounds to the micellar phase described with a distribution constant followed by phase separation at lower temperature to isolate analytes in the surfactant-rich phase.

Micelles can dissolve chemical species with different polarities and size. The hydrophobic core is the binding site in a micelle, which is more widely exploited in CPE. Therefore stable chelate formation is the key step in most methods to extract a few soluble metal chelates from aqueous solution. The following factors should be optimized in this process: (a) the kinetics of the complexation reaction, (b) the phase transfer of chelate or metallic species into the micellar media, and (c) the formation constant of the metal complex. The cloud-point technique employed for the

determination of metals is fairly easy, that is, a few milliliters of concentrated surfactant solution are added to the aqueous sample solution. Based on its solubility a chelating agent solution is added directly to the aqueous sample or in a water-miscible organic solvent. Then, to facilitate the phase separation process, the resulting solution is heated above its cloud-point temperature and then centrifuged.

Following CPE, various instrumental methods are used to analyze metal species, including atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS), hydride generation atomic absorption spectrometry (HGAAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), and spectrophotometry. Using cold vapor atomic fluorescence spectrometry (CVAFS) and CPE, Yuan et al. [13] developed a sensitive method for the determination of mercury at ultratrace levels. The preconcentration process is as follows: (1) Hg(II)-dithizone complex formation (i.e., $[Hg(HDith)_2]$) and (2) micelle-mediated complex extraction using TX114 surfactant. In the surfactant-mediated vapor mercury generation process, foaming was significantly reduced using a reductant (e.g., $SnCl_2$) and a homemade vapor/liquid separator. CPE techniques can be applied to various solid samples. For instance, Zhu et al. [14] proposed a novel technique for the determination of tungsten W(VI) in soil using fluorescence quenching and CPE as separation/preconcentration methods. Under optimized conditions the quenched fluorescence intensity was linearly correlated with W(VI).

Spectrophotometry is an alternative technique for target element detection. A new dithizone-mediated Se(IV) complexation method at a pH smaller than 1 in Triton X-100 micellar media was introduced by Soruraddin et al. [15]. Upon dithizone-mediated complexation, quantitative analyte extraction to the surfactant-rich phase occurs, which was isolated by centrifugation. The analyte was then diluted to 5.0 mL with methanol. The corrected absorbance (i.e., total volume/initial volume × observed absorbance) was used to overcome the problem caused by interference by dithizone ($\lambda_{max} = 434$ nm) that overlaps substantially with the absorbance spectra of the complex ($\lambda_{max} = 424$ nm).

For the determination of cadmium (Cd), a combination of CPE and cold vapor AAS was developed by Manzoori et al. [16]. Preconcentration of cadmium was carried out using the nonionic surfactant (i.e., PONPE 7.5) with no additional chelating agent. Afterward, its determination was performed by cold vapor generation from the surfactant-rich phase at room temperature (i.e., usually in the range of 15–18°C) using a continuous flow-AAS combined system. Using a new polydentate Schiff base extractant, *N*,*N*′-bis(salicylideneaminoethyl)amine (H_2L) from the aqueous sulfate solutions, copper(II) was extracted by CPE using Triton X-100 [17]. In this technique a hydrophobic Cu^{2+}-mediated copper(II)-H_2L complex with an H_2L chelating agent was easily extracted. The maximum recovery was obtained using a 2×10^{-3} mol L^{-1} extractant, (5% by weight of the surfactant) at pH 9 and 65°C.

Sun et al. [18] developed a CPE technique for the preconcentration of chromium species at ultratrace levels in human serum samples using GFAAS. A hydrophobic complex was formed by the reaction of Cr(III) with 1-(2-pyridylazo)-2-naphthol (PAN) and extracted by the surfactant-rich phase, while Cr(VI) remained in the aqueous phase. Hence Cr(III) and Cr(VI) could be separated. Following reduction of chromium(VI) to chromium(III) by ascorbic acid, the determination of total chromium was realized. PAN had two functions in this method: (1) as a chelating reagent in CPE and (2) as a chemical modifier in GFAAS.

In hydride generation (HG), micellar systems have three functions: (1) concentration of reagents, (2) modification of chemical reaction kinetics and thermodynamics, and (3) promotion of the metal and reagent solubilization. Germanium was complexed with quercetin, and the complex extracted from aqueous solution by the micellar phase formed with the nonionic surfactant (Triton X-114) and concentrated in the surfactant-rich phase by bringing the solution to the cloud-point temperature [19]. A preconcentration factor of 200 was obtained. Table 7.1 summarizes the analytical attributes of a few selected examples of CPE for elemental analysis.

7.3.1.2 CPE Coupled to Chromatography

Most CPE publications have described applications to inorganic compounds. Carabias-Martínez et al. [35] investigated CPE as a preconcentration and isolation step for organic compounds prior to HPLC analysis. They observed that elution of surfactant may interfere with the detection of analytes, which occurs when aromatic surfactants are used with ultraviolet-visible absorbance and fluorescence detectors. To avoid this problem, nonaromatic surfactants and electrochemical detection are suitable techniques.

One popular example of CPE extraction coupled to HPLC is the analysis of polycyclic aromatic hydrocarbons (PAHs) in which several nonionic surfactants have been used [36–39]. To minimize the strong absorption of surfactants, the PAHs should be separated from the surfactant using silica-gel cleanup before HPLC analysis [40]. The CPE-assisted PAH extraction from normal human serum with Triton X-100 was investigated by Sirimanne et al. [41] prior to HPLC analysis. To precipitate proteins the macromolecule-rich micellar phase was treated with acetonitrile and the filtrate utilized for HPLC analysis. In the case of vitamins, lipids and other nonpolar coextractants typically require additional sample cleanup and evaporation, which can result in sample loss. Sirimanne et al. [42] used CPE for the extraction of vitamins from human serum and blood, for example, at $\geq$50-μL volumes. Vitamins A and E were extracted from human serum and blood using Genapol X-080 as surfactant under salting-out conditions. To obtain sufficiently large CPE sample volumes, blood and serum samples were diluted with organic-free water. Afterward the surfactant-rich phase was isolated by centrifugation. Furthermore, harmful coextractants were removed by acetonitrile precipitation prior to analysis by HPLC-UV.

TABLE 7.1 Some Recent Application of CPE for Preconcentration of Different Elements

Element	Matrix	Coacervation Agent	Chelating Agent	Cloud-Point Temperature (°C)	Instrument	LOD ($\mu g\ L^{-1}$)	References
Pb	Urine	Triton X-114 and CTAB	4′,4″(5″)-Di-tert-butyldicyclohexano-18-crown-6	23–26	ICP-MS/MS	0.8	[20]
Bi	Soil	Triton X-100	Trioctylamine	70	Spectrophotometer	2.86	[21]
Ag-Au nanoparticle	Water	Triton X-114	–	40	TXRF	0.2–0.3	[22]
As(III) and As(V)	Snow water	Triton X-114	APDC	45	ICP-OES	0.72	[23]
Cd-Pb-Cu	Fish	Triton X-114	Dithizone	55	FAAS	0.056–0.821	[24]
Uranium	Wastewater	Triton X-114	H_2DEH[MDP]	–	ICP-MS	0.01	[25]
Phosphorus (V)	Water	Triton X-114	Ammonium molybdate	–	Spectrophotometer	0.5	[26]
Mn(II)	Food	Triton X-114	Quinalizarin	50	Spectrophotometer	0.8	[27]
Copper	River water	Triton X-100	Dithizone	64–67	FAAS	–	[28]
Al-Zn	Food	Triton X-114	8-Hydroxyquinoline	45	Spectrofluorophotometer	0.79–1.2	[29]
Mercury speciation	Environmental sample	Polyethylene glycol	PAN	35	Spectrophotometer	5.0	[30]

Continued

TABLE 7.1 Some Recent Application of CPE for Preconcentration of Different Elements—cont'd

Element	Matrix	Coacervation Agent	Chelating Agent	Cloud-Point Temperature (°C)	Instrument	LOD ($\mu g\ L^{-1}$)	References
Ag-Cd-Ni	Biological sample	Triton X-114	Dithizone	45	FAAS	0.27–1.12	[31]
V-Mo	Milk	PONPE 7.5	Nile blue A	50	FAAS	0.86–1.55	[32]
Chromium speciation	Blood	Triton X-100	Isopropyl 2-[(isopropoxycarbothiolyl) disulfanyl] ethane thioate	25	ET-AAS	0.005	[33]
As(III)-As(V)	Water	Triton X-114	APDC	40	HGAAS	0.009–0.012	[34]

Organophosphorus pesticides (OPPs) were extracted from water samples using a TX114-based CPE technique prior to HPLC separation with electrochemical detection [43]. The same surfactant was used also for the extraction of fungicides from water samples. In both methods a mobile phase with a low content of organic solvent was employed to minimize the elution of surfactant from the column: to remove surfactant remaining on the column, a washing cycle with acetonitrile was required. Chen et al. [44] used the same approach for the analysis of ergosterol in rat urine and plasma after CPE employing Triton X-114 as surfactant. A reversed-phase separation on an Inertsil ODS-3 column with a water-rich mobile phase (water-methanol 98:2) was used for the analysis by HPLC-UV.

CPE prior to analysis by gas chromatography (GC) is not a widely used technique. This is mainly due to problems caused by direct injection of the surfactant-rich phase into the GC system. To overcome these problems, a few surfactant removal methods have been exploited. Cleanup of the surfactant-rich phase on a silica-gel column with elution of the target compounds by an organic solvent is one such approach. The recoveries of polychlorinated biphenyls (PCBs) using this method compared with liquid-liquid extraction showed both processes gave comparable results for water spiked with the target compounds. For the determination of OPPs in honey by GC-MS, a coacervative microextraction ultrasound-assisted back-extraction (CPE-CME-UABE) method was developed by Altamirano et al. [45]. After CPE the OPPs were back extracted from the surfactant-rich phase into a low-surfactant soluble organic solvent and injected into a GC-MS without damage to the column. Limits of detection were in the low microgram per kilogram range. Table 7.2 summarizes the main analytical features for selected uses of CPE for the extraction of organic compounds.

7.3.1.3 New Trends in CPE

Recently, new trends in CPE have emerged, such as using ultrasonic-assisted extraction (UAE) as an external force. UAE facilitates the different processing stages in sample pretreatment by liquid extraction [53]. The sample solution and solid matrix are affected by the pressure wave and high temperatures derived from the interaction with ultrasonic radiation. In addition, the oxidizing strength of strong acids may lead to a high extractive strength [54]. To overcome the drawbacks of conventional extraction procedures (i.e., in terms of effectiveness of an extraction, number of steps, reagent consumption, and time), an ultrasonic device could be employed as a good choice. Classic and ultrasound-assisted CPE were compared by Wen et al. [55] for the extraction of copper with spectrophotometric detection. Ultrasound had a positive effect on the rate of extraction for CPE. Simitchiev et al. [56] compared the CPE of platinum group metals using a combination of microwave and ultrasound irradiation. In this method, Triton X-100 and 2-mercaptobenzothiazole

TABLE 7.2 Some Recent Application of CPE for Preconcentration of Different Organic Analytes

Analyte	Matrix	Coacervation Agent	Cloud-Point Temperature (°C)	Instrument	LOD ($\mu g\ L^{-1}$)	References
Isoquercitrin	Rat plasma	Tergitol TMN-6	25	HPLC-UV	1.6	[45]
Phenols	Water	Tergitol 15-S-7	50	HPLC-FLD	0.03–8.5	[46]
Antazoline	Human plasma	Triton X-114	45	LC-ESI-MS/MS	<10	[47]
Sulfonamide	Urine	Triton X-114	40	HPLC-UV	3.0–6.2	[16]
Triazine	Milk	Triton X-100	60	HPLC-UV	6.79–11.19	[48]
Flavonoids	Plant	Genapol X-080	55	HPLC-UV	1.2–5	[49]
Formaldehyde	Beer	Triton X-114	60	HPLC-UV	07	[50]
Organophosphorous pesticides	Urine	Triton X-114	50	GC-FID	0.04–0.8	[51]
Trichlorfon	Cabbage	Triton X-100	70	HPLC-UV	2.0	[52]

were used as the nonionic surfactant and ligand, respectively. The effectiveness of the procedure was dramatically improved by microwave irradiation. In the case of ultrasonication of the micellar solution, however, no significant impact was observed. In contrast to ultrasound-assisted CPE, microwave-assisted CPE is characterized by a shorter extraction time and a significant increase in extraction yield.

7.3.2 Mixed micelle mediated extraction

CPE is less attractive for the extraction of metal complexes with low partition constants formed from hydrophilic chelating reagents. Due to the general prevention of phase separation mediated by electrostatic repulsion, the clouding phenomenon seldom happens in the case of charged micelles. A mixed-micelle system containing oppositely charged surfactants is one possible solution. Classic CPE uses individual nonionic surfactant, while for mixed-micelle extraction a combination of two different ionic or nonionic surfactants is used. For polar organic compounds, a cationic-nonionic surfactant combination is expected to lead to an improvement in extraction efficiency [57]. Kenawy et al. [58] extracted Ti(IV) complexed by alizarin red S (ARS) with a mixed-micelle system formed by Triton X-114 and CTAB at pH 3. The neutral chelating agent (i.e., ARS-CTAB) is the result of the sulfonate-mediated reaction between CTAB and ARS. In the Triton X-114-based micellar phase, the hydrophobic complex (i.e., Ti(IV)-ARS-CTAB) was then effortlessly isolated. 4-Aminophenol reacts with dimethylaminobenzaldehyde to form a red-colored Schiff base in acid solution. The colored compound was extracted into a mixed micelle formed with sodium dodecyl sulfate (SDS) and TX-114 (SDS is an anionic surfactant and TX-114 a nonionic surfactant). The addition of the anionic surfactant improved the extraction efficiency of the color product by CPE. The resulting color product-SDS ion pair (the ion-paired complex formed between SDS and the color product) was extracted into the nonionic surfactant, that is, TX-114 [59].

7.3.3 Supramolecular solvents (SUPRASs)

The primary objective of supramolecular chemistry is to develop highly complex chemical systems out of component interactions by noncovalent intermolecular forces. These amphiphilic nanostructured liquids are the result of two sequential and spontaneous coacervation and self-assembly processes, Fig. 7.1. The amphiphiles self-assemble above a CMC into three-dimensional aggregates, depending on amphiphilic structure and solvent characteristics (e.g., aqueous = 3–6 nm, reverse micelles = 4–8 nm, and vesicles = 30–500 nm). During self-assembly an ordered structure is formed by the intermolecular interaction governed by disorganized solitary components via a reversible and spontaneous association. Self-assembly is mediated by a balance between attractive and repulsive forces, namely,

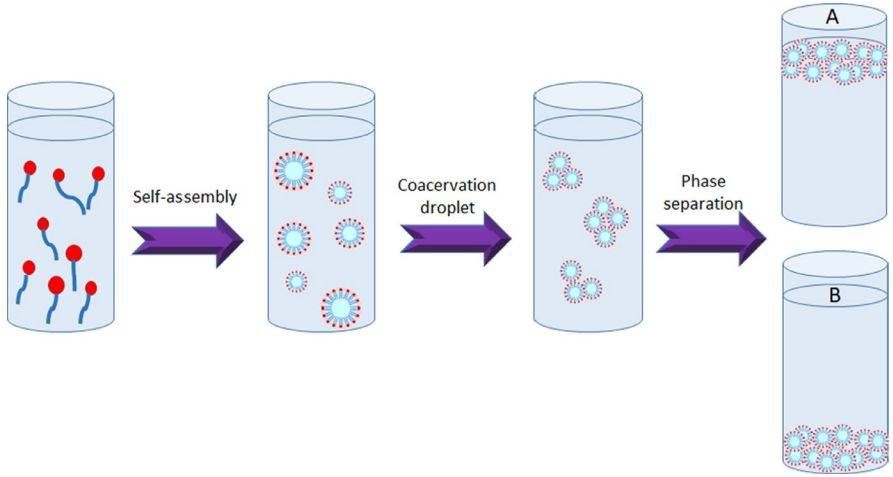

Fig. 7.1 Schematic of supramolecular solvent formation.

the disruptive power of these forces must match the attractive power of the component interactions. Due to the presence of feeble forces in the amphiphilic self-assembly process, a great deal of these soft interactions must produce a rather strong effect to bind the amphiphilic molecules together. In self-assembly processes of amphiphiles, electrostatic interaction, hydrophobic effects, van der Waals forces, and hydrogen bonding are among the major noncovalent forces, which are not as strong as covalent bonds. Table 7.3 summarizes these interactions. The hydration of the polar head groups and insertion of the hydrophobic tail(s) in the solvent leads to a stable aggregation of amphiphiles in solution [60].

TABLE 7.3 Strength of the Main Noncovalent Interaction Involved in Self-Assembly of Amphiphiles

Bonding and Interaction Type	kJ/mol
Covalent bond	100–400
Ion-ion, ion-dipole, dipole-dipole	200–300, 50–200, 5–50
Hydrogen bond	4–120
π-Cation interaction	5–80
π-π interaction	0–50
Van der Waals interaction	<5
Hydrophobic effects	Entropy

The two "opposing forces" must be taken into consideration to be able to forecast the shape and size of the aggregates' structure. The hydrophobic effect-mediated attractive interaction between monomers contributes to the molecular association. However, the repulsive interaction between monomers is mediated by the steric and electrostatic repulsion between head groups. They are required to remain in contact with water. The binding process stops due to the head group repulsion. Equilibrium is then obtained at a particular interfacial area (a_0) per aqueous phase-exposed molecule. Upon determining the volume (v) and length (l_c) of the hydrophobic part of the molecule, the assembly type can be inferred by geometric limitations. From thermodynamic consideration, spherical micelles are preferred to cylindrical micelles or bilayers when there is no other limitation. There are several other important factors that bring about the assembly of some amphiphiles into thermodynamically undesirable bigger structures. According to Fig. 7.2 [61], these factors are included in the packing parameter ($v/(a_0\ l_c)$. The packing parameter is affected by external parameters that govern head group repulsion, for example, concentration and temperature [62].

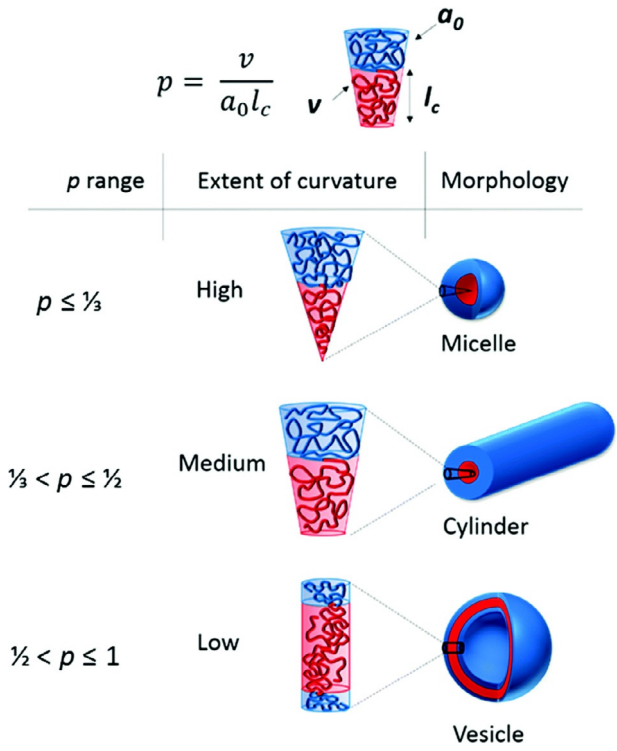

Fig. 7.2 Dependence of aggregate morphologies on the packing factor (the figure is inserted in the book with the permission of author) [61].

External factors such as electrolyte type, pH, temperature, or solvent stimulate the growing aggregates to form bigger aggregates. As a new oily liquid phase (i.e., supramolecular solvent), it separates from the aqueous solution at the top or bottom depending on the density through "coacervation." The coacervation process is characterized by the separation of a colloidal dispersion into two immiscible liquid phases in a common solvent medium. As a colloid-rich dense (high-density) phase, the coacervate is in balance with a comparatively dilute liquid phase. Depending on its density the coacervate phase is characterized by an amorphous suspension of droplets or separation into a two-phase system [63].

An important feature of SUPRASs is that they cannot be mixed with the original solvent, typically water [64]. Unlike ionic and molecular solvents, SUPRASs are generated by noncovalent intermolecular interactions. In response to environmental factors, SUPRASs may reverse or dismantle [65]. SUPRASs have intrinsic properties that make them attractive for extraction processes: ease of synthesis, composition adaptation capability, the development of sample treatment approaches independent of matrices, possible polarity zones contributing to different types of interactions, high amphiphile concentration, higher preconcentration factors induced by low solvent volumes, and nonflammable and nonvolatile solvents [66].

7.3.3.1 Reverse Micelle of Carboxylic Acid as Extraction Phase

Because dipolar aprotic (e.g., acetone, acetonitrile, dioxane, and *N*,*N*-dimethylformamide) and protic (e.g., methanol, ethanol, 1-propanol, and ethylene glycol) solvents are poor solvents for alkyl carboxylic acids (C_8–C_{16} alkanoic and oleic acid), water is added to achieve suitable solubility. For coacervation to occur, solvents capable of dissolving alkyl carboxylic acids and allowing for self-assembly of amphiphiles are required. The phase diagram (i.e., pressure-temperature diagrams) for a ternary solvent system is shown in Fig. 7.3. This system is composed of decanoic acid, water, and acetone (Fig. 7.3A) or ethanol (Fig. 7.3B) at 20°C. This figure demonstrates the presence of three regions: (1) the coacervation region (C), (2) the insoluble region (I), and (3) the homogeneous liquid solution region (L). The phase boundary I-C that is highly solvent-dependent should be small for analytical extractions. The coacervation possibility is greatly influenced by solvent properties, such as polarity and macromolecule solubility. The solvent proportion at which the I-C boundary phase appears for 1% decanoic acid provides a measure of solvent polarity, as shown in Fig. 7.3(C). Furthermore the Hildebrand solubility parameter (δ) (as shown in Fig. 7.3D) is a good indication of solvent miscibility and van der Waals interactions induced by the combined effects of dispersion, hydrogen bond, and polar interaction forces. According to Fig. 7.3C, solvents with $\delta < 25$ do not depend on the solvent percentage for coacervation to occur. For solvents with

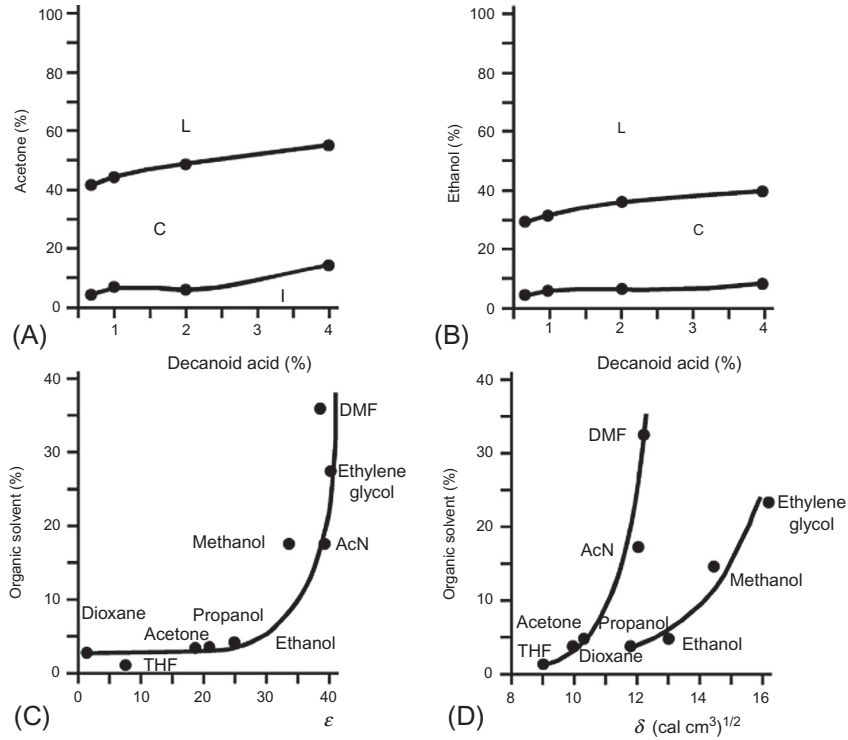

Fig. 7.3 Binary diagrams of phase boundaries in the ternary systems: (A) acetone-decanoic acid-water and (B) ethanol/decanoic acid/water (B). I, C, and L denote the decanoic acid suspension region, the coacervating region, and the single isotropic solution region, respectively. Dependence of the minimum percentage of organic solvent required for the coacervation of 1% decanoic acid as a function of its (C) dielectric constant and (D) Hildebrand solubility parameter (the figure is inserted in the book with the permission of author) [67].

$\delta > 30$, however, the solvent percentage needed to solubilize alkyl carboxylic acid and subsequent coacervation increases markedly. The δ facilitates a more accurate prediction (see Fig. 7.3D). Due to their different hydrogen-bonding capabilities, dipolar aprotic (i.e., moderate hydrogen-bonding liquids) and protic (i.e., strong hydrogen-bonding liquids) solvents constitute two clusters. Hence, to choose an appropriate solvent for coacervation of solvent, alkyl carboxylic acid, and water systems, the Hildebrand solubility parameter is a credible source. For analytical extraction, both aprotic and protic solvents with the smallest values of δ and the highest solvation capability for alkyl carboxylic acids should be selected. Tetrahydrofuran was selected based on this criterion [67].

7.3.3.1.1 Alkyl Carboxylic Acid Based-Reverse Micelles Coupled to Chromatography

Due to the effectiveness of SUPRASs in solubilizing compounds of a wide polarity range, they are of great interest for the extraction of organic compounds and metals from biological, food, and environmental samples. They are well suited to multiresidue methods. Generally, SUPRASs produced via inducing coacervation are typically employed in combination with liquid chromatography with absorption and fluorescence of mass spectrometric detection. For fluorescence and absorption detection, SUPRASs consisting of nonaromatic amphiphiles are mostly used. As a general rule, amphiphiles forming the SUPRASs are directed to waste after chromatographic separation (i.e., only sample components are detected) for mass spectrometric detection. Ion source contamination or ion suppression is minimized in this way [68].

Supramolecular solvent-based extractions employ two common procedures: (1) in situ synthesis of the solvent and (2) ex situ synthesis of the solvent. Due to reversibility of SUPRASs, liquid sample-based extraction process could always be mediated by the first procedure. Pérez-Bendito et al. were the first to introduce a sensitive and convenient extraction method for the determination of bisphenol A and F and their related diglycidyl ethers in river water and wastewater [69]. The extraction phase was composed of reverse micelles formed with decanoic acid-tetrahydrofuran-water and the target compounds detected by fluorescence after separation by liquid chromatography. In a continuation of these studies, SUPRASs were used for the extraction of bisphenol A, ochratoxin A, and benzo(*a*)pyrene from food matrices like beer, coffee, yogurt, soft drinks, tea, vinegar, and wine [70]. The SUPRAS was selected based on its low volume and hydrophobic and hydrogen-bonding analyte-enveloping capacity. The volume of the SUPRAS was not affected by matrix components. There was no need for additional sample cleanup and LOQs for the contaminants (e.g., 562–602 ng L^{-1}, 0.37–0.39 ng L^{-1}, and 14–31 ng L^{-1} for bisphenol A, benzo(*a*)pyrene, and ochratoxin A, respectively) were lower than their established EU threshold limits [70]. A SUPRAS was used for the fast extraction of Sudan dyes from spicy foods [71]. No solvent evaporation or cleanup was needed prior to determination by liquid chromatography with LODs between 2 and 8 μg kg^{-1}. Chlorophenoxy acid herbicides were extracted from water with a decanoic acid-based SUPRAS and determined by LC-MS [72]. A novel pretreatment SPE-SUPRAS combined method was presented by Yamini et al. for the extraction of diclofenac and mefenamic acid from water and urine samples [73]. The SPE-based extraction minimized matrix effects in the determination step. The target compounds were eluted with tetrahydrofuran followed by SUPRAS formation and extraction for LC-UV.

In situ and ex situ SUPRAS syntheses have been investigated for the extraction of solid samples. In situ synthesis consists of an equilibrium solution-mediated solute

extraction and centrifugal phase separation of the three-phase system: solid residue, analyte-containing SUPRAS phase, and the equilibrium solution. This approach is not considered suitable for the extraction of polar compounds due to simultaneous synthesis of a large SUPRAS volume. The ex situ synthesis is performed more easily in practice. To prevent excessive loss of SUPRASs, humidification of samples is recommended [68]. Pérez-Bendito et al. used a decanoic acid reverse micelle-based SUPRAS for the extraction of polar quinolone antibiotics from fish and shellfish [74]. Approximately 400 μL of SUPRASs and 200 mg of sample were blended in this single-step extraction process. In a similar study, ochratoxin A in wheat was extracted with a SUPRAS consisting of a decanoic acid reverse micelle dispersed in a tetrahydrofuran-water continuous phase [75]. A solution of decanoic acid was added to an acidified tetrahydrofuran-water solution at pH 2.7 and then added to the wheat sample to perform the extraction. After centrifugation the target compounds were analyzed in the coacervate phase. The LOQ of this procedure was 1.5 μg kg^{-1}, which was below the accepted ochratoxin A threshold limit value for cereals (i.e., 5.0 μg kg^{-1}) according to the EU directives [75]. As a low-cost and broad-scope strategy, a tetradecanoic acid reverse micelle-based restricted-access supramolecular solvent (SUPRAS-RAM) was used for screening agriculture food products, for example, spices, cereals, and wines for mycotoxins by ELISA [76]. Because of limited access to the SUPRAS, macromolecules like carbohydrates and proteins are not coextracted. In that regard a single-step analyte cleanup and extraction process is possible.

7.3.3.1.2 Reversed Micelles of Alkyl Carboxylic Acids for Metal Ion Extraction

A SUPRAS-based dispersive liquid-liquid microextraction-solidified floating drop (DLLME-SFD) method was used by Pei Liang et al. for preconcentration and determination of Pb(II) by GFAAS [77]. The calibration curve was linear over the range of 0.1–30 ng mL^{-1} for lead with an LOD of 27 ng L^{-1} and an extraction efficiency of 52%. The method was applicable for Pb determination in food and water samples.

7.3.3.2 Vesicle of Carboxylic Acid as Extraction Phase

Due to the prevention of phase separation by electrostatic repulsion, ionic supramolecular assembly-based coacervation is impeded, and clouding usually occurs for nonionic surfactants and seldom for charged molecular aggregates. The alkyl carboxylic acid-based coacervates are most useful for extraction for two reasons: (1) since the polar region of the molecular aggregates is made up of deprotonated and protonated ammonium and carboxylic acid groups, several interactions (e.g., cation-π, electrostatic, and mixed aggregate formation and hydrogen bonds) are available to the analytes (and furthermore some hydrophobic interactions occur in

the hydrophobic region), and (2) on account of the wide variety of solvation sites in vesicles, nonpolar and polar molecules are solubilized in each aggregate at higher concentrations. Mixtures of deprotonated and protonated alkyl carboxylic acid with seven carbon atom chains lead to vesicle generation. The salting-in behavior of tetrabutylammonium ions facilitated solubilization of the suspended carboxylate-alkyl carboxylic acid mixture and subsequent coacervate formation. In addition, the disappearance of the coacervate was mediated by alkyl carboxylic acid precipitation (i.e., $pK_a = -0.5$ to 1) and carboxylate solubilization (i.e., $pK_a = 0.5$–1). Due to the capability of the aggregates to establish a variety of interactions, the analyte extraction process could be mediated by the alkyl carboxylic acid vesicular coacervates for an extensive charge/polarity range. Hydrophobic interactions in the hydrocarbon region of the vesicle contribute to an effective extraction of nonpolar compounds (e.g., PAHs). Hydrogen-bond formation between polar compounds and vesicular coacervates also assists in the extraction of these compounds. Extraction of anionic and cationic compounds can be induced by tetrabutylammonium-carboxylic acid vesicular coacervates by the interaction of polar group with ionic analytes [78].

To simplify the sample preparation process and achieve the necessary sensitivity, Rubio et al. exploited supramolecular solvent-based cleanup/extraction prior to liquid chromatography with fluorescence detection for the determination of ochratoxin A in dried grapes [78]. The extraction phase was generated by a sequential two-step self-assembly process. In the first step, decanoate-decanoic acid vesicles are formed in the aqueous solution. Since hydrophobic forces and hydrogen bonds between carboxylic/carboxylate polar groups contributed to the self-assembly process, maximum stability and volume for these aggregates were achieved at decanoate/decanoic acid molar ratios of 1. In the second step the tetrabutylammonium counterion-induced reduction in the ionic head group repulsion contributed promoting aggregate development. The SUPRAS and dried grapes were blended together (i.e., 400 μL, 300 mg, respectively), and after centrifugation and sonication, the extractant phase was analyzed by liquid chromatography. The recoveries of ochratoxin A were in the 95%–101% range with RSDs ≈3%. The LOQ, 5.3 μg kg^{-1}, was well below 10 μg kg^{-1} (the EU threshold limit accepted for OTA in dried grapes) [78].

Single-drop coacervative microextraction (SDCME) was first described by Pérez-Bendito et al. in 2008 [79]. Chlorophenols were selected as model analytes and vesicular coacervates as the sample solvent. The coacervate droplet dislodging from the tip of the syringe needle at high stirring speeds was considered the major limitation of SDCME leading to an increase in extraction time. A novel liquid-phase microextraction-solidified floating drop (LPME-SFD) method was described by Yamini et al. to mitigate the problem of drop instability in single-drop coacervative microextraction [80]. In this method the extraction solvent was less dense (compared with water), less toxic, with a quasi-room temperature melting point of about 10°C.

A vesicular coacervative droplet was conveyed to the surface of an aqueous sample for extraction of alkylparabens by cation-π, hydrophobic, and hydrogen-bonding interactions. Cold-water immersion was used to cool the sample vessel and the solidified extractant phase removed to a vial to unfreeze for subsequent analysis. Hollow-fiber liquid-phase microextraction (HF-LPME) offers an alternative approach to overcome drop instability of SDCME and improve extraction efficiency. This approach was used with a SUPRAS extraction phase for the extraction of benzodiazepine [81]. HF-LPME employs a porous-walled polypropylene hollow fiber for organic phase stabilization and protection [82]. The high viscosity and low vapor pressure of vesicle-based SUPRASs are compatible with general requirements for use as a liquid membrane. These SUPRASs were generated by coacervation of tetrabutylammonium-mediated decanoic acid aqueous vesicles and used to impregnate the pores of the hollow-fiber walls. Benzodiazepine was extracted from aqueous samples into SUPRAS-impregnated hollow-fiber walls. The extracted benzodiazepines were analyzed by liquid chromatography with absorption detection. The LODs of the target benzodiazepines ranged from 0.5 to 0.7 μg L^{-1} with a linear calibration range from 1.0 to 200 μg L^{-1} for diazepam.

7.3.3.3 Alkanol Aggregates as Extraction Phase

Alkanol-based supramolecular solvents are synthesized by the addition of water to alkyl alcohol (C_7–C_{14}) solutions in tetrahydrofuran. The self-assembly of alkanols with spontaneous formation of oily droplets (coacervate droplets) occurs by the generation of a loose woolly mass of individual-droplet conglomerates. The total density of the solution is partly higher than for the conglomerates, contributing to phase separation (e.g., SUPRAS or coacervate phase) from the bulk solution. Equilibrium is established between the alkanol-rich SUPRAS (i.e., SUPRAS with large amounts of alkanol) and alkanol-poor bulk solution (i.e., the bulk solution with low amounts of alkanol). The structure and composition of the SUPRAS depends on their environment. To change aggregate size and composition, tetrahydrofuran or water is added to the equilibrium solution. Tetrahydrofuran and water in SUPRAS do not mix together; they set up separate, nonpolar and polar microenvironments that equip them with high flexibility as an extraction phase. Furthermore the fact that the aqueous-cavity size in alkanol-based SUPRAS is environment-dependent facilitates their application as restricted-access media for size-selective extractions. In fact, these SUPRAS can be used to improve the extraction selectivity of low-mass ionic and polar compounds over high-mass components in solid matrices. The heterogeneous structure of alkanol-based SUPRAS can facilitate a mixed-mode partition mechanism for nonpolar compounds utilizing dispersion interactions with the hydrocarbon chains of the surfactant and polar solutes by polar and hydrogen-bonding interactions with the surfactant solvated head groups [66].

A restricted-access reverse decanol-based SUPRAS was used for the extraction of endocrine-disrupting compounds from sediments for LC-MS determination [83]. To tailor the aqueous-cavity size, the ratio of tetrahydrofuran and water in the bulk solution where alkanol self-assembly occurs was optimized. These solvents minimize the coextraction of macromolecular compounds, such as humic acids and provide low detection limits and high recoveries of endocrine-disrupting compounds. Restricted access-volatile SUPRAS were used for protein and phospholipid removal from biological fluids for the analysis of low-mass analytes such as bisphenol A by LC-MS [76]. Restricted access-volatile supramolecular solvents (RAM-VOL-SUPRASs) were synthesized spontaneously in urine by adding hexanol dissolved in tetrahydrofuran for removal of proteins by flocculation. The SUPRAS-based phospholipid extraction process was facilitated by hexanol-mediated mixed aggregate formation. The abovementioned macromolecule removal processes significantly reduced the matrix effects in the analysis of bisphenol A by electrospray LC-MS/MS. The LOQ in urine for bisphenol A was 0.025 ng mL^{-1}. A solvent capable of removing the two types of common interferences in LC-MS analysis of biological samples was presented for the first time.

7.3.3.4 Gemini Surfactant Aggregates as Extraction Phase

As a modern synthetic surfactant, gemini surfactants are prepared by insertion of a spacer bonded group between two conventional surfactants. In essence a dimeric surfactant, the dicationic quaternary ammonium compounds known as $C_MC_SC_M$ (Me) (i.e., M stands for the number of carbon atoms in alkyl side chains, while S stands for the number of methylene groups in the spacer) are the most commonly used m-s-m gemini surfactants. Compared with conventional ionic surfactants, the effectiveness of these surfactants is approximately two orders of magnitude more at micelle formation and three orders of magnitude more at surface tension reduction. These astounding features were attributed to the distorted water structure caused by the two hydrophobic tails of the surfactant [84]. A new gemini-based SUPRAS was introduced by Yamini et al. [85] employing a four-component phase of sodium chloride, tetrahydrofuran, dimethylenebis (tetradecyldimethylammonium bromide) ($C_{14}C_2C_{14}$(Me)), and water. The addition of sodium chloride to reduce the electrostatic attraction between the oppositely charged surfactants was made to promote micelle coagulation over precipitation. This SUPRAS is formed at any pH and has multiple regions of different polarity suitable for extracting a wide range of analytes. Because of the low solubility of the gemini surfactant in tetrahydrofuran, the volume range over which SUPRAS was formed was limited, and reproducibility was poor. This problem was solved using 1-propanol to generate the SUPRAS. Applications include the extraction of pyrethroid insecticides [85] and alkylparabens [86]. The recovery of alkylparabens was 92%–108% with LODs ≈ 0.5 μg L^{-1}

(LC-UV). The gemini-based SUPRAS extraction method is compatible with typical separation conditions for reversed-phase liquid chromatography [87]. Problems are arisen for GC from the low volatility and high viscosity of the surfactant causing alteration of column separation properties and the risk of blocking the column. To adapt the gemini-based SUPRAS to GC-MS ultrasound-assisted back extraction from the surfactant-rich phase to *n*-hexane was used for the analysis of phthalate esters [88, 89]. Some of the other applications of SUPRAS are summarized in Table 7.4.

7.4 Emulsification of Organic Solvent by Surfactants

7.4.1 Dispersive liquid-liquid microextraction (DLLME)

A disadvantage of classical DLLME is that the presence of the dispersing solvent in the aqueous phase results in low partition constants for the extraction of polar compounds. A modification of classical DLLME was described that uses a surfactant as the dispersion agent [100]. A mixture of the aqueous solution containing surfactant and extraction solvent is rapidly injected into the sample solution forming an emulsion and subsequent phase separation by centrifugation. Yamini et al. [101] used a combination of surfactant-assisted DLLME (SA-DLLME) and liquid chromatography with absorption detection to determine four chlorophenols in water. No toxic disperser solvent or ultrasound irradiation was used in this approach. A mixture of 1-mL CTAB (0.9 mmol L^{-1}) and 35 μL of 1-octanol was injected rapidly into the 10-mL sample by gastight syringe. Almost immediately a cloudy solution was obtained with phase separation achieved by centrifugation. SA-DLLME with a cationic surfactant was used for the extraction of zinc from environmental water after the formation of a zinc complex with 4-(2-pyridylazo)resorcinol [102].

7.4.2 Ultrasound-based LPME

Ultrasounds affect several aspects of liquid-liquid extraction including emulsification of the two-phase system that leads to a large increase in the contact surface area between phases and faster mass transfer and a large increase in temperature and pressure in the vicinity of collapsing cavities formed throughout the solution. The result is a very effective and rapid analyte extraction [103]. Ultrasound-assisted emulsification-microextraction (USAEME) was employed for the extraction of PCBs from water for determination by GC-MS [104]. Lin et al. [105] combined USAEME and micro-solid phase extraction to preconcentrate Cd and Pb in edible vegetable oils for GFAAS detection. The nonionic surfactant Triton X-100 was used as the emulsifier, the magnetic IL [C_4mim] [$FeCl_4$] as the extractant, and Fe_3O_4

TABLE 7.4 Application of Supramolecular Solvent for the Extraction of Different Analytes

SUPRAS Type	Surfactant	Separation/Detection System	Matrix	Extracted Analyte	References
Alkyl carboxylic acid-based	Decanoic acid	LC-FL	Wastewater and river water	BPA, BPF, BADGE, BFDGE	[69]
		LC-FL	Wine	OTA	[90]
		LC-FL	Canned tea, lemon drinks, wine, must	BPA, BaPY, OTA	[70]
		HPLC-UV	Tomato, vinegar, and meat sauce	Sudan I, II, III, and IV	[71]
		LC-FL	Meat	Sulfonamides	[91]
		LC-MS/MS	Fish	HBCD	[92]
		ELISA	Wine, cereals	OTA, AFB1	[76]
Alkanol-based	1-Decanol	LC/QQQMS-MS	Superficial sediment	EDs	[83]
		FAAS	Tobacco, fertilizer, and water	Ni	[93]
	1-Hexanol	LC-(ESI)-MS/MS	Urine	BPA	[94]
	1-Octanol	GFAAS	Garlic, black tea, and mint	Se	[95]
Tetrabutylammonium-induced vesicular-based	Decanoic acid	LC-FL	Sewage and river water	BPA, BPF, BADGE, and BFDGE	[96]
		HPLC-UV	Cosmetics and water	MP, EP, PP	[97]
		LC-FL	Vine fruits	OTA	[98]
		HPLC-UV	Water	Triazines	[40]
	Octanoic acid	LC-FL	Fish and Meat	PAHs	[99]
Gemini-based	14-2-14	HPLC-UV	Cosmetics and water	MP, EP, PP	[86]
		HPLC-UV	Water and soil	Pyrethroids	[85]
		GC-MS	Water	PEs	[89]

nanoparticles as the sorbent. Ultrasound was used to promote the dispersion of the IL in the sample solution and to speed up the mass-transfer process. Recoveries of the metals ranged from 81% to 104% with LOQs between 2.3 and 4.7 μg kg^{-1}.

7.4.3 Vortex-assisted microextraction (VALLME)

In VALLME, dispersion of the extraction solvent in the aqueous solution is attained by vortex mixing, and no additional dispersion solvent is required. Since the mass-transfer efficiency between sample and extraction phase is slow compared with DLLME, the extraction time is moderately long in VALLME. The addition of surfactant as an emulsifier provides a mechanism to increase the rate of mass transfer in VALLME. The surfactant lowers the interfacial tension between the sample and extraction solvent at the liquid-liquid interface increasing the dispersion efficiency [106]. Lee et al. [107] used vortex-assisted surfactant-enhanced emulsion liquid-liquid microextraction (VALLME) for the extraction of phthalate esters from bottled water for GC-MS analysis. The sample solution was injected into the surfactant (CTAB) and extraction solvent (toluene) mixture forming an emulsion aided by vortex agitation. The toluene extract was collected after phase separation by centrifugation and analyzed by GC-MS. The use of surfactant enhanced the extraction solvent dispersion in the aqueous sample, equilibrium being reached in 1 min. This innovative approach simplifies the use of solvents of low density in DLLME. Gámiz-Gracia et al. [106] used VALLME for the extraction of carbamates from fruit and vegetable juices with analysis by MEKC-MS/MS. before their specification by MEKC-MS/MS for the carbamate pesticide extraction in juice samples [106]. The addition of surfactant (ammonium perfluorooctanoate) in combination with vortex agitation enabled clean extracts to be isolated with a short extraction time. The recovery of the carbamates ranged from 81% to 104% with LOQs between 2.3 and 4.7 μg kg^{-1}.

7.5 Surfactant as Ion Pairing Agent for Liquid Membrane Extraction

7.5.1 Biphasic solvent extraction

The poor extraction capability of DLLME or SA-DLLME for hydrophilic compounds can be mitigated by techniques such as ion pair-based surfactant-assisted microextraction (IP-SAME). The role of the surfactant is to enable ionic species to be extractable by organic solvents by ion-pair formation [6]. IP-SAME was used for the extraction of chloroanilines and nitrophenols from aqueous solution [108]. The solution pH was adjusted so that the chloroanilines were in a neutral form

and the nitrophenols in the deprotonated form. Addition of the cationic surfactant cetyltrimethylammonium bromide allowed the simultaneous extraction of the nitrophenols as ion pairs along with the chloroanilines. The concentration of the cationic surfactant was a critical parameter with the extraction efficiency and extraction rate enhanced in the region of the CMC probably due to more efficient emulsion and ion-pair formation. In a related study the extraction of the drugs ofloxacin and ciprofloxacin was enhanced using a cationic surfactant as a dispersing agent, 1-octanol as extraction solvent, and the quaternary ammonium salt (Aliquat 336) as ion pair-forming regent [109]. The LODs for ofloxacin and ciprofloxacin were 0.06 and 100 ng mL^{-1}, respectively, by liquid chromatography with absorbance detection. The cationic surfactant tetradecyltrimethylammonium bromide was used for emulsification of the two-phase system and for ion-pair extraction of palladium in the presence of iodide ions (PdI_4^- extracted as an ion pair) for the determination of palladium by ICP-AES [6]. Under optimum conditions an enrichment factor as large as 146 was attained. The LOD for palladium in water samples was 0.2 μg L^{-1}.

7.5.2 Triphasic solvent extraction

Hollow-fiber liquid-phase microextraction (HF-LPME) is commonly used to minimize consumption of organic solvent for extraction and to protect the extraction phase from contamination or depletion [110]. For the analysis of polar or ionizable analytes, a three-phase system is frequently used with an organic solvent immobilized in the hollow-fiber pores acting as a liquid membrane separating the donor phase (sample solution) from the acceptor phase (extractant). For ionizable compounds the acceptor phase in the hollow-fiber lumen is often an aqueous solution into which the analytes are back extracted by change of pH, for example. A recent version of this approach is carrier-mediated extraction in which an ion pair or complexing agent is added to the liquid membrane, or sometimes the sample solution, to facilitate the extraction of compounds otherwise difficult to extract by HF-LPME [111]. Yamini et al. [112] used a hollow-fiber liquid membrane incorporating a cationic surfactant (Aliquat 336) for the three-phase extraction of the hydrophilic drugs terbutaline and salbutamol from aqueous solution. The cationic surfactant facilitates migration of the target compounds across the liquid membrane by the formation of neutral ion pairs soluble in the liquid membrane. At the liquid membrane-acceptor solution interface, the ion pairs are dissociated with the negatively charged target compounds abstracted from the organic phase. The driving force for mass transport is the concentration gradient of chloride ions between the donor and acceptor phases. The enrichment factors were 213.1 and 52.9 and LOD 0.5 and 2.5 ng mL^{-1} for terbutaline and salbutamol, respectively.

7.6 Conclusions

Surfactants are amphiphilic, surface-active compounds that assist in solubilizing organic and inorganic compounds in both aqueous and organic solvents and lowering the interfacial surface tension at phase boundaries. The role of surfactants as extractants (CPE and SUPRAS), emulsifiers, and ion-pairing agent in liquid-phase extraction was the focus of this chapter. Although the CPE technique was introduced more than three decades ago, advances are still being made in understanding and applications. Ultrasound irradiation and vortex agitation are becoming increasingly used as methods of dispersion for faster mass transport eliminating the need for a separate disperser solvent in microextraction techniques. The development of supramolecular solvents for more efficient and selective extraction represents a further development of surfactant-based microextraction techniques.

Surfactant-based extraction methods were shown to be efficient approaches for isolating contaminants from a variety of environmental, biological, and agrifood samples. Their adaptation to different microextraction formats, capability for multiresidue analysis, simplicity, and low cost make them suitable alternatives to traditional organic solvents in many analytical scale extractions. Further research on new surfactant-based extraction methods is necessary to identify coacervates that permit different types of interactions with analytes under a variety of experimental conditions.

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Chapter 8

Microextraction With Supported Liquid Membranes

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Chapter Outline

8.1 Introduction

The interest in microextraction in analytical chemistry was initiated by the invention of solid-phase microextraction (SPME) by Pawliszyn and coworkers in 1990 [1]. In SPME, target analytes are extracted into a polymeric coating located on a thin needle. Extraction can be performed directly from an aqueous sample (immersed mode) or from the sample headspace. The coating is typically polydimethylsiloxane (PDMS), but alternatives are also available. After extraction the needle is inserted into a heated injection port of a gas chromatograph (GC), and the extracted material is desorped

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thermally into the GC. SPME has gained significant interest, because the technique is solvent-free, provides soft extraction (equilibrium extraction), is GC compatible, and is automated. Due to the price of SPME fibers, they are normally used several times before they are discarded. In between each sample the needle has to be cleaned carefully, to avoid carryover from sample to sample.

Single-drop microextraction (SDME) represented a different approach and was originally described in 1996 [2, 3]. In SDME, analytes of interest are extracted into a small droplet of organic solvent located at the needle tip of a microsyringe. SDME can be performed in immersed or headspace mode. After extraction the droplet is injected into a GC. Compared with SPME, SDME is less expensive, and since a new droplet is used for every new extraction, SDME is less sensitive to carryover. There has been substantial interest for SDME [4], but the stability of the extraction system is an issue, and the droplet may be lost during extraction.

To avoid this, hollow-fiber liquid-phase microextraction (HF-LPME) was introduced in 1999 [5]. Development of HF-LPME was inspired by pioneering work with SPME [1] and SDME [2, 3] and with supported liquid membrane extraction [6]. The setup and principle of HF-LPME are illustrated in Fig. 8.1. Target analytes are extracted from aqueous sample, into a supported liquid membrane (SLM) and further into an acceptor solution. The SLM comprises a thin film of organic solvent (immiscible with water) immobilized in the pores in the wall of a porous hollow fiber. The acceptor is located in the lumen of the hollow fiber. The major advantage of HF-LPME is that the SLM protects the acceptor from leaking to the sample. Thus, in contrast to SDME, HF-LPME is compatible with complex biological and environmental samples.

The hollow fiber illustrated in Fig. 8.1 is rod-shaped. In the early publications, the hollow fiber was often U-shaped. Based on the principles of HF-LPME, solvent bar microextraction (SBME) was proposed in 2004 [7]. In SBME the lumen of a small piece of hollow fiber is filled with extraction solvent (acceptor). The porous wall is filled with impregnation solvent (SLM), both ends of the hollow fiber are closed, and the solvent bar is located in the sample. The solvent bar moves freely in the sample, which is stirred with a magnetic stirrer.

HF-LPME and SBME are based on passive diffusion, and extractions often have to be performed for 30–45 min to reach equilibrium. To reduce extraction time, electromembrane extraction (EME) was proposed in 2006, where mass transfer is by electrokinetic migration [8]. EME is similar to HF-LPME, but electrodes are located in the sample and acceptor. Using an external power supply, an electric potential is sustained across the SLM. Due to the electric field, charged analyte molecules migrate from the sample, through the SLM, and into the acceptor. Most LPME and EME were accomplished with hollow fibers, but both techniques have also been performed in a 96-well configuration with the SLMs located in filters in a 96-well

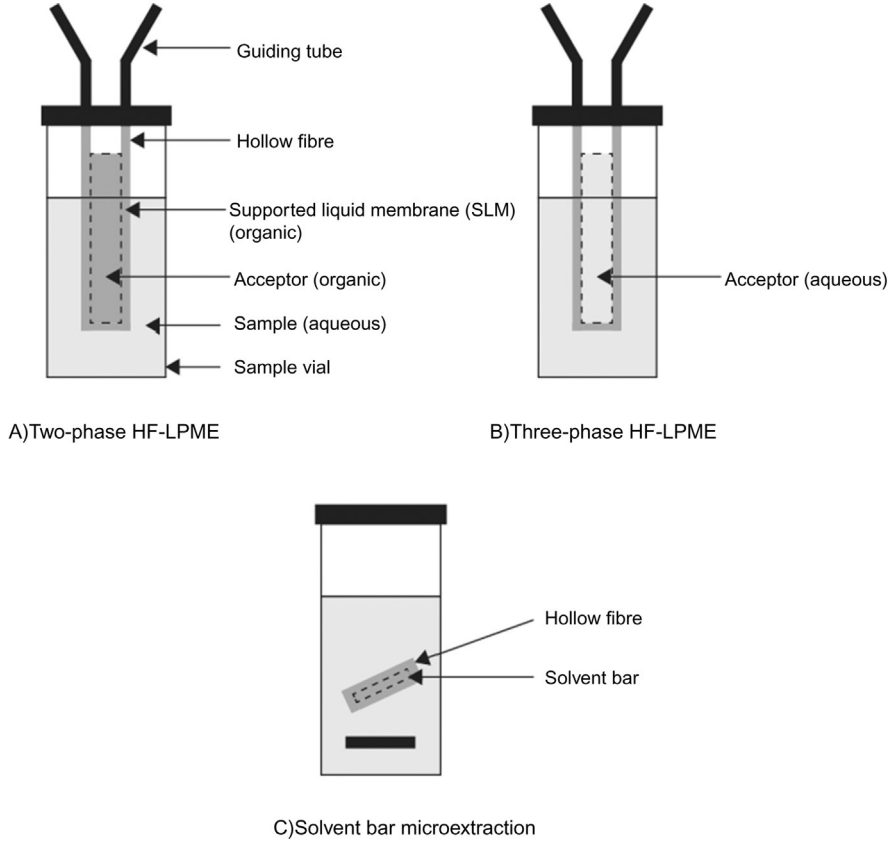

Fig. 8.1 Principle of (A) two- and (B) three-phase hollow-fiber liquid-phase microextraction and (C) solvent bar microextraction (SBME).

filter plate [9, 10]. LPME in 96-well configuration was originally termed parallel artificial liquid membrane extraction (PALME) [9].

This chapter will focus on HF-LPME, SBME, 96-well LPME (PALME), and EME. It is emphasized that other approaches to microextraction with supported liquid membranes have been developed in parallel, such as membrane bag-assisted liquid-phase microextraction [11], solvent-stir bar microextraction [12], in-line supported liquid membrane extraction in capillary electrophoresis [13], hollow-fiber-supported dispersive liquid-liquid microextraction [14], and microfluidic liquid-liquid microextraction [15]. Similar nonmembrane approaches have also been developed such as dispersive liquid-liquid microextraction [16], but these are outside the scope of the current chapter.

8.2 Extraction Principles

The principle for two-phase HF-LPME is illustrated in Fig. 8.1A. The system comprises a sample vial, a hollow fiber (closed in one end), a guiding tube, and a cap. Sample is pipetted into the sample vial. The porous hollow fiber is dipped into an organic solvent immiscible with water, such as 1-octanol. Due to capillary forces the solvent rapidly penetrate and immobilize in the pores in the wall of the porous hollow fiber. Next the acceptor solution is injected into the lumen of the hollow fiber. In two-phase HF-LPME the acceptor is an organic solvent, normally the same solvent as used for the SLM. The hollow fiber with the SLM and acceptor is placed into the sample, and the entire system is agitated during extraction. Extraction is performed for typically 30–45 min, and after this the acceptor is collected with a microsyringe. Because the acceptor is a small volume of organic solvent, it can be injected directly into a GC system. Extraction in two-phase LPME is based on partition, and mass transfer is by passive diffusion. Agitation is performed during HF-LPME, to promote convection in the sample solution. This is highly important to avoid excessive extraction times. Two-phase HF-LPME is preferred for neutral analytes, aimed for analysis by GC. Typical applications are within environmental analysis.

Alternatively, HF-LPME can be performed using an aqueous acceptor, and extraction is then accomplished in a three-phase system (Fig. 8.1B). Three-phase HF-LPME is used for basic or acidic analytes, and extraction is forced by a pH gradient across the SLM. For basic analytes the sample is made alkaline prior to extraction, to make sure that analytes are in a neutral form. In neutral form the analytes enter the SLM, and they diffuse across the SLM. The acceptor is acidic, and in contact with this, the analytes becomes protonated, and by such, they are prevented from reentering the SLM. For acidic analytes, the pH gradient is reversed. Thus the sample is made acidic, while the acceptor solution is neutral or alkaline.

Recently the principle of three-phase HF-LPME was transferred to 96-well plates and termed parallel artificial liquid membrane extraction (PALME) or 96-well LPME [9]. This is illustrated in Fig. 8.2. Ninety-six-well LPME takes advantage of commercially available 96-well plates intended for filtration, and the equipment comprises a sample plate, a filter plate (acceptor plate), and a lid. Samples are pipetted into wells in the sample plate (step 1). The filter plate comprises 96 filters of polyvinylidene fluoride (PVDF), and these are holding the SLMs. Thus 3–5 μL organic solvent is pipetted into each filter (step 2). Acceptor solutions are pipetted in wells above the filters in the filter plate (step 3). The sample plate and the filter plate are clamped, and extraction is facilitated by agitation of the whole assembly (step 4). Finally, acceptors are collected and analyzed, typically by LC-MS/MS (step 5). PALME is a three-phase system similar to three-phase HF-LPME, and basic and acidic analytes can be extracted based on pH gradients across the SLM. The advantage of 96-well LPME is that the plates are commercially available, and extraction can be automated in a high throughput mode.

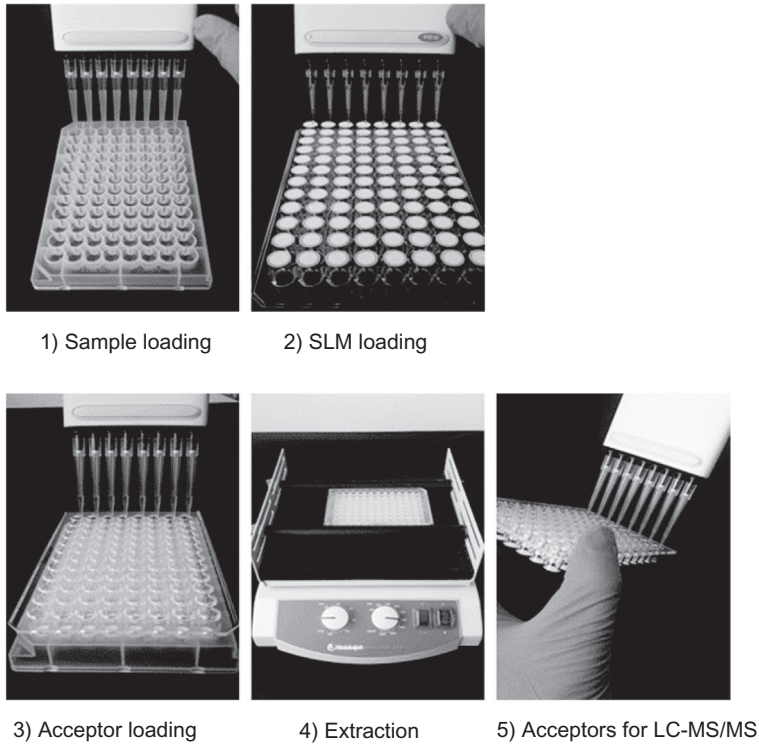

Fig. 8.2 Photo of 96-well liquid-phase microextraction and schematic illustration of the extraction system in one well.

Solvent bar microextraction is very similar to HF-LPME and can be operated in both two- and three-phase modes [7]. Thus the extraction chemistry is the same as in HF-LPME, extraction is based on partition, and mass transfer is by passive diffusion. The main difference between SBME and HF-LPME is that the piece of hollow fiber in SBME is floating freely in the sample and is thus tumbling during extraction because the sample is stirred.

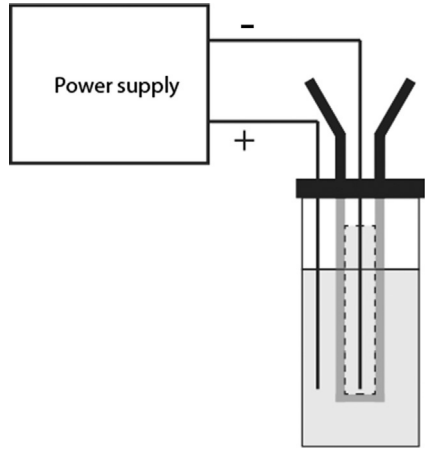

Fig. 8.3 Principle of electromembrane extraction.

The setup for electromembrane extraction (EME) is similar to three-phase LPME as illustrated in Fig. 8.3. However, in EME, electrodes are inserted into the sample and acceptor, and these are coupled to an external power supply.

By application of a dc potential across the SLM, charged analytes are extracted from the sample, through the SLM, and into the acceptor solution. During EME the whole assembly is agitated to promote convection in the sample. For extraction of basic analytes, neutral or acidic conditions are used in the sample and acceptor. The cathode (negative electrode) is located in the acceptor, and the anode is in the sample. For extraction of acidic analytes, the direction of the electric field and pH conditions are changed. Thus samples and acceptor are neutral or alkaline, and the anode (positive electrode) is now located in the acceptor. For rapid mass transfer across the SLM, the entire assembly is agitated during extraction.

8.3 Mass Transfer

A considerable effort has been devoted to the fundamentals of LPME, SBME, and EME. Equations for mass transfer have been developed, and these are important because they give the techniques a scientific anchor. In addition, such equations identify the major operational parameters and explain how they affect mass transfer across the SLM and into the acceptor. For three-phase LPME the mass transfer equation in Fig. 8.4 describes the concentration of analyte in the acceptor ($C_A(t)$) as function of time (t):

The LPME mass transfer equation predicts that the analyte concentration in the acceptor is proportional to the original concentration of analyte in the sample. This is

$$C_A(t) = \frac{V_D C_D^0 - C_D^0 \cdot exp\left(-\frac{A_f D_m K_d \cdot t}{V_D h}\right)(V_D + K_d V_m)}{V_A}$$

C_D^0 Original analyte concentration in sample

Device parameters

A_f Surface area of SLM
h SLM thickness
V_m SLM volume

Operational parameters (analyte dependent)

V_D Sample volume
V_A Acceptor volume
t Time
K_d Sample-SLM distribution coefficient for analyte
D_m Analyte diffusion coefficient in SLM

Fig. 8.4 Mass transfer equation for LPME [17].

fundamental for obtaining linear calibration curves. The mass transfer equation also predicts that geometry and design of the extraction device impact extraction efficiency. Thus extraction efficiency is increased with a larger surface area for the SLM and with decreasing thickness and volume of the SLM. Operational parameters, which are optimized during method development, include sample volume and time. Analyte enrichment increases with a larger sample volume and extraction time and decreasing acceptor volume. The selection of SLM is very important, as both analyte distribution into the SLM and analyte diffusion across the SLM are dependent on the organic solvent selected as SLM.

The mass transfer equation for EME is similar (Fig. 8.5). Therefore several device-related and operational parameters are the same. However, the driving force in EME is an electric potential, and therefore the EME mass transfer equation is more complicated. Extraction efficiency increases with a higher voltage and longer time. In addition the selection of organic solvent is critical and controls the SLM permeability and analyte distribution parameter. The latter is voltage dependent, and therefore EME offers an additional parameter for system control and selectivity:

8.4 Method Optimization

In two-phase HF-LPME (and SBME), traditional method optimization includes selection of the organic solvent used as SLM and acceptor, sample volume and pH, agitation rate, and extraction time. The organic solvent is often 1-octanol or toluene, but many alternative solvents have also been explored including o-xylene, 1-heptanol, nonanoic

$$C_A(t) = \frac{V_D \cdot C_D^0 - C_D^0 \cdot \exp(-\frac{A_f \cdot P^{D\to A}}{V_D} \cdot t)\left(V_D + \exp(\frac{z \cdot F}{RT}(\Delta_o^w\varphi - \Delta_o^w\varphi^0) \cdot V_m)\right)}{V_A}$$

C_D^0	Original analyte concentration in sample

Constants

R	Gas constant
F	Faradays constant

Device parameters

A_f	Surface area of SLM
V_m	SLM volume

Operational and analyte dependent parameters

V_D	Sample volume
V_A	Acceptor volume
$\Delta_o^w\varphi$	Galvani potential difference across SLM
t	Time
T	Absolute temperature
z	Analyte charge
$P^{D\to A}$	SLM permeability coefficient for analyte
$\Delta_o^w\varphi^0$	Analyte distribution parameter

Fig. 8.5 Mass transfer equation for EME [18].

acid, and different ionic liquids [19]. Generally the organic solvent should be immiscible with water to avoid leakage into the sample. In addition the viscosity should be low to promote fast analyte diffusion across the SLM. Optimization typically involves testing of a few different solvents and selecting the solvent providing highest recovery. Prior to the loading of organic solvent into the hollow fiber, the hollow fiber is cleaned in acetone (or a similar type of volatile organic solvent) to remove contaminants present in the polymeric hollow fiber.

Sample volumes in two-phase HF-LPME vary depending on the application and can range from 1 to 1000 mL [19]. Extraction of neutral compounds can be performed directly without manipulation of the sample. For acidic or basic analytes, pH adjustment is required to promote analyte distribution into the organic phase. For basic analytes the sample is made alkaline, while samples are acidified for extraction of acidic analytes. Often salt is added to the sample, to enhance partition into the SLM based on the salting-out effect. Typical, sodium chloride or sulfate is used in amounts of 3%–5% (w/w) [19]. Stirring or agitation of the sample is important to promote convection, and to avoid that mass transfer in the bulk sample is limiting the extraction kinetics. Extraction recovery increases with increasing stirring or agitation rate, and the optimum is typically at 500–1000 rpm (Fig. 8.6).

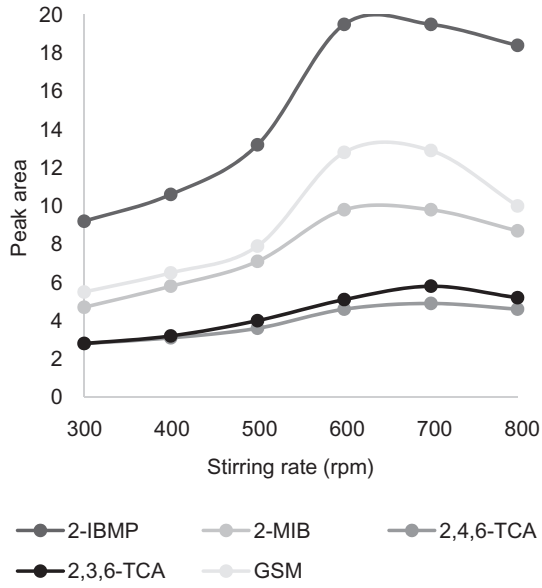

Fig. 8.6 Extracted amount versus stirring rate in two-phase HF-LPME. *2-IBMP,* 2-isobutyl-3-methoxy pyrazine; *2-MIB,* 2-methylisoborneol; *2,4,6-TCA,* 2,4,6-trichloroanisole; *2,3,6-TCA,* 2,3,6-trichloroanisole; *GSM,* geosmin. *(Data adopted from Yu S, Xiao Q, Zhu B, Zhong X, Xu Y, Su G, Chen M. Gas chromatography–mass spectrometry determination of earthy–musty odorous compounds in waters by two phase hollow-fiber liquid-phase microextraction using polyvinylidene fluoride fibers. J Chromatogr A 2014;1329:45–51.)*

In most cases, two-phase LPME is not an exhaustive extraction method, but is an equilibrium system. Recovery increases with time until a certain point and levels off during prolonged extraction (Fig. 8.7). The equilibrium time is typically in the range 15–45 min, depending on the analytes and the geometry of the setup [19].

For three-phase HF-LPME and 96-well LPME, optimization is similar to two-phase extraction. Agitation and extraction time optimization follow the same principles as discussed earlier. The organic solvent serves as SLM in a three-phase HF-LPME, and the solvent is selected based on extraction recoveries obtained experimentally during method optimization. Solvents such as 1-octanol, dodecyl acetate, dihexyl ether, and isopentyl benzene are frequently used [20]. These are organic solvents with high boiling point and very low solubility in water (2.5×10^{-3}–1.2 g/L). They are not prone to evaporation or leakage during extraction.

Three-phase HF-LPME and 96-well LPME are used for basic and acidic analytes, and therefore pH in the sample and acceptor plays an important role. For extraction of acidic analytes, the sample should be acidified, preferably to a pH value 2–3 units

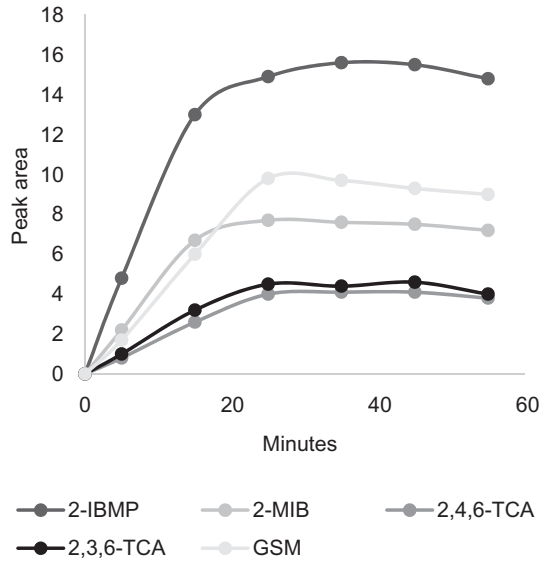

Fig. 8.7 Extracted amount versus extraction time in two-phase HF-LPME. *2-IBMP*, 2-isobutyl-3-methoxy pyrazine; *2-MIB*, 2-methylisoborneol; *2,4,6-TCA*, 2,4,6-trichloroanisole; *2,3,6-TCA*, 2,3,6-trichloroanisole; *GSM*, geosmin. *(Data adopted from Yu S, Xiao Q, Zhu B, Zhong X, Xu Y, Su G, Chen M. Gas chromatography–mass spectrometry determination of earthy–musty odorous compounds in waters by two phase hollow-fiber liquid-phase microextraction using polyvinylidene fluoride fibers. J Chromatogr A 2014;1329:45–51.)*

lower than the pK_a value of the analytes [20]. Under such conditions, the analytes are neutral, and they readily partition into the SLM. On the other hand, the acceptor should be neutral or alkaline for efficient ionization of the analytes. By such, analyte molecules are trapped in the acceptor. For extraction of basic analytes, the pH gradient is reversed, with neutral or alkaline conditions in the sample and with acidic conditions in the acceptor. Neutral conditions are typically obtained using phosphate buffers, while hydrochloric acid or formic acid is often used for acidification. High pH is normally obtained by adding dilute solutions of sodium hydroxide [20].

For optimization of EME the composition of the SLM is very important. For EME of basic analytes with log $P > 1.5$ (octanol-water partition coefficient), 2-nitrophenyl octyl ether (NPOE) is preferred as SLM [21]. NPOE is highly efficient for extraction of basic substances, is immiscible with water, and is of low volatility. Furthermore the current in the EME system is low with NPOE, typically at the 1–5 μA level. This is important, because at high current levels electrolysis may occur in the sample and acceptor, resulting in formation of small bubbles and drifting pH.

For polar basic analytes (log $P < 1.5$), mass transfer is strongly limited with pure NPOE. In such cases, the addition of an ion-pair reagent to the SLM solvent is required to obtain high extraction recoveries. Often di(2-ethylhexyl) phosphate (DEHP) is used for polar basic analytes [21]. Recently, alternative SLMs for polar analytes have been suggested, including bis(2-ethylhexyl) hydrogen phosphite and tributyl phosphate that have been operated without the addition of ion-pair reagents [22]. For acidic analytes with log $P > 1.5$, long-chain alcohols such as 1-octanol and 1-nonanol are used as SLM, and for polar acidic analytes (log $P < 1.5$) the SLMs are modified with an appropriate ion-pair reagent such as Aliquat 336 [21]. More research is expected in the near future on the development of new SLMs for different EME applications.

When the SLM has been selected, optimization of voltage is normally the next step in EME method development. According to the EME mass transfer equation in Fig. 8.5, the EME efficiency increases with increasing voltage. However, as exemplified in Fig. 8.8, this effect levels off at higher voltages. Above this point, the voltage is no longer the rate-limiting step. The optimal voltage is dependent on the analyte, the composition of the SLM, and the geometry of the setup and is established based on experimental data. Voltages exceeding 300 V are not used in EME, because

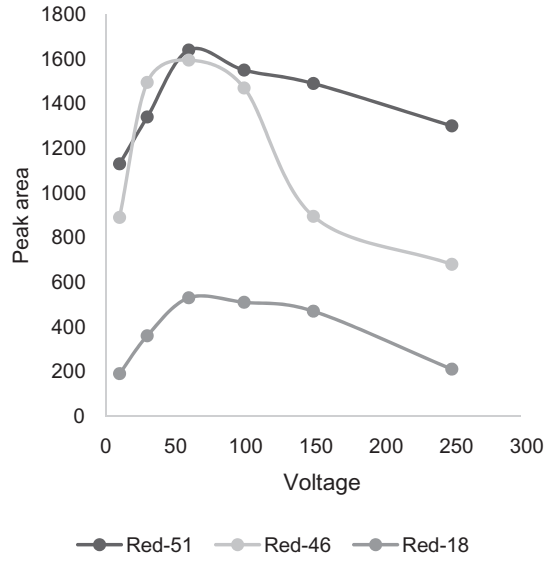

Fig. 8.8 Extracted amount versus voltage in EME. *Red-51*, azo dye Basic Red 51; *Red-46*, azo dye Basic Red 46; *Red-18*, azo dye Basic Red 18. *(Data adopted from Nojavan S, Tahmasebi Z, Bidarmanesh T, Behdad H, Nasiri-Aghdam M, Mansori S, Pourahadi A. Electrically enhanced liquid-phase microextraction of three textile azo dyes from wastewater and plant samples. J Sep Sci 2013;36:3256–3263.)*

at high voltage the EME system is prone to dielectric breakdown with a major loss of stability.

Selection of sample and acceptor pH is another very important part of EME method development. The pH value in the acceptor is critical, due to local pH effects at the acceptor/SLM interface [23]. Thus, for extraction of basic analytes, pH is higher close to the SLM than in the bulk acceptor, and to compensate for this, pH in the acceptor should be at least 2–3 units below the pK_a value of the analyte. For basic analytes, pH in the sample should also be low, but here pH is less critical, and often analytes are extracted with high efficiency even at pH values close to their pK_a. Similarly, extraction of acidic analytes is performed from neutral or alkaline samples and into an alkaline acceptor. Acidification of samples and acceptor in EME is typically with hydrochloric acid, acetic acid, or formic acid [24]. Alkaline conditions are obtained using ammonia or using dilute solutions of sodium hydroxide [24]. The final steps in EME method optimization include experiments with different extraction time and agitation rate. This is similar to HF-LPME, except that extraction times are shorter in EME (typically 5–10 min).

8.5 Selected Applications

In this section, selected applications are discussed in detail to illustrate (a) typical experimental conditions and (b) performance with two- and three-phase HF-LPME, SBME, 96-well LPME (PALME), and EME. For an overview of applications, readers should refer to review articles such as Refs. [19], [20], [24], and [25].

The first application is summarized in Table 8.1. Here two-phase hollow-fiber liquid-phase microextraction (HF-LPME) was combined with LC-MS/MS for monitoring emerging pollutants in river water [26]. Emerging pollutants included drugs, personal care products, synthetic hormones, industrial additives, and pesticides. Commercially available hollow fibers were used, and prior to LPME the hollow fibers were cut into 5.5 cm pieces. Hollow fibers of this length housed 60 μL of acceptor phase. In addition the hollow fibers were cleaned by 5-min sonication in acetone and then dried prior to use. The purpose of this was to remove polymeric contaminants and avoid contamination of the acceptor solution.

Samples were 1000 mL of river water adjusted to pH 7.0. Furthermore, sodium chloride was added to each sample to a concentration corresponding to 3% (w/w). Sample pH and the amount of sodium chloride were optimized experimentally. The analytes comprised a mix of neutral, acidic, and basic substances, and for the acids and bases, sample pH affected extraction efficiency. Optimal pH differed for acidic and basic analytes, and pH 7.0 was selected as a compromise. This pH value was not optimal for all substances, but served to avoid recovery variations with sample pH. The addition of sodium chloride increased the extraction efficiency due to the salting-out effect.

TABLE 8.1 Experimental Conditions and Performance for Two-Phase HF-LPME of Emerging Pollutants in River Water [26]

Experimental Conditions	
Configuration	Hollow fiber
Analytes	27 emerging pollutants including drugs, personal care products, synthetic hormones, industrial additives, and pesticides
Hollow fiber	Polypropylene, 5.5 cm length, 600 μm id, 200 μm wall, 0.2 μm pores
	Sonicated in acetone for 5 min and dried prior to use
Sample	1000 mL river water +3% NaCl, adjusted to pH 7
SLM	1-Octanol
Acceptor	60 μL 1-octanol
Stirring	100 rpm
Time	30 min
Performance	
LC-MS compatibility	Acceptors injected directly in LC-MS
Enrichment	6–4177
Linearity	$r > 0.99$
Repeatability	Within 15% RSD
Accuracy	81%–128%

Five different organic solvents were tested under method optimization, namely, 1-octanol, isooctane, toluene, n-hexane, and diethyl ether. Isooctane and n-hexane were not properly retained in the fiber during extraction, and among the three other solvents, 1-octanol was superior in terms of recovery. Therefore 1-octanol was selected and used both as SLM liquid and as acceptor. First the SLM was loaded into the pores in the porous wall of the hollow fiber. This was accomplished by dipping the entire piece of hollow fiber into 1-octanol, and the fiber was kept there for 1 min. After this, 60 μL of 1-octanol was pipetted into the lumen of the hollow fiber, and this aliquot served as the acceptor. The hollow fiber was then placed in the sample, and this was stirred at 100 rpm for 30 min. Extraction recoveries increased with increasing extraction time up to approximately 30 min. With longer extraction times, recoveries decreased, probably due to long-term instability of the extraction system. The stirring rate was relatively low as compared with most two-phase HF-LPME applications, and this was because the hollow fiber was open. With sealed hollow fibers, the stirring rate can be increased. After extraction, 20 μL of the acceptor phase was pipetted from the hollow fiber and transferred for LC-MS/MS analysis. Although the acceptors were not miscible with the mobile phase, acceptors were injected directly

into the LC-MS/MS system. This was feasible when the injection volume was limited to 5 μL.

As seen from Table 8.1, enrichment factors varied from 6 to 4177 depending on the analyte. Analytes with a high log *P*-value (nonpolar) were enriched more efficiently than the compounds with a low log *P* (polar). Based on the sample-to-acceptor volume ratio (1000/0.060 mL), enrichments of 16,667 times were theoretically possible. As an example, the pesticide ametrine was enriched by a factor of 4177, and this corresponded to 25% recovery. The method combining two-phase HF-LPME and LC-MS/MS was validated for the 27 emerging pollutants. Linearity with $r > 0.99$ was obtained for all analytes in the range 1–100 μg/L. Accuracy was reported between 80.6% and 127.8% for spiked river water samples, and precision was better than 15% RSD in all cases. The limits of quantification were within 2–125 ng/L depending on the analyte. Both differences in recovery and differences in electrospray ionization efficiency contributed to this major variability.

Solvent bar microextraction (SBME) is very similar to two-phase HF-LPME, except that the piece of hollow fiber containing the organic solvent is tumbling freely in the stirred sample solution. A typical SBME application is summarized in Table 8.2. Here SBME was combined with GC-MS/MS for detection of polyaromatic hydrocarbons (PAHs) in seawater [27]. PAHs are neutral and nonpolar substances and are suited for two-phase SBME. Extractions were performed from 1000 mL seawater samples, using hexane as the organic solvent. Thus hexane was present in the pores and in the lumen of the hollow fiber. The volume of hexane present in the lumen was 100 μL prior to extraction, while 33 ± 4 μL was recovered after extraction. Thus a substantial fraction of the organic solvent leaked into the aqueous sample. This is a general challenge using microliter volumes of organic solvent for extraction of large volumes of aqueous sample. Although the solvents used are immiscible with water, still small amounts dissolve in large volumes of aqueous sample. For quantitative purposes therefore internal standards are important to correct for such variability. Extraction was performed for 60 min and was supported by stirring at 500 rpm. After extraction, 1 μL of acceptor was collected from the solvent bar and injected directly into GC-MS/MS. Enrichment factors were in the range 45–163. With this level of enrichment, the PAHs were detected down to the 0.21–0.82 ng/L by GC coupled with tandem mass spectrometry.

Three-phase HF-LPME is featured in Table 8.3. In this example, LPME was combined with HPLC and fluorescence detection for the determination of sulfonamides in influent and effluent water from sewage treatment plants [28]. The method was based on in situ derivatization with fluorescamine, and eight different sulfonamides were covered by the method. A polypropylene hollow fiber was used with an internal diameter of 1800 μm, a length of 2.0 cm, and a wall thickness of 450 μm. Mechanically this is a very robust hollow fiber, but due to the thick wall, extraction kinetics were relatively slow, and 60 min was required to obtain equilibrium. In addition,

TABLE 8.2 Experimental Conditions and Performance for Solvent bar Microextraction (SBME) of Polyaromatic Hydrocarbons in Seawater [27]

Experimental Conditions	
Configuration	Hollow fiber, sealed
Analytes	Naphthalene, acenaphthene, acenaphthylene, anthracene, phenanthrene, fluorine, fluoranthene, benz(a)anthracene, chrysene, pyrene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, benzo (g,h,i)perylene, indene
Hollow fiber	Polypropylene, 5.0 cm length, 1200 μm id, 450 μm wall, 0.2 μm pores
	Sonicated in acetone for 15 min and dried prior to use
Sample	1000 mL seawater
SLM	Hexane
Acceptor	100 μL hexane
Stirring	500 rpm
Time	60 min
Performance	
LC-MS compatibility	Acceptors injected directly in GC-MS/MS
Enrichment	45–163
Detection limits	0.21–0.82 ng/L

because the fiber was relatively short, the contact area with the sample was somewhat limited.

Water samples of 8 mL were used for extraction. Derivatization was accomplished directly in the sample solution, followed by three-phase HF-LPME. Sample solutions were acidified to pH 3.5. The optimal pH was found by testing pH values in the range 2.5–4.5. The derivatized sulfonamides were zwitterionic, and at optimal pH the net charge was close to zero. Optimization of the SLM was performed by testing 1-octanol, dihexyl ether, toluene, and undecane. Highest recoveries were obtained with 1-octanol, and this solvent was selected for the application. The three other solvents were not effective, due to low polarity. The acceptor was a dilute solution of sodium hydroxide with pH 12.5. This was found by testing different acceptors between pH 11 and 13. At optimum pH the sulfonamide derivatives were negatively charged, and therefore they were trapped efficiently in the acceptor. Extraction was performed for 60 min (as discussed earlier) with 600 rpm stirring. The optimization also tested the impact of temperature, and for this application the extraction efficiency increased significantly with temperature up to 45°C. Thus, in the final method, 45°C was used.

TABLE 8.3 Experimental Conditions and Performance for Three-Phase HF-LPME of Sulfonamides in Influent and Effluent Water From Sewage Treatment Plants [28]

Experimental Conditions	
Configuration	Hollow fiber
Analytes	Sulfadiazine, sulfacetamide, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfachloropyridazine, sulfamethoxazole, and sulfisoxazole
Hollow fiber	Polypropylene, 2.0 cm length, 1800 μm id, 450 μm wall, 0.2 μm pores
Sample	8 mL water sample adjusted to pH 3.5 with HCl + 0.2% (w/v) fluorescamine (derivatization reagent)
SLM	1-Octanol
Acceptor	30 μL of NaOH in water pH 12.5
Temperature	45°C
Stirring	600 rpm
Time	60 min
Performance	
Enrichment	14–60
Linearity	$r^2 > 0.99$ (0.05–5 μg/L)
Detection limits	3.1–11.2 ng/L
Repeatability	3%–19% RSD
Accuracy	56%–113%

The three-phase HF-LPME method provided 14–60 times enrichment. Linearity was obtained for all sulfonamides in the range 0.05–5 μg/mL, detection limits were in the range 3.1–11.2 ng/L with fluorescence detection, repeatability is <19% RSD, and accuracy ranged between 56% and 113% in wastewater.

Ninety-six-well LPME is exemplified and featured in Table 8.4. In this application, eight major antidepressant drugs were extracted from samples of human blood plasma by 96-well LPME and subsequently measured by LS-MS/MS [29]. The antidepressant drugs are basic substances of low polarity and are ideally suited for three-phase LPME. First, 125 μL plasma samples were pipetted into a 96-well sample plate. Second, 115 μL 40 mM sodium hydroxide in water was pipetted into each sample. At high pH the analytes were neutral, and this enhanced their partition into the SLM. Third, 10 μL solution of internal standard (fluoxetine-d_5) was pipetted to each sample. In step 4 the SLMs were loaded. Each SLM comprised 5 μL of dodecyl acetate containing 1% trioctyl amine (TOA). TOA served to avoid nonspecific binding

TABLE 8.4 Experimental Conditions and Performance for 96-well LPME of Psychoactive Drugs [29]

Experimental Conditions	
Configuration	Ninety-six-well
Analytes	Venlafaxine, *O*-desmethylvenlafaxine, citalopram, norfluoxetine, fluvoxamine, fluoxetine, sertraline, paroxetine
Sample matrix	Human blood plasma
Sample	125 μL plasma +115 μL 40 mM NaOH+10 μL internal standard
SLM	5 μL 1% trioctyl amine in dodecyl acetate
Acceptor	50 μL 20 mM HCOOH
Agitation	900 rpm
Time	60–120 min
Performance	
Throughput	96 samples extracted simultaneously in 60 min
LC-MS compatibility	Acceptors injected directly in LC-MS
Recoveries	60%–100%
Repeatability	Within 15% RSD
Evaluation	Precision, linearity, accuracy, and matrix effects in compliance with EMA guidelines[a]

[a] European Medicines Agency.

of analyte to the polyvinylidene fluoride (PVDF) filter. The SLMs were loaded by pipetting 5 μL SLM liquid directly into PVDF filters in a 96-well filter plate. The organic SLM liquid quickly penetrated and filled the entire porous volume of the filters and was immobilized there by capillary forces. In step five, 50 μL of acceptor phase was pipetted into the reservoirs above the PVDF filter (and SLM). Formic acid at 20 mM in water was used as acceptor. The two 96-well plates were clamped, a lid was placed on the top to avoid evaporation of the acceptor phase, and the entire assembly was agitated for 60 min at 900 rpm to facilitate extraction. After this the acceptor phases were transferred into an autosampler and analyzed directly by LC-MS/MS.

The concept enabled extraction of 96 samples simultaneously in 60 min. With a 96-tip pipette the time to load samples, SLMs, and acceptors was about 5–10 min, and this has potential for automation. Because the acceptor phase was aqueous, the extracts were analyzed directly by LC-MS/MS. Thus evaporation and reconstitution in LC mobile phase, which is a common procedure after liquid-liquid

extraction (LLE) or solid-phase extraction (SPE), were avoided with 96-well LPME. The consumption of organic solvent per sample was 5 μL, and therefore 96 samples were extracted with a total consumption of <500 μL of organic solvent. The two 96-well plates are reasonably priced, and total costs were about 30 euro cent per sample.

Recoveries ranged between 60% and 100% after 60-min extraction, and after 120 min, all eight antidepressants were extracted exhaustively. Repeated extractions varied <15% RSD and complied with official guidelines for bioanalytical method validation used by the pharmaceutical industry. Due to the nonpolar nature of the SLM, proteins, salts, phospholipids, and many other endogenous compounds remained in the sample during extraction, and therefore very clean extracts were obtained with 96-well LPME [30]. The entire method, based on 96-well LPME and LC-MS/MS, was evaluated, and data complied with European Medicines Agency guidelines. This application illustrates a significant potential for 96-well LPME, for extraction of basic and acidic substances with log $P > 1.5$ from relatively small sample volumes. Such applications are typical for bioanalysis of drug substances, which are carried out in the pharmaceutical industry, at hospitals, and in doping and forensic toxicology laboratories. In addition, because 96-well plates are commercially available, 96-well LPME is expected to be a valuable tool in the future.

In Table 8.5, EME is featured. In this example, nine major benzodiazepines were extracted from human blood plasma [31]. Benzodiazepines are very weak bases ($pK_a < 4$) and are drugs used as tranquilizers. EME was conducted in a 96-well system comprising a 96-well sample plate, a 96-well filter plate, and a top plate with 96 electrodes. The filter plate was commercially available and identical to the one used above for 96-well LPME (Table 8.4). The two other plates were laboratory made. The sample plate was made of stainless steel and comprised 96 sample wells. The sample plate was conducting and used as the positive electrode (anode). The top plate was made of aluminum and with 96 small stainless steel electrodes that extended into the acceptor during operation. The top plate was used as negative electrode (cathode). Extraction was controlled by an external dc power supply connected to the sample and top plates.

The samples were loaded into the sample plate. The samples comprised 50 μL plasma mixed with 40 μL 20 mM formic acid in water and 10 μL solution of internal standard (mixture of deuterated benzodiazepines). Formic acid was added to the samples for acidification and served to protonate the benzodiazepines. This was mandatory in order for the analytes to migrate in the electric field. The SLMs were pipetted into the PVDF filters of the 96-well filter plate and were immobilized by capillary forces. Each SLM was 3 μL, and 2-nitrophenyl octyl ether (NPOE) was used as the SLM solvent. As discussed earlier, NPOE is superior as SLM for basic analytes with log $P > 1.5$, such as benzodiazepines [21]. The acceptor was 100 μL 250 mM trifluoroacetic acid (TFA) in water and was pipetted into the wells in the filter plate (above the SLMs). The acceptor was highly acidic, and this was required

TABLE 8.5 Experimental Conditions and Performance for EME of Benzodiazepines [31]

Experimental Conditions	
Configuration	96-well
Analytes	7-Aminoclonazepam, nitrazepam, clonazepam, flunitrazepam, oxazepam, alprazolam, *N*-desmethyldiazepam, phenazepam, diazepam
Sample matrix	Human blood plasma
Sample	50 µL plasma +40 µL 20 mM HCOOH +10 µL internal standard
SLM	3 µL 2-nitrophenyl octyl ether (NPOE)
Acceptor	100 µL 250 mM trifluoroacetic acid (TFA)
Voltage	20 V
Agitation	900 rpm
Time	15 min
Performance	
Throughput	96 samples extracted simultaneously in 15 min
LC-MS compatibility	Acceptors injected directly in LC-MS
Recoveries	45%–100%
Repeatability	Within 15% RSD
Evaluation	Precision, linearity, accuracy, and matrix effects in compliance with EMA guidelines[a]

[a] European Medicines Agency.

to extract the benzodiazepines with very low pK_a values (p$K_a < 4$). For analytes with p$K_a > 4$, 250 mM, trifluoroacetic acid can be replaced with less acidic solutions such as 20 mM formic acid. After loading the liquids the three plates were clamped and placed on an agitator. EME was performed for 15 min with 20 V and 900 rpm. After this the acceptors were transferred for direct analysis by LC-MS/MS.

Compared with LPME, extractions are faster in EME due to the electric field. The electric field was coupled to all 96 wells through the sample plate and the top plate, and therefore up to 96 samples can be processed simultaneously in 15 min. Performance data were very similar to those reported in Table 8.4 with 96-well LPME. EME is interesting for the future due to the possibility for tuning extraction selectivity based on (a) direction and (b) magnitude of the electric field, (c) the chemical composition of the SLM, (d) pH in the sample, and (e) pH in the acceptor. This may facilitate unique applications in the future such as the combination of microchip EME and smartphone detection as published recently [32]. However, EME is less mature, and more fundamental research is required.

8.6 Outlook

Microextraction with supported liquid membranes was proposed nearly 20 years ago [5]. For two decades, scientists around the world have developed different technical configurations and applications, and it has been a very active field of research. In spite of this, SLM-based microextraction has mainly been done in academia and still has not been implemented in routine laboratories. There are probably several reasons for this, but one is the lack of commercial equipment and consumables.

For two- and three-phase HF-LPME and SBME, the hollow fibers used are commercially available, but they have not been tailored for microextraction purposes. Thus the hollow fibers have to be cut in appropriate length, sealed, and connected to some type of guiding tube prior to use. Commercial products in this area will most probably appear in the future. This is justified by the fact that HF-LPME and SBME offer the following advantages:

- Consumption of organic solvent is reduced to a few microliters per sample.
- Very high enrichment can be obtained.
- Evaporation of extraction solvent and reconstitution is not required.

Thus microextraction techniques represent a green chemistry approach to sample preparation, and next-generation analytic scientist will give this aspect high priority. Both HF-LPME and SBME can provide very high enrichment factors, which are especially important in trace environmental analysis. Thus, in one example, antidepressant drugs were enriched 27,000 times from seawater by three-phase HF-LPME [33]. Since the acceptor can be either organic (two-phase system) or aqueous (three-phase system) in HF-LPME or SBME, evaporation and reconstitution are eliminated.

For 96-well LPME, equipment is commercially available. Although this is not developed for LPME (but for filtration), 96-well LPME can now be performed with consumables of industrial standard and with a high degree of automation. Ninety-six-well LPME is especially suited for extraction of basic and acidic drugs with log $P > 1.5$ from small volumes (<250 μL) of biological fluids. Such applications are typically within the pharmaceutical industry, in hospitals, and in forensic toxicology and doping laboratories. Ninety-six-well LPME offers the following advantages:

- Low price (approximately 30 Euro cent per sample).
- Acceptor is LC-MS/MS compatible.
- Consumption of organic solvent is reduced to a few microliters per sample.

Compared with protein precipitation (PP), which is commonly used with human plasma and serum samples, 96-well LPME provides much better sample cleanup. This is highly beneficial with LC-MS/MS, to avoid ion suppression and to reduce the contamination of the mass spectrometer. Compared with solid-phase extraction

(SPE), 96-well LPME is superior in terms of price, LC-MS/MS compatibility, and low consumption of organic solvent. The two latter points are also valid in comparison with liquid-liquid extraction (LLE). Thus 96-well LPME will likely be implemented in routine laboratories in the near future. The major limitation now is that no company supports the commercial 96-well plate for LPME use.

In recent years, there has been substantial interest for EME in academic institutions. A company in Norway is currently developing a prototype device for EME, which will be commercially available in the near future. This is important, and with such a device, future research in this area can be performed with standardized equipment. EME will likely be implemented in the future for challenging applications, such as extraction of very hydrophilic analytes and biomolecules. To reach this point however, more fundamental research is required, to understand how the SLM should be tailored for specific applications. EME also has potential for use with smartphones, microfluidics, and handheld devices, which are expected to be important tools in next-generation analytical chemistry.

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Chapter 9

Totally Organic Biphasic Systems

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Chapter Outline

9.1 Introduction

Solvent extraction is a venerable technique used routinely for matrix simplification in analysis and as an isolation technique in organic synthesis. In its simplest form, it requires a minimal amount of glassware and is easily scaled to accommodate samples of varying size. What has not changed significantly in more than a century of use

Liquid-Phase Extraction. https://doi.org/10.1016/B978-0-12-816911-7.00009-8

is that most practical applications of liquid-liquid extraction utilize water as the main component of one phase of biphasic systems. One practical reason for this is that water forms the largest number of biphasic systems with organic solvents than any other solvent [1, 2]. Also, water is a ubiquitous component of most biological and environmental samples and a common component among food and industrial products. Thus samples containing water as a major component are common and favor extraction techniques that are compatible with water. Water is also the least expensive of common laboratory solvents and the least restricted by disposal regulations an important consideration for applications at a large scale. On the other hand the distribution properties of aqueous biphasic systems are dominated by the characteristic properties of water. Water is the most cohesive and hydrogen-bond acidic of the common laboratory solvents and is competitive in terms of its dipolarity/polarizability and hydrogen-bond basicity with typical polar organic solvents [1]. Any solvent that is likely to be competitive with water for these interactions is also likely to be miscible with water [3]. This limits the possible selectivity space available for aqueous biphasic systems. For solvents of low mutual solubility, the classification of selectivity for a large database of aqueous-organic solvent biphasic systems indicated that the available selectivity space could be adequately sampled with just a few countersolvents identified as an n-alkane (e.g., n-heptane), an aromatic hydrocarbon (e.g., toluene), a haloalkane (e.g., dichloromethane or chloroform), an ether or ester (e.g., diethyl ether or ethyl acetate), and an n-alkyl alcohol (e.g., n-butanol or n-octanol) [1, 3, 4]. Since no organic solvent is as dominant as water in controlling selectivity, totally organic biphasic systems might be expected to provide complementary and more varied distribution properties compared with aqueous biphasic systems.

A second reason for exploring the use of totally organic biphasic systems is that water-based systems are of little use for the extraction of compounds and matrices of low-water solubility and cannot be used at all for compounds that are water unstable or react with water. There is thus a practical need to identify suitable nonaqueous extraction systems to complement water-based systems for extraction. Major applications are the extraction of oil-based samples of natural and synthetic origin, cosmetics, pharmaceutical creams and depositories, spices, and fat- and oil-based food products.

9.2 Totally Organic Distribution Systems and Their Properties

It is convenient to consider totally organic biphasic systems as composed of a polar solvent and a low-polarity countersolvent optimized to minimize mutual solubility. Typical polar solvent are methanol, 3,3,3-trifluoroethanol, ethylene glycol,

ethanolamine, acetonitrile, dimethyl sulfoxide, *N*,*N*-dimethylformamide, formamide, and propylene carbonate. For the countersolvent an n-alkane (typically n-heptane), isopentyl ether, 1,2-dichloroethane, or n-octanol is typically used and less commonly triethylamine.

9.2.1 Mutual solubility and stability

n-Heptane (or n-hexane) forms the largest number of binary biphasic systems of low mutual solubility, followed by isopentyl ether, with a few systems formed with 1,2-dichloroethane, n-octanol, and triethylamine, as summarized in Fig. 9.1 [5]. The room-temperature mutual solubility for some binary biphasic systems is given in Table 9.1 [6]. The relatively high solubility of triethylamine in propylene carbonate would make this biphasic system an unlikely candidate for practical applications [5]. Primary alcohols and phenols form formyl esters as by-products in the triethylamine-formamide biphasic system [5]. Schiff base adducts are formed with aldehydes and ketones, and aromatic esters are degraded in the triethylamine-ethanolamine biphasic system [5]. In addition, triethylamine enhances inlet activity and causes column degradation in gas chromatography. These factors limit its use as a countersolvent for some applications. Although formamide has a weak response to the flame ionization detector, it forms a by-product of higher response at typical injection temperatures for gas chromatography [7]. This by-product might interfere in the analysis of some compounds if their separation from the formamide degradation product

Polar solvent	Counter solvent				
	Heptane	1,2-DCE	IPE	OcOH	TEA
Acetonitrile	▨				
Dimethylformamide	▨		▨		
Dimethyl sulfoxide	▨		▨		▨
Ethylene glycol	▨	▨	▨		
Ethanolamine	▨		▨		▨
Formamide	▨	▨	▨	▨	▨
Hexafluoroisopropanol	▨				
Propylene carbonate	▨				
Methanol	▨		▨	▨	▨
Trifluoroethanol	▨				

Fig. 9.1 Mutual solubility of totally organic biphasic systems containing two solvents. Biphasic systems with low mutual solubility are indicated by the shaded boxes. Clear boxes indicate miscible systems.

TABLE 9.1 Mutual Solubility for Some Totally Organic Biphasic Systems at Room Temperature

		Mutual Solubility % (v/v)	
Solvent 1	**Solvent 2**	**Solvent 1 in 2**	**Solvent 2 in 1**
n-Hexane	Acetonitrile	13.2	2.7
n-Heptane	Methanol	5.2	1.2
n-Heptane	*N,N*-Dimethylformamide	3.3	5.3
n-Heptane	Dimethyl sulfoxide	1	0.32
Triethylamine	Dimethyl sulfoxide	1.09	0.98
n-Heptane	Ethylene glycol		0.02
n-Heptane	3,3,3-Trifluoroethanol	2.8	1.3
Isopentyl ether	Ethanolamine	1.19	0.73
Triethylamine	Ethanolamine	5	0.43
Triethylamine	Propylene carbonate	89.7	0.54

cannot be achieved by adjusting the column temperature. We have not found this to be particularly troublesome problems for the analysis of varied volatile organic compounds. n-Heptane is preferable to n-hexane for small-scale liquid-liquid partition systems since its lower volatility simplifies laboratory manipulations and storing of extracts. 1,1,1,3,3,3-Hexafluoroisopropanol is a relatively expensive solvent with a clearing temperature close to room temperature requiring special handling [8]. It also presents a specific safety concern (eye damage).

Fluorous solvents have been used for the extraction of fluorine-containing compounds from reaction mixtures and more complex matrices [9–13]. Applications involving nonfluorinated compounds are not common. Modest size partition constant databases for organofluorine compounds are available for perfluoro(methylcyclohexyl)-toluene [14] and perfluorodecalin-acetonitrile [15]. The poor solvation of organic compounds and preference for highly fluorinated compounds of fluorous solvents has more to do with the relatively weak dispersion forces between fluorous solvents and hydrocarbon-like compounds than a strong affinity for highly fluorinated compounds.

9.2.2 Solvation properties

Systematic studies of the solvation properties of totally organic biphasic systems are based on the solvation parameter model [6, 16–19]. This model provides a framework for comparing biphasic systems in terms of fundamental intermolecular

interactions and for the selection of biphasic systems for specific applications. It is founded on the parameterization of the cavity model of solvation and is set out below in a form suitable for the distribution of neutral compounds in a biphasic solvent system:

$$\log K_p = c + e\mathrm{E} + s\mathrm{S} + a\mathrm{A} + b\mathrm{B} + v\mathrm{V} \tag{9.1}$$

where K_p is the liquid-liquid partition constant. The lowercase letters in *italics* are the system constants describing the complementary interactions of the system with the solute. The solute descriptors are indicated by the capital letters and are defined in Table 9.2. Solute descriptors are available for several thousand compounds, and further values can be either measured or estimated by software if needed [15, 18]. They do not concern us here since all information for the solvation properties of the biphasic systems is described by the system constants. These are determined by multiple linear regression analysis utilizing experimentally determined partition constants for varied compounds with known descriptor values. There are both chemical and statistical constraints that must be fulfilled to obtain meaningful model coefficients independent of solute identity, which are then suitable for predicting the distribution properties of further compounds [18, 19]. The descriptors for the selected solutes define the descriptor space, which defines the range of individual descriptor values over which the model equation provides a suitable description of the distribution process, and for calibration, it is preferable that the selected solutes have descriptor values that evenly fill the descriptor space. Cross correlation between each series of descriptor values should be low to obtain a globally reliable model for the distribution process. More stable models are expected when the range of

TABLE 9.2 Descriptors Used in the Solvation Parameter Model and Their Measurement [16–18]

Symbol	Description	Measurement
E	Excess molar refraction	For liquids, calculated from the refractive index and characteristic volume. For solids determined experimentally or calculated from an estimated refractive index
S	Dipolarity/ polarizability	Determined experimentally from (usually) chromatographic liquid-liquid partition or solubility measurements
A	Hydrogen-bond acidity	Determined experimentally from (usually) chromatographic liquid-liquid partition or solubility measurements
B	Hydrogen-bond basicity	Determined experimentally from (usually) chromatographic liquid-liquid partition or solubility measurements
V	McGowan characteristic volume	Calculated by summing atom fragment constants and correcting for the number of bonds

experimentally measured distribution constants is reasonably large.

The system constants for the biphasic systems represent the difference in the solvation environment attracting a solute from one phase to the other. The *e* system constant represents the difference in n- and π-electron lone pair interactions between phases (or the additional dispersion interactions possible for compounds with loosely bound polarizable electrons); the *s* system constant the difference in interactions of a dipole type (induction and orientation) between phases; the *a* system constant the difference in hydrogen-bond basicity between phases and the *b* system constant the difference in hydrogen-bond acidity between phases; and the *v* system constant the difference in cohesion (cavity formation) between phases and any additional dispersion interactions that are not self-canceling when the solute is transferred from one phase to the other. The thermodynamic cycle for the transfer of a solute from one phase to the other requires the formation of a cavity in the receptor phase of sufficient size to accommodate the solute, which requires disruption of solvent-solvent interactions specific to that phase, accompanied by the setup of solute-solvent interactions in the same phase favorable for the transfer with the simultaneous collapse of the cavity in the donor phase and depletion of the solute-solvent interactions in that phase. The driving force for the distribution mechanism is the overall change in free energy for the process at equilibrium.

The application of Eq. (9.1) to totally organic binary biphasic systems requires the availability of a sufficient number of experimental distribution constants for varied compounds. For totally organic biphasic systems, these are collated in a series of

TABLE 9.3 Databases for Distribution Constants for Totally Organic Biphasic Systems

Biphasic System	Reference
n-Hexane-acetonitrile	[20]
n-Heptane-methanol	[21]
n-Heptane-*N*,*N*-dimethylformamide	[21]
n-Heptane-3,3,3-trifluoroethanol	[8]
n-Heptane-1,1,1,3,3,3-hexafluoroisopropanol	[8]
n-Heptane-formamide	[22]
n-Heptane-propylene carbonate	[23]
n-Heptane-dimethyl sulfoxide	[24]
n-Heptane-ethylene glycol	[25]
n-Heptane-ethanolamine	[26]

TABLE 9.3 Databases for Distribution Constants for Totally Organic Biphasic Systems—cont'd

Biphasic System	Reference
Isopentyl ether-formamide	[7]
Isopentyl ether-propylene carbonate	[23]
Isopentyl ether-dimethyl sulfoxide	[24]
Isopentyl ether-ethylene glycol	[25]
Isopentyl ether-ethanolamine	[26]
1,2-Dichloroethane-formamide	[7]
1,2-Dichloroethane-ethylene glycol	[25]
Octan-1-ol-formamide	[7]
Octan-1-ol-propylene carbonate	[23]
Triethylamine-dimethyl sulfoxide	[5]
Triethylamine-formamide	[5]
Triethylamine-ethanolamine	[5]

databases identified in Table 9.3 [5, 7, 8, 20–26]. A database containing distribution constants for additional compounds is provided in Ref. [6]

9.2.2.1 *Binay Biphasic Systems Containing n-Alkane Solvents*

Alkanes saturated with small amounts of a polar solvent can be classified as low-polarity solvents with favorable cohesion properties for the transfer of solutes from polar solvents but with minimal capacity for polar interactions. Thus the n-alkane-containing biphasic systems in Table 9.4 all have positive v system constants in the range from 0.49 to 2.23 with the larger values associated with the more cohesive polar countersolvents. Except for n-heptane-ethanolamine the e system constant is either zero (n-heptane-dimethyl sulfoxide) or positive with the largest values associated with the fluorine-containing countersolvents. The latter reflects the characteristic weaker electron lone pair interactions between perfluoroalkane and alkane groups compared with the same interactions between alkane groups [5]. It is not surprising that interactions of a dipole-type and hydrogen-bonding interactions favor transfer to the polar countersolvents. For solvents with low mutual solubility in n-heptane, the s system constants covers the range from -0.73 to -2.1 with most between -1.5 and -2.1. Thus the selectivity space available for the extraction of compounds differentiated by their dipolarity/polarizability is not large for these biphasic systems. In contrast the range of system constants for hydrogen-bonding

TABLE 9.4 System Constants and Model Statistics for n-Alkane-Organic Solvent Biphasic Systems

Polar Solvent	System Constants		Statistics[a]							
	e	*s*	*a*	*b*	*v*	*c*	*r*	SE	*F*	*n*
Acetonitrile	0.406 (0.019)	−1.516 (0.031)	−1.647 (0.030)	−0.832 (0.028)	0.657 (0.011)	0.178 (0.020)	0.997	0.086	5492	189
Methanol	0.226 (0.019)	−0.733 (0.036)	−1.145 (0.029)	−0.914 (0.029)	0.589 (0.019)	−0.136 (0.027)	0.993	0.074	1148	93
N,N-Dimethylformamide	0.043 (0.021)	−1.405 (0.032)	−2.099 (0.039)	−0.573 (0.026)	0.486 (0.010)	0.259 (0.021)	0.998	0.080	5251	130
3,3,3-Trifluoroethanol	0.914 (0.013)	−1.582 (0.020)	−1.283 (0.023)	−2.857 (0.023)	1.297 (0.009)	−0.005 (0.016)	0.998	0.077	12,953	229
1,1,1,3,3,3-Hexafluoroisopropanol	0.912 (0.056)	−1.576 (0.078)	−0.67 (0.058)	−2.337 (0.112)	1.127 (0.049)	−0.281 (0.064)	0.987	0.126	586	83
Propylene carbonate	0.435 (0.013)	−2.078 (0.022)	−2.701 (0.026)	−0.463 (0.023)	0.784 (0.011)	0.558 (0.022)	0.998	0.073	9528	166
Dimethyl sulfoxide	0	−1.764 (0.024)	−3.239 (0.048)	−1.148 (0.034)	1.156 (0.017)	0.317 (0.039)	0.997	0.119	5670	143
Formamide	0.521 (0.020)	−2.142 (0.032)	−3.389 (0.041)	−1.652 (0.036)	2.23 (0.024)	0.203 (0.034)	0.997	0.107	6141	166
Ethylene glycol	0.113 (0.015)	−1.498 (0.023)	−3.761 (0.026)	−1.573 (0.028)	2.113 (0.018)	0.283 (0.024)	0.999	0.085	12,920	186
Ethanolamine	−0.220 (0.044)	−1.141 (0.064)	−4.536 (0.066)	−1.302 (0.062)	1.981 (0.064)	−0.206 (0.087)	0.997	0.138	2438	82

The countersolvent is n-hexane for acetonitrile and n-heptane for all other polar solvents.
[a] r = multiple correlation coefficient, SE the standard error of the estimate, F the Fisher statistic, and n = number of compounds with partition constants entered into the model.

interactions is considerably wider from −0.67 to −4.5 for *a* and from −0.46 to −2.8 for *b*. Small values for the *a* system constant and large values for the *b* system constant are associated with the fluorine-containing alcohols as polar countersolvents. Even so, there is a considerable variation in the ratio of the *a*/*b* system constants, which facilitates the fine-tuning of extraction systems for the separation of compounds differentiated by their capability for hydrogen-bonding interactions. The n-alkane solvent systems most favorable for exploiting dipole-type interactions are n-heptane-propylene carbonate and n-heptane-formamide. For the extraction of hydrogen-bond acids, n-heptane with either ethanolamine, ethylene glycol, formamide, or dimethyl sulfoxide is a reasonable choice. For the extraction of hydrogen-bond bases, n-heptane-3,3,3-trifluoroethanol is the preferred system. Carboxylic acids form oligomers (mainly dimmers) in n-alkane solvents, and their extraction properties are not correctly predicted by the models in Table 9.4 [20].

9.2.2.2 Binary Biphasic Systems Containing Isopentyl Ether

Isopentyl ether is a more polar solvent than the n-alkanes and is expected to be more competitive in interactions of a dipole-type and as a hydrogen-bond base (it has no hydrogen-bond acidity). The system constants for the five isopentyl ether-containing biphasic systems are summarized in Table 9.5. Compared with the same biphasic system containing n-heptane, the *a* system constants are smaller (less negative), and the *s* system constants retain their negative sign but are not reduced as much as the *a* system constants. There are only small changes in the *v* system constant (except for isopentyl ether-ethanolamine) suggesting that isopentyl ether and n-heptane (saturated with polar organic solvent) have similar cohesive energy. There is no trend for the *b* system constant, which has similar values to the n-heptane systems but with moderate positive and negative differences. With the exception of isopentyl ether-ethylene glycol, electron lone pair interactions are weak and not strongly influenced by the change in countersolvent. For ethylene glycol the *e* system constant is small with a positive sign for n-heptane and a negative sign for isopentyl ether as countersolvents. The standout feature is the change in hydrogen-bond basicity when n-heptane is replaced by isopentyl ether.

9.2.2.3 Binary Biphasic Systems Containing 1,2-Dichloroethane and Octan-1-ol

Increasing the general polarity of the countersolvent results in the formation of a smaller number of biphasic systems with their system constants summarized in Table 9.5. For the octan-1-ol-containing biphasic systems, electron lone pair interactions and dipole-type interactions are about the same for formamide and propylene carbonate, and selectivity differences result from changes in the *v*, *a*, and *b* system constants. The formamide-rich phase is considerably more cohesive than the

TABLE 9.5 System Constants and Model Statistics for Biphasic Totally Organic Solvent Systems Containing Isopentyl Ether, 1,2-Dichloroethane, Octan-1-ol, or Triethylamine

Biphasic System	System Constants						Statistics			
	e	*s*	*a*	*b*	*v*	*c*	*r*	SE	*F*	*n*
Isopentyl ether-dimethyl sulfoxide	0	−1.445 (0.028)	−2.159 (0.050)	−1.003 (0.050)	1.118 (0.022)	0.159 (0.047)	0.996	0.124	2903	108
Isopentyl ether-formamide	0.571 (0.022)	−1.726 (0.036)	−1.307 (0.035)	−1.435 (0.038)	2.000 (0.023)	0.141 (0.036)	0.997	0.098	3416	120
Isopentyl ether-propylene carbonate	0.287 (0.023)	−1.463 (0.032)	−0.757 (0.034)	−0.534 (0.041)	0.703 (0.017)	0.367 (0.034)	0.995	0.089	1869	100
Isopentyl ether-ethylene glycol	−0.119 (0.022)	−1.088 (0.031)	−1.551 (0.036)	−1.937 (0.038)	2.100 (0.025)	0.380 (0.034)	0.996	0.103	3892	148
Isopentyl ether-ethanolamine	−0.304 (0.039)	−0.558 (0.051)	−3.296 (0.059)	−0.723 (0.059)	1.215 (0.058)	−0.048 (0.072)	0.994	0.128	1449	91
1,2-Dichloroethane-ethylene glycol	0.105 (0.024)	0	2.441 (0.044)	1.011 (0.060)	−1.253 (0.048)	−0.74 (0.062)	0.993	0.112	1622	97
1,2-Dichloroethane-formamide	−0.092 (0.023)	0.418 (0.033)	2.011 (0.037)	1.269 (0.041)	−1.635 (0.030)	−0.293 (0.043)	0.995	0.099	2187	120
Octan-1-ol-Formamide	0.269 (0.032)	−1.046 (0.044)	−0.34 (0.037)	−0.872 0.066	1.308 (0.045)	0.266 (0.064)	0.986	0.095	552	83
Octan-1-ol-propylene carbonate	0.266 (0.027)	−1.104 (0.037)	0.212 (0.041)	0	0.347 (0.019)	0.332 (0.046)	0.978	0.107	482	91
Triethylamine-dimethyl sulfoxide	0.085 (0.054)	−1.325 (0.084)	−1.215 (0.095)	−0.716 (0.081)	1.046 (0.052)	−0.167 (0.093)	0.977	0.187	329	86
Triethylamine-Formamide	0.561 (0.043)	−1.100 (0.079)	−0.377 (0.142)	−1.601 (0.084)	1.733 (0.052)	−0.089 (0.093)	0.984	0.171	448	86
Triethylamine-octan-1-ol	−0.394 (0.083)	−0.640 (0.123)	−1.340 (0.119)	−1.282 (0.108)	1.406 (0.101)	−0.067 (0.145)	0.968	0.230	228	82

propylene carbonate-rich phase, more hydrogen-bond acidic than the octan-1-ol-rich phase, and more hydrogen-bond basic. For the 1,2-dichloroethane-containing biphasic systems, selectivity differences for formamide and ethylene glycol as countersolvents are quite small with only modest differences for all system constants. The formamide-rich phase is slightly more cohesive, hydrogen-bond acidic, and dipolar/polarizable than ethylene glycol saturated with 1,2-dichloroethane and a weaker hydrogen-bond base.

9.2.2.4 Binary Biphasic Systems Containing Triethylamine

Triethylamine is a weak cohesive and dipolar/polarizable solvent, moderately hydrogen-bond basic and non-hydrogen-bond acidic. The triethylamine-dimethyl sulfoxide biphasic system has similar selectivity to the isopentyl ether-propylene carbonate biphasic system, the triethylamine-formamide system to octan-1-ol-formamide, and the triethylamine-ethanolamine biphasic system to 1,2-dichloroethane with either ethylene glycol or formamide as countersolvents. None of these systems are selectivity equivalent, but the differences in system constants are small. For the triethylamine-dimethyl sulfoxide biphasic system, the significant difference in cohesive properties to isopentyl ether-propylene carbonate favors the distribution of larger solutes to the triethylamine-rich phase and those with significant hydrogen-bond acidity to dimethyl sulfoxide. For the triethylamine-formamide biphasic system, larger solutes have a slight preference for transfer to the triethylamine-rich phase compared with octan-1-ol, and hydrogen-bond bases are selectively extracted into the formamide-rich layer, since triethylamine is not competitive with octan-1-ol as a hydrogen-bond acid. The earlier discussion refers to neutral compounds only. Triethylamine would be expected to selectively extract protonic acids on account of its proton basicity. There are more instances of the formation of catalytic transformation products for triethylamine than for the other countersolvents in Tables 9.4 and 9.5 and column stability problems for gas chromatographic analysis, which may limit practical applications [5].

9.2.2.5 Classification of Totally Organic Biphasic Systems

Hierarchical cluster analysis of representative aqueous and totally organic biphasic systems with their system constants as variables affords a simple visualization of the range of extraction properties available for these systems, Fig. 9.2 [3, 6]. The dominant feature on the plot is the grouping of the aqueous biphasic systems as a separate cluster with little in common with the totally organic biphasic systems. This is a strong indication of the complementary nature of the two types of biphasic systems. The variation of the system constants for the water-based systems is not large, and the examples indicated on the plot cover the available selectivity range for aqueous biphasic systems reflecting the dominant control of selectivity by water [1, 3, 4].

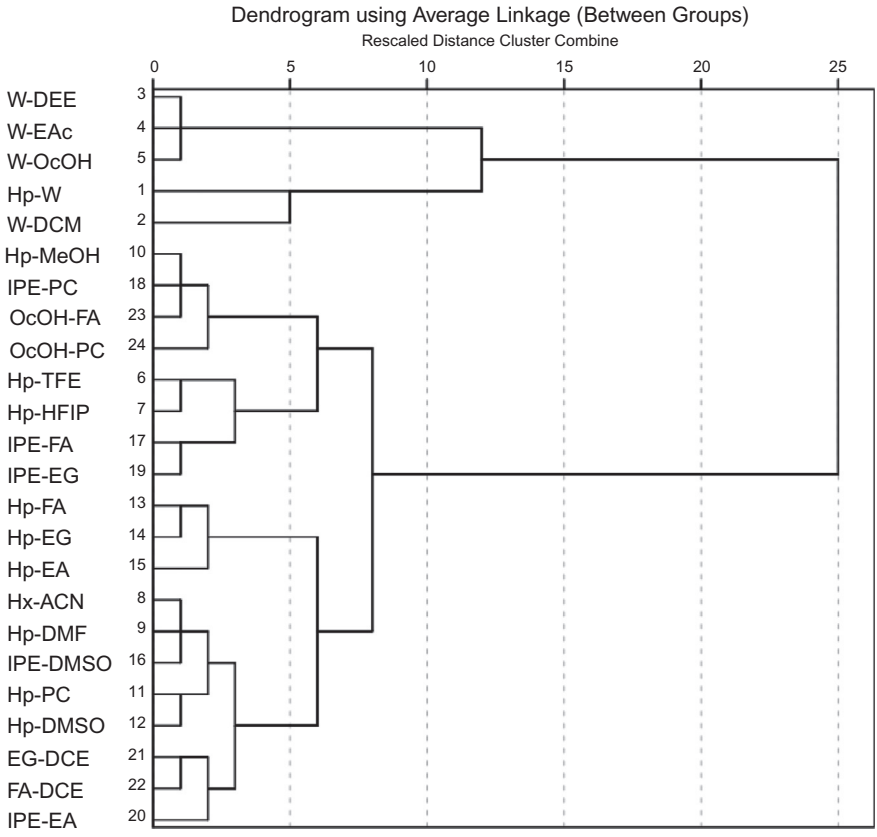

Fig. 9.2 Average-linkage agglomeration cluster dendrogram for representative water-based and totally organic biphasic systems with the system constants of the solvation parameter model as variables. Solvent abbreviations: *ACN*, acetonitrile; *DCM*, dichloroethane; *DEE*, diethyl ether; *DMF*, *N,N*-dimethylformamide; *DMSO*, dimethyl sulfoxide; *EA*, ethanolamine; *EAC*, ethyl acetate; *EG*, ethylene glycol; *FA*, formamide; *HFIP*, 1,1,1,3,3,3-hexafluoroisopropanol; *Hx*, n-hexane; *Hp-*, n-heptane; *IPE*, isopentyl ether; *MeOH*, methanol; *OcOH*, octan-1-ol; *PC*, propylene carbonate; *TFE*, 3,3,3-trifluoroethanol; and *W*, water. *(Source: Reprint from reference Poole CF. Partition constant database for totally organic biphasic systems. J Chromatogr A 2017;1527:18–32.)*

None of the polar organic solvents are as cohesive as water for which the v system constant for aqueous biphasic systems is about 4, while for the totally organic biphasic systems, it is generally ≤ 2.2. None of the polar organic solvents are competitive with water as a hydrogen-bond acid with b around -4 for the aqueous biphasic systems and < -2 for the totally organic biphasic systems except for the totally organic biphasic systems formed by fluorine-containing alcohols with values

between −3 and −2. A characteristic feature of the aqueous biphasic systems is the separation into two narrow ranges for interactions of a dipole-type and as a hydrogen-bond base. For low-polarity countersolvents the *s* system constant is close to −2 and for more competitive countersolvents close to 0. For the *a* system constant, values around −4 are observed for low-polarity countersolvents and close to 0 for low-miscibility polar organic solvents with significant hydrogen-bond basicity. In contrast a wider variation for the *s* and *a* system constants from 0 to −2.1 and from 0.2 to −4.5, respectively, is observed for the totally organic biphasic systems. Similarly the totally organic biphasic systems exhibit a broader range of values for the *e* system constant (−0.2 to 0.9) with a narrower overlap of values with the aqueous biphasic systems.

Four major clusters can be identified in Fig. 9.2 for the totally organic biphasic systems. Each cluster, however, is composed of near neighbors with some variation in selectivity best described as similar rather than equivalent systems in terms of selectivity. A solvent system taken from each group could be adopted for general screening and within group systems evaluated for further optimization. A reasonable selection of totally organic biphasic systems for screening purposes is isopentyl ether-propylene carbonate, n-heptane-3,3,3-trifluoroethanol, n-heptane-ethylene glycol, and n-heptane-dimethyl sulfoxide.

9.3 Applications

Contemporary methods of instrumental analysis tend to utilize small sample sizes in the form of a solution. Solvent extraction is a common preliminary step in sample preparation to dissolve, simplify, or concentrate samples, with some initial form of sample preparation required for most chemical analysis. If a solvent is employed for trituration, extraction, or dissolution, further sample simplification or concentration can be conveniently achieved by partition or adsorption in which a fraction of the sample of interest is isolated from a matrix by transfer to a different solvent or sorbent. Organic solvents are suitable for the extraction of low-polarity analytes from water or the dissolution of low- to medium-polarity samples, such as fats, oils, and plant materials, with subsequent transfer to an immiscible solvent to selectivity enhance the concentration of target analytes and minimize contamination from the matrix. Totally organic biphasic systems are suitable for these operations but historically have been limited by the scale commonly employed. The trend in contemporary sample preparation procedures to minimize sample size and solvent consumption facilitates the use of a wider range of more expensive and less volatile or viscous solvents avoided for general use in the past. Small-scale extraction methods are not limited to solvents easily evaporated to concentrate extracts and provide a better match between solvent use and instrument utilization.

9.3.1 n-Alkane-acetonitrile

One of the most studied totally organic biphasic systems is the use of n-hexane-acetonitrile for the extraction of residues of pest control compounds from agricultural products and plant materials. It provides efficient extraction of pesticides from plant-based oils and fatty foods prior to gas or liquid chromatographic analysis. Plant materials are usually blended with acetonitrile and coextracted low-polarity contaminants removed by washing with n-hexane. Fats and oils are typically diluted with n-hexane and more polar analytes extracted into acetonitrile [27, 28]. Zayats et al. determined the partition constant for 150 widely used pesticides [29] and 19 triazole and imidazole fungicides [30] in the n-hexane-acetonitrile biphasic system. Noteworthy is that virtually, all partition constants as log K_p are negative supporting the use of this extraction system for the group extraction from hydrocarbon solutions or oil/fat matrices poorly soluble in acetonitrile. The extraction of triazole and imidazole fungicides was enhanced by using anhydrous acetonitrile containing 0.1-M perchloric acid. Inadequate matrix removal for oils and fats can result in column contamination and matrix-induced response enhancement for the analysis of polar pesticides by gas chromatography [29, 31]. The latter is usually minimized by using matrix-matched calibration standards. Low-temperature precipitation and gel permeation column cleanup are suitable alternatives for matrix simplification for acetonitrile extracts [30, 32]. Polar additives were efficiently and cleanly extracted from power transformer mineral oils diluted with n-hexane by acetonitrile [33].

In the 1960s Bowman and Beroza introduced the extraction *p*-value to assist in the identification of compounds (mainly pesticides, insect chemosterilants, and industrial chemicals) separated by gas chromatography [31, 34]. The *p*-value was defined as the fractional amount of solute partitioning into the low-polarity phase of an equivolume biphasic system and is thus directly related to the partition constant. Several biphasic systems were trialed for this application including some partially aqueous biphasic systems, but the n-hexane-acetonitrile system offered the most discriminating power for compounds of intermediate polarity. Quite large databases of *p*-values mainly for pesticides in common use at the time were developed [3, 20]. The limited accuracy of experimental *p*-values and the inability to measure *p*-values for compounds with extreme values of the partition constant and later the development of affordable hyphenated gas chromatographic-spectroscopic and spectrometric detectors conspired to confine this method to historical interest. Later, Berezkin et al. improved the precision of the method naming the technique the partition-chromatographic method [35]. These authors introduced the use of an internal standard of known properties for the direct calculation of partition constants, which subsequently led to its general use for the determination of partition constants of volatile compounds by the gas chromatographic method [20–26]. The values determined in this way do not depend on the phase ratio and the volume of injected phase and are only weakly dependent on small variations in temperature. Zenkevich

and coworkers [36, 37] developed the general approach further for the identification of pesticides, industrial chemicals and chemical warfare degradation products in environmental samples utilizing retention index values, and n-hexane-acetonitrile partition constants determined simultaneously by gas chromatography requiring two separations. Isidorov and coworkers [38–42] adopted a similar approach to characterize the composition of essential oils and for the determination of aromatic hydrocarbons, aromatic esters, and some polar compounds as trimethylsilyl derivatives. In later studies, partition constants in n-hexane-nitromethane [43, 44], n-hexane-3,3,3-trifluoroethanol [45], and n-hexane-octan-1-ol [46] were utilized to expand the scope of the method. Together with the partition constants contained in the WSU database [6], the references cited earlier provide a large collection of partition constants for varied compounds in the n-hexane-acetonitrile biphasic system and related systems mentioned here.

9.3.2 n-Alkane-dimethyl sulfoxide

A common method for the isolation of polycyclic aromatic hydrocarbons [47–52] and polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans [53, 54] from samples, such as petroleum products, lipids, and vegetable oils typically dissolved in an n-alkane or cyclohexane, is their extraction into either dimethyl sulfoxide or *N*,*N*-dimethylformamide. The extraction conditions have been optimized for different analyte and matrix combinations by the addition of a small amount of a third solvent, such as acetonitrile or water. Three successive extractions of a pentane solution by dimethyl sulfoxide or *N*,*N*-dimethylformamide followed by three successive back extractions of the polar phase after dilution with water (1:1 or 1:2) with fresh n-pentane are typical conditions for the isolation of polycyclic aromatic compounds (Fig. 9.3). The original n-pentane solution retains the hydrocarbons and other low-polarity matrix compounds, such as neutral lipids. The water-polar solvent phase retains alcohols, phenols, and low-mass aliphatic and aromatic carboxylic acids. The polycyclic aromatic hydrocarbons and high-mass aliphatic acids and other neutral compounds are recovered in high yield from the aqueous-polar organic solvent phase by back extraction into n-pentane. Jones [55] reported partition constants for several pesticides in the n-heptane-*N*,*N*-dimethylformamide biphasic system that were subsequently used to optimize the conditions for their extraction from wool wax.

It is generally assumed that favorable interactions between the aromatic rings of the polycyclic aromatic compounds and the electron lone pairs on the oxygen atoms of dimethyl sulfoxide or *N*,*N*-dimethylformamide are responsible for their selective extraction into the polar phase and that the suppression of these interactions by the addition of water through hydrogen-bonding with the oxygen atoms of the polar solvent, for their ease of recovery by back extraction into n-pentane [47, 50]. There is, however, an alternative explanation that seems more reasonable [3, 24]. The reason that

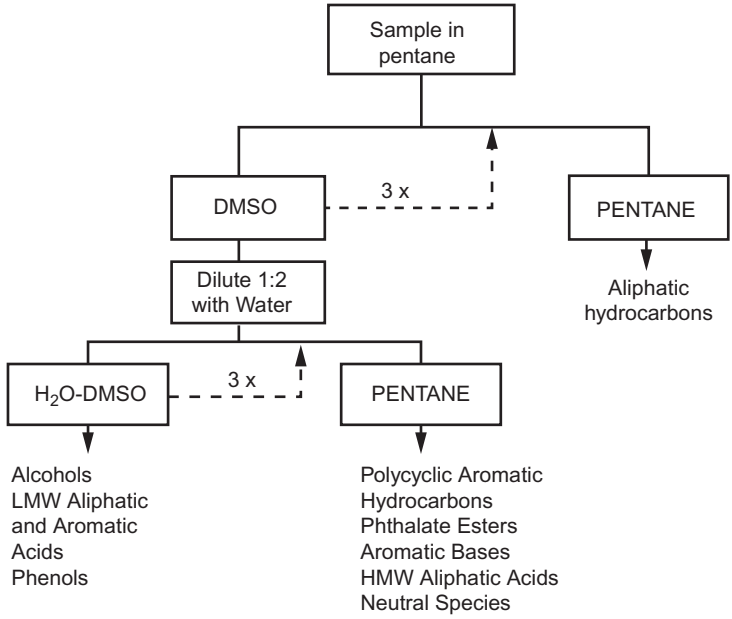

Fig. 9.3 Scheme for the isolation of polycyclic aromatic compounds by liquid-liquid distribution between n-pentane and dimethyl sulfoxide.

the n-heptane-dimethyl sulfoxide or n-heptane-*N*,*N*-dimethylformamide biphasic systems are effective for the isolation of polycyclic aromatic hydrocarbons is that the free energy barrier to transfer from the n-alkane phase to the polar solvent represented by the cavity term is sufficiently high to minimize transfer of low-polarity hydrocarbons but not so high that it cannot be overcome by dipole-type and hydrogen-bonding interactions for the polycyclic aromatic hydrocarbons in the polar solvents. For the polycyclic aromatic hydrocarbons, the driving force for transfer to the dimethylsulfoxide and *N*,*N*-dimethylformamide layer is their dipolarity/polarizability supplemented by their hydrogen-bond basicity that exceed the opposing contribution from cavity formation. While it is true that polycyclic aromatic hydrocarbons have a relatively large capacity for electron lone pair interactions, these are not important for their transfer to the polar solvents since neither dimethyl sulfoxide nor *N*,*N*-dimethylformamide have a significant capability for the complementary interaction as indicated by the system constants for the biphasic systems in Table 9.4 in which the *e* system constants are essentially zero. The n-heptane-dimethyl sulfoxide and n-heptane-*N*,*N*-dimethylformamide biphasic systems cannot be expected to provide high selectivity for the separation of polycyclic aromatic hydrocarbons and polycyclic aromatic compounds with polar functional groups since both types of compounds have favorable partition constants for transfer to the polar solvents. They are only differentiated by the range of values for their partition constants.

9.3.3 Further biphasic systems

The n-heptane-methanol biphasic system was used for the extraction of pesticides from foods [56] and fatty acids from lipids [57]. Correlation plots for the partition constants in n-heptane-methanol and n-heptane-*N*,*N*-dimethylformamide and n-hexane-acetonitrile indicate selectivity differences for the three biphasic systems [21]. Halobenzenes are selectively extracted by *N*,*N*-dimethylformamide, while aliphatic ketones, alcohols, and phenols have similar partition constants in both n-heptane-*N*,*N*-dimethylformamide and n-heptane-methanol. Comparing n-heptane-methanol with n-hexane-acetonitrile, alcohols are selectively extracted by methanol, but no family behavior was observed for other functional groups. Peterson et al. [58] determined partition constants for 11 peptides and 20 aromatic compounds in the n-heptane-ethylene glycol biphasic system. The partition constants were used to build a correlation model for the permeability coefficient for transport through cell membranes. These partition constants with additional experimental and estimated partition constants were used by Abraham et al. [59] to assign the system constants for the solvation parameter model for the n-heptane-ethylene glycol biphasic system.

Totally organic biphasic systems are increasingly used in countercurrent chromatography but not as frequently as water-based systems [60–65]. They provide a more convenient range of partition constants for compounds of low-water solubility. This area is growing as countercurrent chromatography enhances its reputation as a complementary technique to column liquid chromatography for the fractionation of mixtures and new opportunities are being realized for low-polarity matrices. One advantage of countercurrent chromatography over traditional extraction techniques is the feasibility of using gradient elution for the fractionation of mixtures with a wide range of partition constants. Berthod et al. [66] determined partition constants by countercurrent chromatography for several n-alkylbenzenes and polycyclic aromatic hydrocarbons in the n-heptane-methanol and n-heptane-*N*,*N*-dimethylformamide biphasic systems.

9.3.4 Estimation of physicochemical properties

Any free energy-related property can be estimated using the solvation parameter model, Eq. (9.1), if the system constants for the model are known (determined from available experimental data) and the descriptor values for the compounds whose properties are to be estimated are either known, are easily determined, or can be estimated [16–18]. Ideally the physicochemical properties for compounds to be estimated will be more reliable if their descriptor values fall within the descriptor space represented by the calibration compounds used to construct the model.

The classic approach for the measurement of descriptors employs chromatographic, liquid-liquid partition, and solubility data to set up a series of equations that are solved

TABLE 9.6 Descriptors for Cholesterol ($V = 3.4942$ and Assigned Values $E = 1.362$, $S = 1.084$, $A = 0.235$, and $B = 0.504$) Estimated From Chromatographic and Liquid-Liquid Partition Constant Measurements in Totally Organic Biphasic Systems

Biphasic System	Experimental	Calculated
n-Heptane-propylene carbonate	0.775	0.805
n-Heptane-trifluoroethanol	2.332	2.340
n-Heptane-dimethylformamide	−0.261	−0.264
n-Heptane-dimethyl sulfoxide	1.114	1.181
n-Heptane-methanol	0.729	0.637
n-Hexane-acetonitrile	0.672	0.603
Isopentyl ether-dimethyl sulfoxide	1.514	1.482
Isopentyl ether-propylene carbonate	1.081	1.135
Triethylamine-dimethylsulfoxide	1.482	1.520
Octan-1-ol-water		11.27
n-Heptane-water		11.74
Dichloromethane-water		12.57
Diethyl ether-water		11.81
Ethyl acetate-water		11.14

The values for aqueous-organic solvent partition constants are model estimates.

simultaneously to estimate the descriptor values. Aqueous-organic biphasic systems have become a common component in these calculations for estimating the S, A, and B descriptors since several suitable aqueous biphasic systems with relatively large values for the complementary system constants are available [16]. A problem arises, however, for compounds of low-water solubility and for compounds unstable in water. These compounds have partition constants that are inaccessible to routine laboratory techniques. In this case, totally organic biphasic systems represent a suitable alternative and facilitate the measurement of descriptors for compounds such as organosiloxanes [67, 68], fragrance compounds [69], plasticizers (phthalate and alkyl esters) [70], polycyclic aromatic compounds [71], steroids, triglycerides, and hydrogen-bonding compounds [6, 72]. A case in point is the estimation of descriptors for cholesterol, Table 9.6. Partition constants in the totally organic biphasic systems fall into the range log $K_p = 0.60$–2.50 and are amenable to measurement by typical laboratory techniques, while the estimated values for the aqueous biphasic systems fall into a range log $K_p = 11.5$–12.6 and would be exceedingly challenging to measure. In addition, typical poly(siloxane) and polyethylene glycol stationary phases used for gas chromatography are non-hydrogen-bond

acids, and the totally organic biphasic systems are particularly useful for estimating the B descriptor for compounds of low-water solubility.

An important application of descriptors is their use to predict equilibrium properties for compounds in systems for which experimental values are unavailable and perhaps experimentally inaccessible. This includes the estimation of properties such as solubility, partition constants, biopartition constants, environmental fate modeling, and nonspecific toxicity [18, 73–75]. The octan-1-ol-water partition constant (log P_{OW}) is widely used as a measure of lipophilicity and to estimate the distribution and fate of compounds in biological and environmental systems. On account of the large chemical inventory, only a small fraction of known compounds have experimental log P_{OW} values, and for some compounds, it may not be possible to determine experimental values by routine measurements. Gas chromatography and partition constants in totally organic biphasic systems have been used to estimate log P_{OW} for compounds of low-water solubility. The correlation plot in Fig. 9.4 is representative of results obtained for estimating log P_{OW} for compounds of low-water solubility. The coefficient of determination for the correlation model with experimental values is 0.993, and at the 95% confidence level, the slope includes 1 and the intercept 0. Thus there is no bias in the estimated values for these difficult-to-measure log P_{OW} values, and this approach is a viable option when direct measurements are difficult. A similar approach can be used to estimate the solubility of organosiloxanes, phthalate esters, and polycyclic aromatic compounds of low-water solubility (Table 9.7) [67, 70, 71]. The general approach presented earlier is applicable to hundreds of processes that can be described by a linear free energy relationship model for physicochemical, biopartition, or environmental systems [18, 73–75].

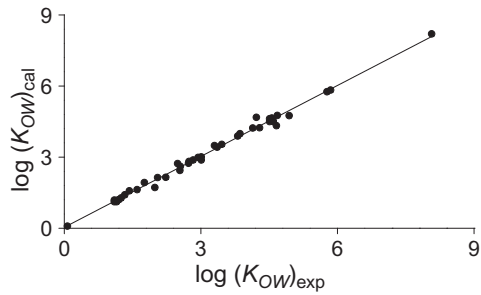

Fig. 9.4 Correlation plot of experimental and estimated octan-1-ol-water partition constants (log K_{OW}) for compounds of low-water solubility (phthalate esters, polycyclic aromatic compounds, steroids, terpenes, etc.). The estimated partition coefficients are calculated from retention factors determined by gas chromatography and partition constants in totally organic biphasic systems.

TABLE 9.7 Comparison of Experimental and Estimated Aqueous Solubility for Some Low-Solubility Nonelectrolytes

	$-\log S_W$ (mol/L)	
Compound	**Experimental**	**Calculated**
Hexamethyldisiloxane	5.24	4.95
Octamethyltrisiloxane	6.84	6.39
Decamethyltetrasiloxane	7.66	7.57
Decamethylcyclopentasiloxane	7.34	8.17
Di-n-butyl phthalate	4.40	4.23
Di-n-octyl phthalate	7.88	7.64
Butyl benzyl phthalate	5.04	4.98
Phenanthrene	5.21	4.95
Benzo[*e*]pyrene	7.69	7.14
Dibenz[*a*,*h*]anthracene	8.79	8.09
1-Nitropyrene	7.62	6.43
3,3′-Dichlorobenzidine	4.91	4.46
Carbazole	5.27	4.58

9.4 Conclusions

Totally organic biphasic systems cannot be described as new but are certainly underutilized. The reliance on extraction methods employing large volumes of solvent historically contributed to this situation on account of the higher purchase and disposal costs of less common organic solvents. In addition, a number of potentially useful solvents for the formation of totally organic biphasic systems are only poorly suited to commonly used preconcentration methods employing evaporation. Modern approaches to liquid-phase extraction typically utilize small solvent volumes and minimize preconcentration steps such as gas blowdown or distillation facilitating the use of a wider range of organic solvents for extraction purposes. Totally organic biphasic systems are complementary to aqueous biphasic systems and essential for the fractionation of compounds or matrices of low-water solubility and are the only approach possible for the extraction of compounds unstable to water. In this chapter, we have summarized the available literature for the totally organic biphasic system to provide a starting point for their wider exploitation in liquid-phase extraction for problems poorly handled using aqueous biphasic systems. Totally organic biphasic systems are also suitable for determining descriptors for compounds difficult to

measure by conventional approaches enabling subsequent estimation of physicochemical properties otherwise only accessible to direct measurement with considerable difficulty.

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Chapter 10

Countercurrent Chromatography—When Liquid-Liquid Extraction Meets Chromatography

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10.1 Principle, Instrumentation, Basic Parameters and Terms

Countercurrent chromatography (CCC) is an all liquid-based sample separation technique that elegantly links the principle of liquid-liquid extraction with the separation power of chromatographic techniques. Due to its (semi)preparative nature, CCC is preferably used in the field of natural product isolation and for the initial separation of raw extracts and complex mixtures.

10.1.1 History and principle of CCC

CCC takes advantage of the distribution of sample compounds between two immiscible liquid phases according to Nernst's distribution law [1]. Basically, CCC is related to liquid-liquid extractions (LLE) in a separatory funnel. The fundamental parameter of LLE and CCC is the partition constant P (typically specified as $P_{U/L}$) of a compound in a two-phase solvent system (Eq. 10.1):

$$P_{U/L} = \frac{c_U}{c_L} \tag{10.1}$$

with c_U being the concentration of the compound in the upper phase and c_L being the concentration of the compound in the lower phase [2].

The two immiscible liquid phases can be obtained by combinations of two or (typically) more components, and the result is named *solvent system* (Section 10.2). There are two basic features in which CCC differs from LLE. First of all, CCC is a chromatographic technique that is based on many settings of equilibriums (while LLE is based on only one). This is achieved by the transport of one phase (mobile phase), while the other phase is kept stationary in the "column" by means of centrifugal forces (see succeeding text). Second, LLE aims to transfer the analyte exclusively into one phase (while other compounds in the sample should preferably stay in the other phase). In contrast for CCC an equal distribution of analytes between both phases is more beneficial [3].

For illustration an analyte with $P_{U/L} < 0.01$ (virtually) partitions solely into the lower phase and analytes with $P_{U/L} > 100$ solely into the upper phase (i.e., the goal of LLE). Solvent systems resulting in such $P_{U/L}$ values are useless in CCC. In these extrema the analyte either would move very fast through the column (if it is predominantly soluble in the mobile phase) or would not be eluted (if the analyte is predominantly soluble in the stationary phase). Hence the distribution of analytes between

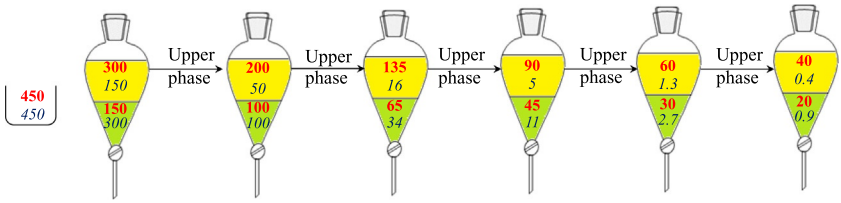

Fig. 10.1 Scheme of the repeated liquid–liquid fractionation ($n = 6$) of two compounds with $P = 2$ (compound A, red/bold) and $P = 0.5$ (compound B, black/italic), respectively, in a biphasic solvent system. Numbers (selected to result in quotients with full numbers, values slightly rounded) refer to the abundance ratio (equal abundance at start) from step to step. After each equilibration, upper phase is transferred to the next flask that already contained lower phase (without analyte). After six steps the purity of compound A is increased to 99%, but the yield is only ~9%.

the two liquid phases should be close to one ($P_{U/L} = 1$) so that they partition equally between both phases. In practice, best CCC separations are obtained within the so-called sweet spot of $P_{U/L}$ ~0.4–2.5 [4]. Hence the thorough selection of the biphasic solvent system is the major task in CCC (Section 10.2).

The limited separation power of LLE of one theoretical plate [5] can be improved by performing several LLE procedures in separatory funnels with fresh aliquots of the extracting solvent (comparable with the mobile phase) that results in extremely tedious (and laborious) procedures. Fig. 10.1 shows the result of six LLE steps with the same amount of two compounds A and B (450:450) having $P_{U/L}$ values of 2 and 0.5, respectively, in a (not specified) solvent system. Poured into a separatory funnel, the A/B ratio will be 2 (300:150) and 0.5 (150:300) in upper and lower phase. When only the upper phase is transferred to the next separatory funnel that contains the same volume of lower phase, the A/B ratio will be 4 (200:50) and 1 (100:100) (Fig. 10.1). Repetition of the procedure for another four times leads to an A/B ratio of 100 (40:0.4) and a purity of 99% for A in the upper phase (Fig. 10.1). Despite the excellent purity a major disadvantage of repeated LLE is that the yield drops to ~9% due to the partial distribution of A into the lower phase. As CCC represents a continuous process, the yield is quantitative, and the separation power is better. Furthermore, CCC is fully automated, so excessive lab work can be avoided.

The first successful attempt to overcome the drawbacks of LLE by automation was introduced by Lyman C. Craig in the 1940s. Craig serially linked a high number of glass chambers (cells) in a frame and developed a multichamber LLE apparatus (Fig. 10.2A) [6–8]. After LLE in the first glass chamber, one phase is mechanically transferred into the second tubes by tilting the tubes, a.s.o. Since the early 1950s the so-called Craig apparatus or countercurrent distribution

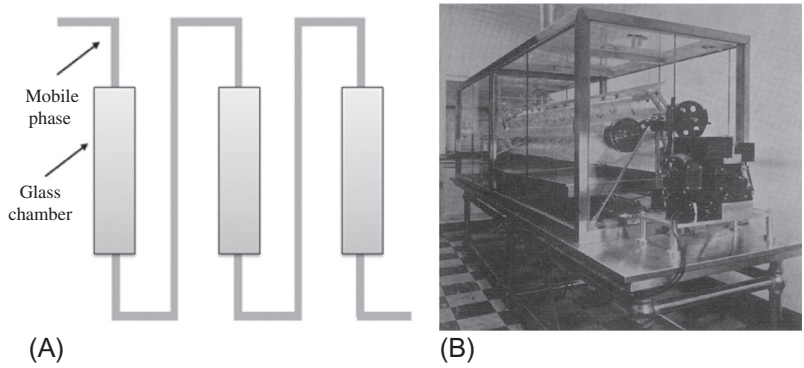

Fig. 10.2 (A) Scheme of a multichamber liquid-liquid extractor (Craig or countercurrent distribution apparatus) with three connected glass tubes and (B) a photo of a Craig apparatus with 220 glass tubes arranged on a frame and connected in series. *(Reproduced from Craig LC, Hausmann W, Ahrens Jr. EH, Harfenist EJ. Automatic countercurrent distribution equipment. Anal Chem 1951;23:1236–44.)*

(CCD) apparatus was used for the purification of peptides and small molecules (e.g., penicillin, Fig. 10.2B) [9, 10]. Despite the perfect partition equilibrium that was obtained between the two liquid phases, the number of theoretical plates (one per chamber) was low [11]. This necessitated the use of >200 glass chambers in the Craig apparatus so that (i) separation times were very long (up to many days) and (ii) solvent consumption was high [6, 12]. Consequently the invention and immediate success of solid support-based (partition) liquid chromatography (LC) by Martin and Synge in 1941 [13] effectively superseded CCD that became a niche field for very few applications such as peptide purification [14].

In the late 1960s Yoichiro Ito introduced a new instrument that featured a coil planet centrifuge with a rotating, sealed, helical tubing in which the mobile phase formed droplets passing through the stationary phase (Fig. 10.3) [15–17]. This separation technique was initially named liquid-liquid partition chromatography but subsequently became known as CCC in 1970 [18]. CCC was more efficient than CCD and also benefited from the avoidance of irreversible adsorption of sample material preventing degradation of (macromolecular) analytes. For instance, separation of lymphocytes according to their size and density had not been achieved with other techniques by this time [19]. However, the novel method still suffered from a number of different drawbacks that inhibited its breakthrough (i.e., low retention of the liquid stationary phase in the coil, laminar peak broadening, and limited mixing and mass transfer rates between the liquid phases) [15].

Most of these drawbacks were overcome by Ito's second masterstroke, that is, the invention of the so-called J-type setup for the coil planet centrifuge [20, 21]. This planetary setup takes advantage of two processes whereof the first pertains to the

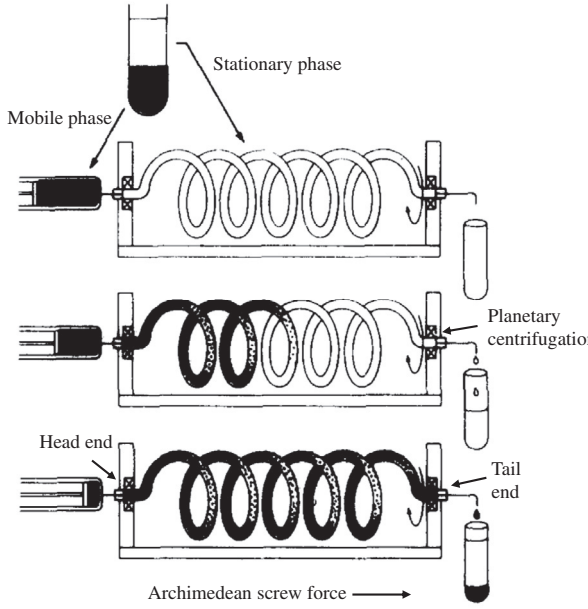

Fig. 10.3 Scheme of the initial coil planet centrifuge that consisted of a rotating coiled tube that was used for the separation of subtypes of lymphocytes. *(Reproduced from Ito Y. Countercurrent chromatography. J Biochem Biophys Methods 1981;5:105–29.)*

transport and the second to the separation of analytes (Section 10.1.2). Due to the use of two axes of rotation, CCC belongs to the so-called hydrodynamic methods, while those with only one axis (as found in centrifugal partition chromatography, CPC) are named hydrostatic methods [17].

In the original J-type scheme, polytetrafluoroethylene (PTFE) tubing was wound in several layers (coaxially) around a coil holder hub (Fig. 10.4). This new CCC device proved to be robust and simple and could achieve efficient separations within a few hours using mobile phase flow rates in the (low) milliliter-per-minute range. This gain in time meant a tremendous improvement compared with the old CCD instruments, and consequently the instrument was termed high-speed CCC (HSCCC). Also the J-type setup enabled rapid mixing and settling steps of the biphasic solvent system inside the tube that resulted in good stationary phase retention. The most striking and crucial difference between CCD and CCC is that Ito transferred the discontinuous process in CCD into the continuous process of CCC. Discontinuous processes are always time-consuming (see Soxhlet extractions), and Ito's new CCC method made liquid-liquid chromatography competitive again with other LC methods.

Soon after Ito's invention the first commercial HSCCC instrument was marketed in the United States [22]. Contemporary commercial instruments are still based on

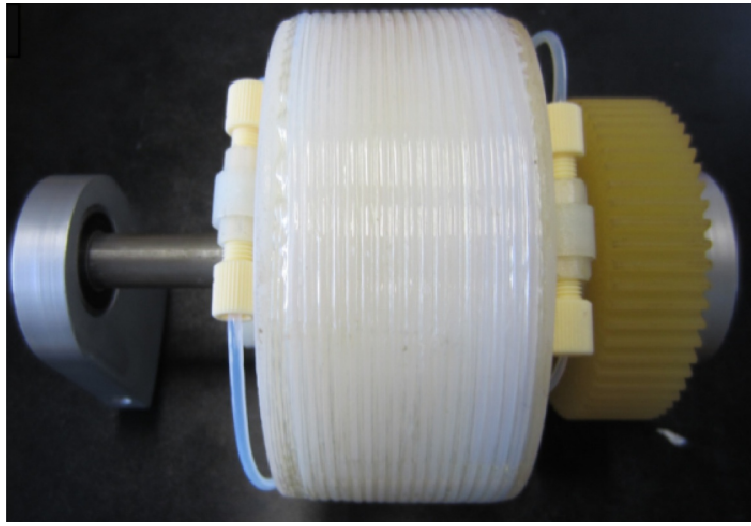

Fig. 10.4 Photo of a HSCCC coil with PTFE tubing (105-mL volume) wound in multiple layers around a coil holder hub. *(Courtesy of M. Englert.)*

the J-type scheme of Ito, but they are equipped with modern bobbin designs and offer the opportunity to generate and tolerate higher centrifugal forces than the first prototype [23]. Due to the universal use of the J-type (or closely related designs), there is no longer a need to distinguish between HSCCC and CCC. Eventually, the term CCC replaced HSCCC (similarly as GC has replaced HRGC because of its almost exclusive use since the 1980s). Ito's original HSCCC instrument of 1981 included one multilayer coil equipped with a 45-m and 1.65-mm internal diameter PTFE tubing column that corresponded with a coil volume of ~100 mL which was rotated at a speed of 1000 rpm [20]—a setup not very far from the dimensions used in modern commercial instruments.

The centrifuge (Section 10.1.2) is the heart of the modern CCC system. The other components of lab-scale CCC systems are the same as in a (semi)preparative high-performance liquid chromatography (HPLC) system, wherein the CCC centrifuge substitutes the HPLC column (Section 10.1.2, Fig. 10.5). Hence modern CCC systems consist of a pump for solvent delivery, a low-pressure injection valve equipped with a sample loop, a suitable detector with a data-recording unit, and a fraction collector. Besides the centrifuge, there is only one additional component compared with HPLC systems, that is, a switching valve for *head*-to-*tail* or *tail*-to-*head* mode selection (Section 10.1.2), which enables solvents to be pumped from both sides into the system (which could also be beneficial if present in HPLC systems). Typical CCC instruments (with HPLC periphery) are designed for injections of (up to) ~1 g sample. Coil volumes are in the range of 50–300 mL, which is equal to the solvent

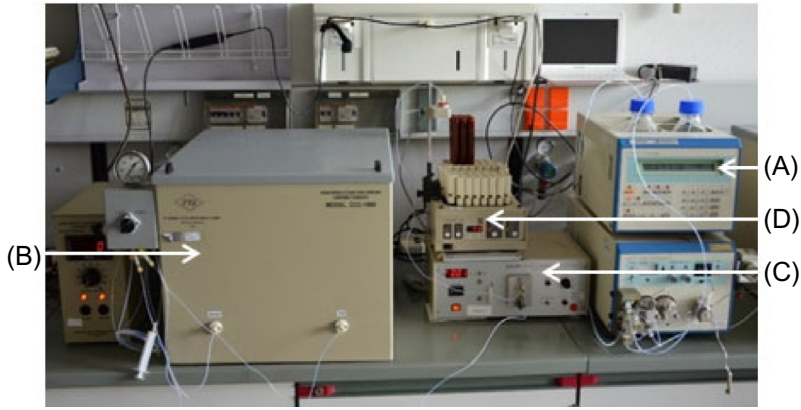

Fig. 10.5 Photo of the CCC-1000 setup with (A) pump; (B) CCC centrifuge; (C) UV/Vis detector; and (D) fraction collector. *(Courtesy of M. Englert.)*

consumption to elute a compound with $P_{U/L} = 1$ as this mathematically corresponds exactly with one coil volume (Section 10.1.3.1). Based on the principles of LLE, CCC separations and elution volumes are easy to perform and predict (Section 10.1.3.1). Hence upscaling of an existing CCC method is easily achieved by increasing the coil dimensions, as wider inner diameters are increasing the sample capacity [24, 25]. Large CCC machines were built at the Brunel Institute for Bioengineering (London, United Kingdom) with coil volumes of 4.6 L and 18 L that enabled the injection and isolation of kilogram amounts [26, 27].

CPC, a sister technique to CCC, was introduced by Murayama in 1982 [28]. The hydrostatic CPC technology (one axis of rotation, see previous text) was optimized to fulfill the criteria for the pharmaceutical industries, and Margraff et al. developed a 25-L machine suited to separate large amounts of sample [29]. Lately, industrial CPC instruments were announced to manage isolations of kilogram amounts per day [30]. In CPC instruments the coils are substituted with a rotor that consists of metal disks stacked on each other. Each disk carries punched chambers (~0.1 mL in a semipreparative instrument [31]) that are serially connected by narrow bore ducts as are the individual disks. The rotor is mounted on the axis and rotated to typically higher speeds than for CCC instruments. However, in the following, this article will focus on CCC only.

Both the low solvent consumption and the good predictability of separations have contributed to the fact that CCC is increasingly used especially for the isolation of natural products from (plant) extracts. The number of matches on the CCC term in Scopus, starting from the introduction of CCC in 1981, verifies the steadily increasing interest and number of publications containing the term CCC (Fig. 10.6).

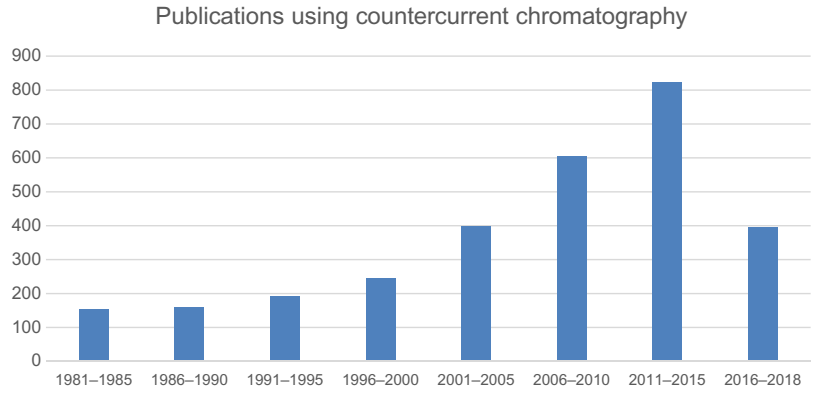

Fig. 10.6 Number of matches for the terms "countercurrent chromatography," "counter current chromatography," and "counter-current chromatography" in Scopus in 5-year periods since 1981 and for the publications from 2016 to mid-2018.

10.1.2 The CCC centrifuge and operation modes

The centrifugal field of modern CCC instruments provides the required retention of the liquid stationary phase in the coil, while mobile phase can be transported through it [32]. The planetary motion of the centrifuge is generated by means of a fixed central gear that is driven by an electric motor (Fig. 10.7). Laterally completed by flanges the bobbin is equipped with multiple layers of PTFE or stainless-steel tubing wound in

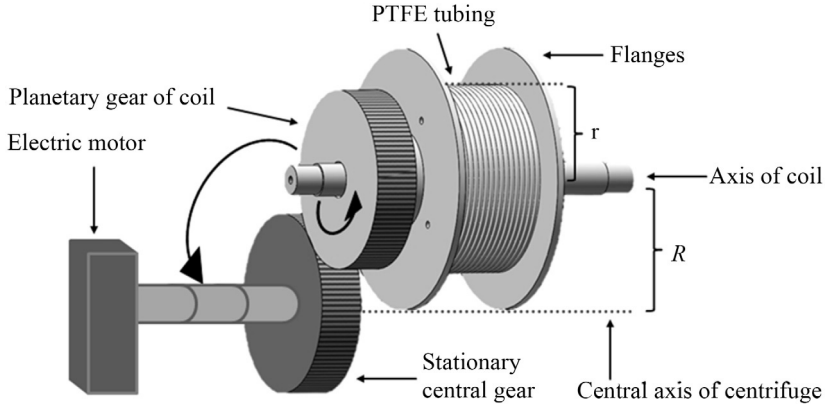

Fig. 10.7 Scheme of a CCC multilayer coil in a J-type instrument with *r*, the distance between the axis of the coil and the outer layer of tubing, and *R*, the distance of the central axis of the centrifuge to the axis of the coil. The direction of rotation of the coil and the revolution of the coil around the stationary central gear are the same.

several layers to a coil [33]. The 1:1 coupling between central and planetary gear results in the same rotational speed and direction of rotation of bobbin and rotor [20]. Nontwisting connections of rotating bobbin(s) and the static solvent reservoir and detector during the planetary motion are obtained by so-called flying leads [34, 35]. These are short coupling tubes which are additionally protected with seath tubing to prevent damage. Due to the fast planetary movement, these connections are the weakest part of the CCC system (Fig. 10.8) [21]. In practice, strong forces arise at these sensitive connections so that they may occasionally break (duration ~1 or 2 years), depending on the conditions and frequency of use. Typically, (broken) flying leads can be changed within minutes by users, but practical training is recommended.

When the coil is filled with a liquid and rotated, the liquid is pushed toward one end of the coil, and this end is by definition named *head*. This motion is caused by the Archimedean screw effect [36, 37]. In general the direction of the flow is determined by the direction of the rotation that is usually invariant in a CCC centrifuge. When two immiscible phases of a solvent system are introduced into a rotating coil, the phase with lower density will be moved toward *head*, while the heavier phase is pushed toward the opposite end that is named *tail* [20]. Under the impact of the centrifugal field, one phase can be kept stationary if the other phase is pumped in the direction that corresponds to the Archimedean screw effect. In practice the coil is first filled with stationary phase, and then mobile phase is pumped in its natural direction. In this way the retention of the stationary phase can be kept high that is essential for separations (Section 10.1.3) [20]. Hence, if upper phase—the one with lower density—is used as mobile phase, it has to be introduced at the *tail* end (and

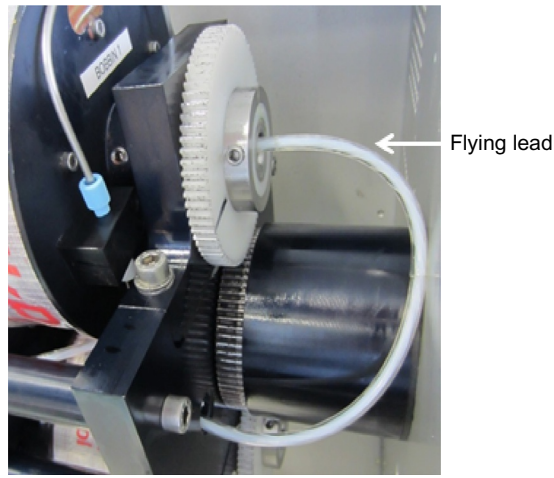

Fig. 10.8 Photo of a flying lead with a short coupling tubing inside the sheath tubing. *(Courtesy of M. Englert.)*

TABLE 10.1 Operation Modes of a CCC Apparatus

Properties	Head-to-Tail	Tail-to-Head
Mobile phase	Lower phase	Upper phase
Stationary phase	Upper phase	Lower phase

then will be moved toward the *head* end) to ensure that the lower phase will be kept stationary by the forces arising from the planetary motion [36–38]. This mode is named *tail*-to-*head* mode (upper phase mobile). Vice versa, if the lower (denser) phase is mobile, it should be introduced from the head end (*head*-to-*tail* mode) (Table 10.1).

The second effect of the planetary motion generates areas of low- and high-centrifugal force fields with varying directions that act on the biphasic solvent system (for details, see description in [3]). When the centrifugal force is high, the two liquid phases are separated, whereas when the centrifugal field is low, the two phases are vigorously mixed. This process of mixing and settling occurs in each rotation cycle of the centrifuge [38, 39]. Hence the planetary motion generates mixing and settling zones within a coil that are responsible for the separation and achievable resolution. Importantly the hydrodynamic behavior is depending on the ratio between the distance of the axis of the coil and the outer layer of tubing (r) and the distance of the central axis of the centrifuge to the axis of the coil (R) (Fig. 10.7). This ratio is expressed in the form of the β value (Eq. 10.2). β-Values should preferably be between 0.5 and 1 since this range provides hydrodynamic stability and good retention of the stationary phase for a broad range of solvent systems with different physicochemical properties [40–44]:

$$\beta = \frac{r}{R} \tag{10.2}$$

The typical rotation speed of a preparative CCC instrument is about 850–2300 rpm [45, 46]. Faster rotation speeds of the coils typically lead to a higher volume of the stationary phase retained in the CCC. Compared with CCC, car wheels (~50-cm diameter) turn at ~1000 rpm at a speed of 100 km/h. Accordingly the electrical motors required for CCC are standard and not particularly demanding.

Due to the strong forces in the planetary rotating centrifuge, CCC coils must be counterbalanced. In its initial prototype, Ito actually used a counterweight for this purpose [15]. Later on and up until today, one or two additional coils are used in commercial instruments. These coils must not necessarily have the same volume but rather the same weight. Likewise, some instruments are equipped with analytic and (semi)preparative coils with the first one being suggested for method development because solvent can be saved in this way. However, the setup of CCC instruments is usually inflexible, and this can be inconvenient for users. For instance, compounds that could be separated with the volume of one coil must still be run through the whole system (all coils). As will be shown later in Section 10.3, time

can be saved in such a situation by the application of different modes. However, solvent consumption is higher without benefit. Therefore different options have been reported. For instance, Berthod et al. used only one coil in a three-coil CCC machine [11]. The way of modification was not reported, but most likely the exit of a flying lead connection between two coils (Fig. 10.8) was used to lead the effluent directly to the detector. In any case, unused coils also need to be filled with solvent to properly balance the system. Englert constructed self-made bobbins with Teflon tubes corresponding to one-third of the volume of the standard system [47]. Depending on the separation problem, the small or larger coils could be installed in the centrifuge. The option to freely select the number of coils (and hence the volume) to be used is also offered in at least one commercial instrument. Depending on the problem the user can perform a CCC run on one, two, three, or four coils by individually connecting them in series [47]. This setup is also convenient for the implementation of special modes (Section 10.3).

10.1.3 Stationary phase retention (S_f value) and prediction of elution times (volumes) from *P* values

The volume ratio of mobile and stationary phase inside the CCC column is typically expressed by means of the stationary phase retention (S_f value) (Eq. 10.3):

$$S_f = V_s / (V_s + V_m) = V_s / V_c \tag{10.3}$$

with V = volume and indeces s = stationary phase, m = mobile phase (both in the coil), c = coil volume, and sd = displaced stationary phase. S_f is usually given in S_f values in percent (Eq. 10.4):

$$S_f \text{value}\ (\%) = V_s/V_c \times 100 \tag{10.4}$$

In stable solvent systems, S_f values are frequently determined as follows: First the coil is filled with stationary phase. Then, rotation is started, and mobile phase is pumped into the system, and the effluent from the coil is collected in a graduated flask until mobile phase breaks through. Then or a little later, the volume of stationary phase displaced (V_{sd}) is measured, and together with the known coil volume (V_c), the S_f value can be determined (Eqs. 10.5 and 10.6):

$$V_s = V_c - V_m = V_c - V_{sd} \tag{10.5}$$

$$S_f \text{ value}\ (\%) = (V_c - V_{sd})/V_c \times 100 \tag{10.6}$$

Alternatively, S_f can be determined after the separation by clearing all solvents from the coil (typically by flushing with nitrogen) followed by volumetric measurements of V_s. Ideally, S_f values (%) should be as high as possible and typically range from 95% down to 70% [3], even though separations may also be achieved with lower S_f values (%).

10.1.3.1 Calculation of Elution Volumes From P Values and S_f Values

The elution volume of the analyte (EV) is proportional with P (Eq. 10.7):

$$EV = V_m + PV_s \tag{10.7}$$

Insertion of Eq. (10.3) allows V_s to be substituted by $S_f V_c$ (Eq. 10.8):

$$EV = V_m + PS_f V_c \tag{10.8}$$

Because of the constant coil volume, the elution volume and the volume of mobile phase can be related to $V_c = 1$ (Eq. 10.9):

$$EV/V_c = V_m/V_c + PS_f \tag{10.9}$$

Plots of P value over elution volume (EV) result in straight lines with the slope increasing with S_f (Fig. 10.9). For analytes with $P = 1$, Eq. (10.6) is reduced to $EV = (V_m + V_s) = V_c$. Hence they elute exactly after one coil volume from the

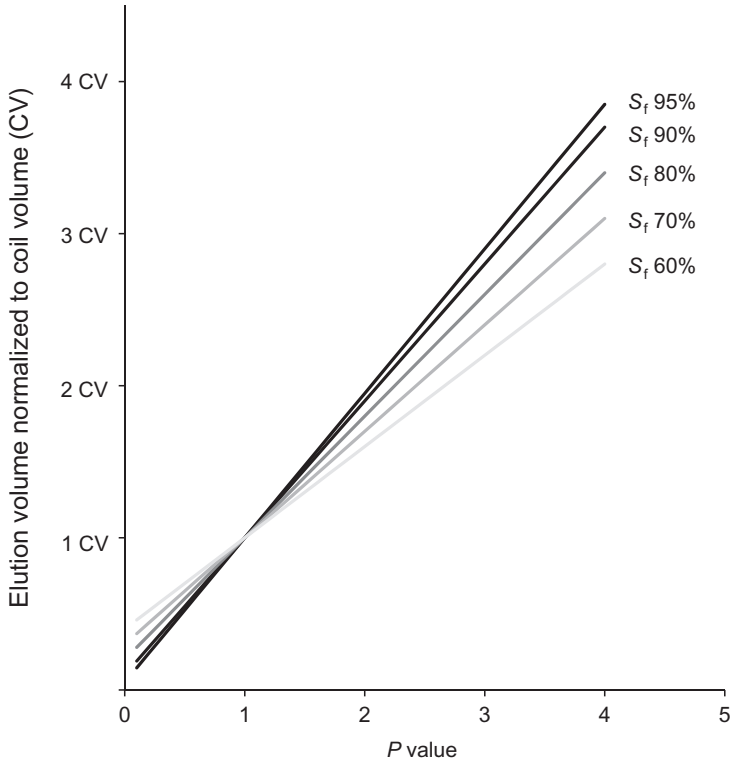

Fig. 10.9 Plot of S_f values in dependence of elution volume (expressed in factors of the coil volume, CV) and P value.

CCC system, irrespective of the S_f value (Fig. 10.9). Due to the interdependency of V_s and V_m, elution volumes of all other P values are dependent of the S_f value. For instance, compounds with $P = 2$ require between ~1.4 ($S_f = 60\%$) and 1.9 ($S_f = 95\%$) coil volumes for their elution (Fig. 10.9).

From the higher slope of the lines with higher S_f values, it follows that high S_f values extend the corresponding elution range of analytes in the sweet spot. For instance, the sweet spot covers 52–195 mL at 95% S_f but only 70–160 mL at 60% S_f (Fig. 10.9). Despite additional parameters that have an impact on peak width, high S_f values provide a better dispersion of peaks. The impact of S_f values could be the reason why some experts define the sweet spot between 0.5 and 2 [3] and others between 0.4 and 2.5 [4]. Generally, higher S_f values are in favor of the resolution between peaks (Eq. 10.10):

$$R_s = \frac{2 \bullet (EV_2 - EV_1)}{w_{b2} + w_{b1}} \tag{10.10}$$

with EV being the elution volume and w_b being the peak width of the second (index 2) and first (index 1) eluting peaks. Considering typical peak widths in CCC, approximately seven compounds can be baseline-separated with the sweet spot in a solvent system with a suitable S_f value. Fig. 10.10 shows the CCC separation of six peaks that all eluted into the sweet spot [48]. The chromatogram also gives an impression of the steadily increasing peak width in CCC. In either case, high S_f values will improve the resolution.

S_f values of solvent systems can roughly be predicted from settling times, that is, the time it takes until a mixed solvent system separates into two distinct phases when untouched [3]. The settling time can be estimated by placing the same (small) volume of both phases in a test tube and shaking it vigorously. Then the time is measured

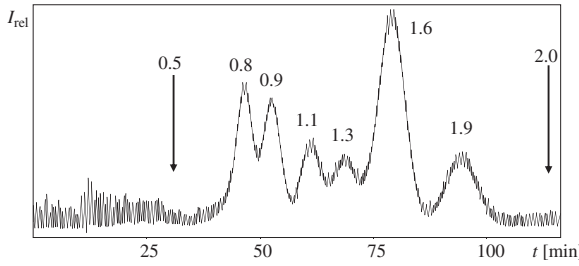

Fig. 10.10 CCC/UV chromatogram of six peaks originating from tocochromanols (solvent system *n*-hexane/BTF/acetonitrile). *(Modified from Vetter W, Müller M, Sommer K, Schröder M, Hammann S, Development of equivalent chain length (ECL) rules for lipid compounds.* J Chromatogr A *2019.* https://doi.org/10.1016/j.chroma.2019.04.042)

until both phases have settled again. Settling times of <20 s indicate that the S_f value of this solvent system will be appropriate [3]. Typically, S_f values of solvent systems can be improved by reducing the flow rate of the mobile phase and by increasing the revolution speed of the CCC centrifuge [3]. Accordingly, higher mobile phase flow rates can be applied at increased rotation speeds [49, 50]. Hence the high rotation speeds of modern instruments of up to 2300 rpm (271 g) facilitate the application of higher flow rates while retaining sufficient stationary phase and thus enable separations in minutes rather than hours at the same resolution [51–53].

The absence of a solid phase in CCC enables the injection of crude plant extracts and solid material into the instrument [54–56]. Special cleaning steps between the runs are usually unnecessary, and the whole liquid content can be pushed out, for example, by applying nitrogen (~5-bar pressure) at the inlet of the CCC.

10.2 Solvent Systems

The most important variable for method development is the solvent system. This is achieved by mixing the individual components of a biphasic system (even three-phase solvent systems can be used in CCC, Section 10.3.1.5). Hence both the stationary phase and mobile phase are flexible but linked to each other, and *both* have to be modified to find a suitable solvent system for a given separation problem. Likewise the requirement that both phases need to be liquid does not limit the number of components. Biphasic mixtures can be created with two compounds (binary mixtures), but most suitable solvent systems mostly contain three (ternary mixtures), four (quaternary mixtures), and even more components [57]. Notably, components used in solvent systems need not necessarily be liquids but can also be solids that (preferably) dissolve in one phase. Hence the liquid phase can be considered as a homogeneous space that is defined by physicochemical properties (e.g., density and viscosity).

After mixing of the components and settling to a biphasic solvent system, specific proportions of each solvent can be found in the upper and the lower phase due to the mutual solubility of the solvents. For example, in an equilibrated *n*-hexane/acetonitrile solvent system, 0.5% acetonitrile will be found in the upper phase and 1.2% *n*-hexane in the lower phase [58]. Moreover the solubility of the components in the "other" phase depends on the temperature. Hence an increase in temperature during a CCC run will alter the composition of both phases that is typically a disadvantage (and can even lead to monophasic systems). Accordingly, any change in one phase will also alter the other phase, and the phases of a solvent system cannot be treated independently [3].

Selection or creation of a suitable biphasic solvent system is the most important task for a new CCC separation/isolation problem that often takes up to 90% of the

total time [59]. Typically a first idea can be derived from searching the literature for similar compounds or by estimating the polarity from the structure. In practice the most frequently used solvent system selection method for a target compound is a partitioning study called the shake-flask test [3]. For this purpose an aliquot of the sample is dissolved in equal volumes of upper and lower phase of the biphasic solvent system. After vigorous shaking and settling of the phases, the same volume of each layer is removed, and the concentration of the compound(s) of interest is determined, for example, by LC, GC, UV/Vis spectroscopy, or any other quantitative method [60]. The accuracy of this determination of $P_{U/L}$ values is crucial for the prediction of the subsequent elution volume of the analyte in CCC. Alternatively the suitability of a solvent system can be estimated from thin-layer chromatography (TLC) measurements [4]. In a TLC approach known as the "generally useful estimate of solvent systems" (GUESS) method, the retention factor (R_f) of the analyte on a silica TLC plate using the lesser polar phase of the CCC solvent system is correlated with the P value [4, 60]. The GUESS method aims to ascertain the suitable polarity range of the solvent system. In addition, analytic CCC instruments equipped with very small coils can be used for the required tests [61–63].

The preparative nature of CCC implies not only a rather even distribution of the analyte in the solvent system but also its good solubility [64]. Hence the polarity difference of the two phases of the solvent system should not be large but as similar as possible (without becoming monophasic). Therefore appropriate solvent systems need to dissolve the target compound in both phases. When the $P_{U/L}$ value of the analyte is not in the anticipated sweet spot range the solvent system needs to be modified by adjusting its composition. For instance, if the $P_{U/L}$ value is too high (here, stronger partitioning into the less polar upper phase), the polarity of the solvent system should be shifted into the desired direction. Typically, one polar and one immiscible, less polar solvent are a good starting point to which a third component can be added (Table 10.2). The biphasic solvent system can also be modified by the addition of a so-called modifier (e.g., buffer salts) to adjust the pH value [2, 65] by complexing agents [66–68] or by surfactants [69]. Yet, nonsystematic approaches are usually cumbersome and inefficient. For this reason a more goal-driven approach is to group several solvents logically in a so-called solvent system family [70]. Typically, solvent families consist of four or five solvents that form stable biphasic mixtures in a wide range of proportions. The resulting solvent systems can be numbered and grouped in tables. For an easier use, they are abbreviated with one, two, or three letters. Examples for solvent system families (and solvent abbreviations) are the *n*-hexane/ethyl acetate/methanol/*n*-butanol/water (HEMBWat) family [71], the ethyl acetate/*n*-butanol/water (EBWat) family, the chloroform/methanol/water (ChMWat) family [4], the so-called Arizona family (*n*-heptane/ethyl acetate/methanol/water, HpEMWat), and the *n*-hexane/ethyl acetate/methanol/water (HEMWat) family [72].

TABLE 10.2 Examples for Solvents of Different Polarity Used in the Best Solvent Approach in CCC to Generate Two-Phase Solvent Systems [64]

Less Polar Solvent	Best Solvent	More Polar Solvent
n-Heptane	Methanol	Water
Toluene	Methanol	Water
tert-Butyl methyl ether	Acetonitrile	Water
Ethyl acetate	Acetonitrile	Water
Ethyl acetate	*n*-Butanol	Water
n-Heptane	Tetrahydrofuran	Acetonitrile
n-Heptane	Ethanol	Acetonitrile

The HEMWat system consists of four components, two of which are rather nonpolar (*n*-hexane and ethyl acetate) and two are rather polar (methanol and water). Different compositions of a solvent family are usually arranged in tables with the least polar composition being listed on top. In the HEMWat system, this is *n*-hexane (the least polar of the nonpolar components) and methanol (the least polar of the polar components) in a 1:1 mixture (Table 10.3). Typically, the solvent system composed of equal amounts of all solvents (e.g., HEMWat 0, Table 10.3) serves as an excellent starting point when searching for the best "member" of the solvent system family for a specific separation problem. Then the share of both *n*-hexane and methanol is gradually reduced (or enhanced) in favor of the other two components, that is, ethyl acetate and water (Table 10.3). This leads to a broad range of polarity that increases from top to bottom of the table. Especially in the central medium range, further compositions are added where only either the polar or the nonpolar components are changed (i.e., ±4, ±2, and ±1; Table 10.3). This procedure results in a total of 17 compositions of the HEMWat family, which are labeled with numbers representing the continuously varying polarity, where −8 represents the least polar system and +8 the most polar system of the family (Table 10.3) [57, 73]. Without previous knowledge the user may start with number 0 in Table 10.3. Depending on the result, subsequent adjustment is obtained by moving up or down the numbers until the $P_{U/L}$ is appropriate. The column on the right of Table 10.3 also lists several natural compounds and the corresponding solvent system that provides P values in the sweet spot range.

Next to appropriate $P_{U/L}$ values and high solubility of analytes, the solvent system should also provide a high retention of the stationary phase (Section 10.1.3) and stability during the CCC run. The S_f value mainly depends on the physicochemical properties of

TABLE 10.3 Numbering of the *n*-Hexane/Ethyl Acetate/Methanol/Water (HEMWat) Family With Relative Volume Portions of the Solvents and Examples of Compounds of the So-Called GUESS Mix [4, 70]

HEMWat System Number	*n*-Hexane	Ethyl Acetate	Methanol	Water	GUESS Mix Compounds With *P* Value in the Sweet Spot Area
−8	10	0	10	0	Carvone, β-ionone, cholesterol
−7	9	1	9	1	Carvone, β-ionone
−6	8	2	8	2	Carvone, β-ionone
−5	7	3	7	3	Carvone
−4	7	3	6	4	Salicylic acid, carvone
−3	6	4	6	4	Coumarin, salicylic acid, carvone
−2	7	3	5	5	Coumarin, estradiol, salicylic acid, carvone
−1	6	4	5	5	Coumarin, estradiol, salicylic acid
0	5	5	5	5	Naringenin, coumarin, estradiol, salicylic acid
+1	4	6	5	5	Quercetin, umbelliferone, aspirin, vanillin, naringenin, coumarin, estradiol, salicylic acid
+2	3	7	5	5	Reserpine, ferulic acid, quercetin, umbelliferone, aspirin, vanillin, naringenin, coumarin, estradiol, salicylic acid
+3	4	6	4	6	Reserpine, ferulic acid, quercetin, umbelliferone, aspirin, vanillin, coumarin
+4	3	7	4	6	Reserpine, ferulic acid, umbelliferone, quercetin, aspirin, vanillin
+5	3	7	3	7	Chlorogenic acid, reserpine, ferulic acid, umbelliferone, aspirin, vanillin
+6	2	8	2	8	Caffeine, chlorogenic acid, ferulic acid
+7	1	9	1	9	Caffeine, chlorogenic acid, ferulic acid
+8	0	10	0	10	Caffeine, chlorogenic acid

the solvent system, that is, interfacial tension and difference in densities and viscosities of the upper and lower phase [74–76]. For the characterization of a solvent system, the organic solvent amounts in the upper and lower phases can be measured by GC, and even small portions of water can be determined accurately by Karl Fischer titration [58]. When phase compositions are known, both phases can be prepared individually to reduce solvent consumption. In addition, collected effluents after a CCC run can be analyzed directly or after (azeotropic) distillation, and the missing share of a component can be added and the phases can be re-used. This is especially important for industrial applications where high volumes are consumed during a CCC run [77, 78].

In general, the HEMWat family is best suited for weakly to moderately polar analytes, while the EBWat family has its merits for the separation of moderately to highly polar analytes. In fact the five solvents in the HEMWat and EBWat system, water, ethyl acetate, methanol, *n*-hexane, and *n*-butanol, represent the top five solvent choices [32, 57, 79] although many more solvents are in principle suitable for use in CCC. Further commonly used solvents are acetonitrile and *t*-butyl methyl ether [57]. Chloroform and dichloromethane are special because of their high density. Their use can lead to the rather unique case that the lower phase is the less polar phase. In most cases, however, the least polar solvent is represented by a hydrocarbon with a strong preference for *n*-hexane or *n*-heptane, while *iso*octane or cyclohexane is seldom used. Likewise, mixtures of hydrocarbons have been scarcely reported in CCC [58, 77, 80].

While HEMWat and EBWat (and other) solvent families are well suited for moderately polar compounds, they are less suited for lipid compounds. Isolation of lipophilic compounds often necessitates the application of nonaqueous solvent systems. This can be challenging as most of the available organic solvents are miscible and therefore do not form biphasic systems (recent reviews indicated that only 3.7% of the CCC articles feature nonaqueous solvent systems [57, 79]). The compositions of some nonaqueous solvent systems used for the isolation or enrichment of highly lipophilic compounds are summarized in Table 10.4.

The simplest nonaqueous solvent system used for lipophilic compounds is *n*-hexane/acetonitrile (1:1, v/v), which has been used for the enrichment of fatty acids (Table 10.4). However, very lipophilic compounds still solely partition into the upper phase ($P_{U/L}$ values >100) [90]. Better results were achieved by using benzotrifluoride (BTF) as a modifier in the solvent system *n*-hexane/BTF/acetonitrile (10/3.5/6.5) [89]. The reason for the improved separations is that BTF distributes evenly between the lower and the upper phase and therefore bridges the polarity gap in a manner that both phases become more similar. This solvent system has been successfully used for tocopherols, sterols, and carotenoids (Table 10.4) [89, 91, 92].

Ionic liquids are interesting components in solvent systems [93–99]. Room-temperature ionic liquids are salts with melting points at or below room temperature that typically consist of organic cations (usually *N*-heterocycles) and organic or

TABLE 10.4 Examples for the Usage of Nonaqueous Solvent Systems

Solvent System	Target Compound	Source
n-Heptane/chloroform/acetonitrile (10:3:7, v/v/v) [81]	Lutein	Marigold flower petals
n-Hexane/acetonitrile (1:1, v/v) [82]	Anacardic acid homologues	Cashew nuts
n-Hexane/acetonitrile (1:1, v/v) [83]	Sciadonic acid (20:3Δ5,11,14) and juniperonic acid (20:4Δ5,11,14,17)	*Podocarpus falcatus*
n-Heptane/methanol (3:2, v/v) [84]	Fucosterol	*Pelvetia siliquosa*
n-Hexane/chloroform/acetonitrile (6:2:5, v/v/v) [85]	β-Caryophyllene	*Vitex negundo*
n-Heptane/*n*-butanol/acetonitrile (1.8:0.7:1.4, v/v/v) [86]	γ-Oryzanol	Rice bran oil
n-Hexane/ethyl acetate/ acetonitrile (5:2:5, v/v/v) [87]	Aphyllodenticulide	*Gypothamnium pinifolium*
n-Heptane/dichloromethane/ acetonitrile (12:3.5:7, v/v/v) [88]	Coenzyme Q10	Fermentation broth extract
n-Hexane/benzotrifluoride/ acetonitrile (10:3.5:6.5, v/v/v) [89]	α-/β-Carotene	Carrots

inorganic anions [100]. Ionic liquids provide unique solvating properties and thermal stability; in addition, their viscosity and electrochemical window can be adjusted to specific requirements [99]. One disadvantage of ionic liquids is their negligible vapor pressure. Hence their removal from CCC fractions can be challenging and time-consuming. Similar to ionic liquids, deep eutectic solvents (DES) have been used as phase modifiers in CCC (and CPC) solvent systems [101, 102]. Next to such phase modifiers, uncharacteristic solvents have also been used in solvent systems. For instance, limonene was used to substitute *n*-heptane in the Arizona system [103–105], and even sunflower oil was used as a stationary phase [106]. Both support the principle of green chemistry processes because they are natural compounds and biologically degradable solvents.

10.3 Taking Advantage of the Liquid Nature of the Stationary Phase

The most significant advantage of CCC is that the stationary phase is stationary by will and not by principle (actually in GC the stationary phase is also a liquid—i.e., a

film—but it is immobile). Accordingly, in CCC the stationary phase can be moved. Likewise, liquid mobile and stationary phases can be interchanged during a CCC run [11]. Users can take advantage of this opportunity in the case of two scenarios. On the one hand the sample may contain analytes eluting out of the sweet spot range (Section 10.3.1). On the other hand, analytes may elute into the sweet spot but may not be sufficiently resolved (Section 10.3.2).

10.3.1 Methods suited to elute compounds with *P* values outside the sweet spot range

Sample extracts may contain several compounds of interest whose structures and polarites require different ideal solvent systems for their effective isolation. Elution of compounds with high *P* values (>5) is strongly delayed and results in broad peaks and/or unnecessarily extends the run time without any benefits (Fig. 10.11A). Different elution modes have been developed to overcome problems related to analytes with nonideal *P* values. Samples containing analytes of different polarity are difficult to analyze in normal CCC mode. In HPLC, such problems are dealt with by means of gradients. While gradients can also be used in CCC (Section 10.3.1.4), other options exist (Fig. 10.12).

10.3.1.1 Elution Extrusion and Back Extrusion Modes

The most common and probably most convenient use of the liquid nature of the stationary phase is the elution-extrusion mode (Fig. 10.12B), which was introduced by Conway [11, 108]. After compounds with suitable retention times are eluted, compounds still in the CCC system can be extruded, by pumping stationary instead of mobile phase through the coil. Fresh stationary phase will displace stationary phase loaded with sample that thus will be eluted. Basically the remaining sample is flushed out without separation. As an alternative, removal of loaded stationary phase can also be performed in the opposite flow direction (back-extrusion mode, Fig. 10.12C) [11, 108]. For example, if a CCC separation is operated in *head*-to-*tail* mode with the lower phase serving as the mobile phase, back extrusion can be achieved when lower phase is pumped in the *tail*-to-*head* direction [109, 110]. Noteworthy, no further separation is achieved during extrusion steps; however, the separation obtained up to this point still remains. Berthod et al. developed the theoretical base of elution-extrusion and back-extrusion modes [11]. The CCC run was divided into two steps, that is, normal CCC mode followed by extrusion. The calculations showed that the number of theoretical plates can be very high, especially for compounds with high elution volumes during the first step [11]. The elution-extrusion mode has been used in many CCC applications [111–116].

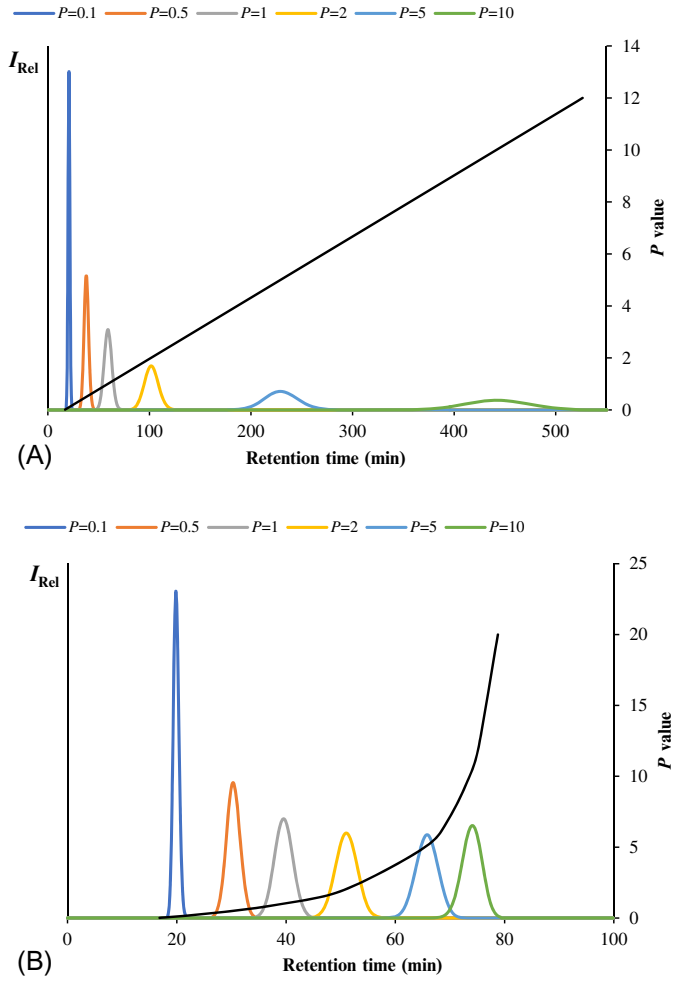

Fig. 10.11 Simulated CCC separations (ProMISE 2, V.1.2.0.1, Joost de Folter, 2014 [107]) of six hypothetical compounds with partition coefficients (P) of 0.1 (dark blue), 0.5 (orange), 1 (gray), 2 (yellow), 5 (light blue), and 10 (green). (A) Separation in conventional CCC with a mobile flow rate of 4 mL/min. (B) The same separation in co-current mode with an additional flow of the stationary phase at 2 mL/min. The function of the partition coefficient (of eluted compounds) over the retention time showing linear relationship in conventional CCC and exponential relationship in cocurrent CCC.)

10.3.1.2 Dual Mode

In the dual mode (Fig. 10.12D) the role of mobile and stationary phase and also the flow direction is reversed during the separation [11]. Hence analytes remaining in the system are moved in opposite directions, while they are still separated [11]. This

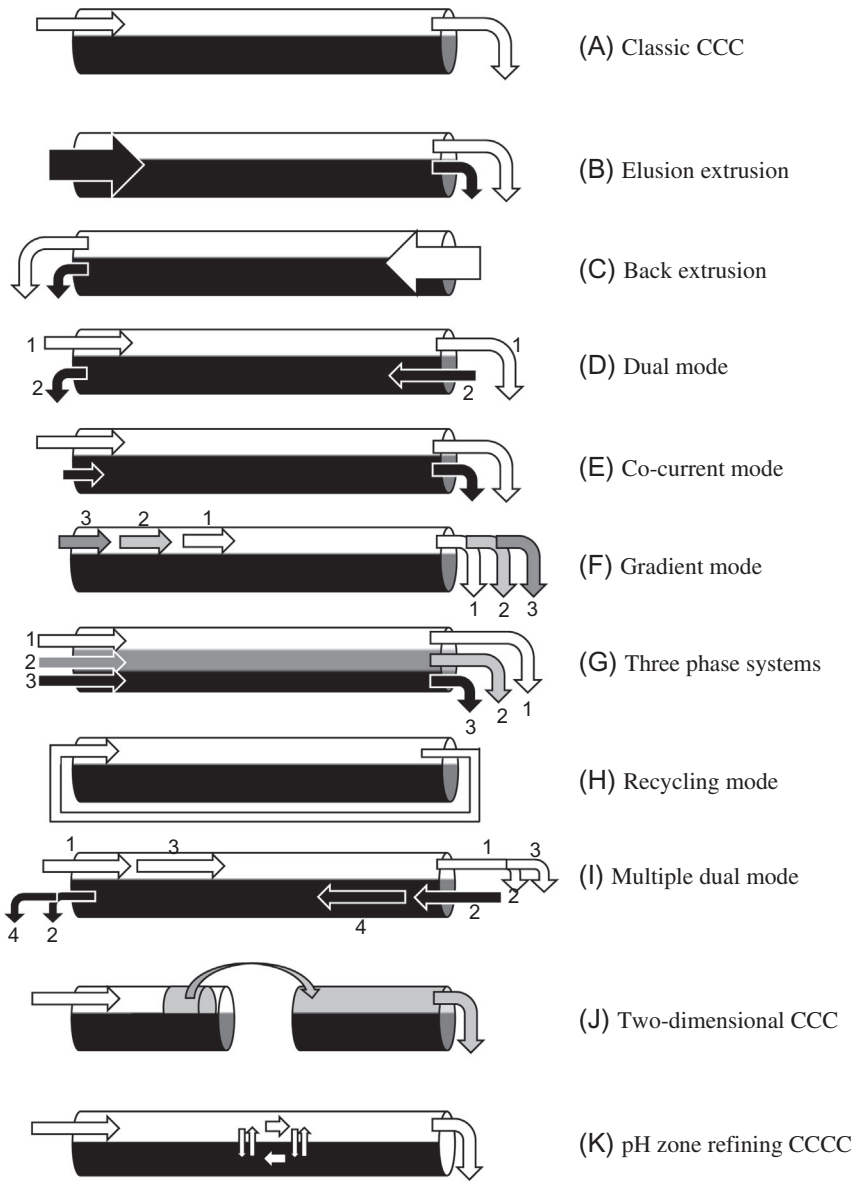

Fig. 10.12 Schemes of different application modes of CCC. Numbers refer to different steps, and arrows (and their colour) denote which phase is moved. (A–K) Different elution modes which are described in this chapter.

mode was introduced by Gluck et al. [117] and Menges et al. [118] and further developed by Agnely and Thiébaut [119].

In practice, at a free selectable point of a regular CCC separation in *head*-to-*tail* mode (lower, mobile phase is introduced at the head end), the flow is stopped. Then, upper phase (former stationary phase) is pumped into the system via the *tail* end. As pointed out by Berthod et al., the required stop during the change leads to a discontinuous process [11]. Since the P values are inversed when the stationary phase and mobile phase are switched, compounds strongly retained until this point will now be eluted within a short time. Likewise, fast-moving analytes, which were close to the end of the coil before the switch, will now be moved slowly in the opposite direction. Both effects eliminate the risk of introducing overlaps for compounds already separated during the initial separation. Typically the dual mode is used when the sample contains analytes with a wide range of P values. Berthod et al. showed that resolution factors are almost 1/4 compared to the classical CCC mode [11]. We suggest that this is at least partly due to the S_f value. While S_f is high in the normal mode, reversal of stationary and mobile phase leads to a low S_f value that is detrimental for the CCC separation (Section 10.1.3). Nevertheless the dual mode has been successfully used for the separation of complex mixtures [86, 120].

10.3.1.3 Co-Current Mode

Introduced in 1984 by Sutherland et al. [121], the co-current mode (Fig. 10.12E) is used to accelerate the elution of compounds, especially those with high P values (long elution volumes). Hence it can also be used to extend the P value range in CCC by accelerating the elution of highly retained compounds. This is accomplished by keeping the stationary phase not stationary but moving it in the same direction as the mobile phase. Practically, this is achieved by pumping "stationary" phase together with mobile phase through the column. Consider two persons who have to walk a longer distance from point A to B in an airport or subway tunnel. Persons walking with different speed will be "separated," but it may take a long time to reach B. The movement can be increased by means of conveyor belts. Then the "ground" (conveyor belt that corresponds to the stationary phase) is additionally moved and the movement (elution) will be accelerated. Berthod et al. [122–124] theoretically investigated the method with the goal to define the best relative velocity of the mobile phase (Eq. 10.11):

$$P = \left(\frac{t_R \times F_m - V_m}{V_s - t_R \times F_s} \right) \tag{10.11}$$

where t_R is the retention time, F_m/F_s are the flow rates of mobile/stationary phase, and V_m and V_s are the volumes of mobile/stationary phase in the coil.

For compounds with similar (and small) P values, the effect of the co-current phase migration is small but generally detrimental for peak resolution. Strongly retained compounds with high P values (people standing on a conveyor belt) will be moved toward the end of the coil. Berthod et al. showed that the relationship between retention time and partition ratio is not linear anymore but follows an exponential curve (Fig. 10.11B) [123, 124]. Accordingly, analytes with high P values can be eluted from the system within reasonable time (e.g., in about 90 min in the example in Fig. 10.11B). For implementation of the co-current mode, the CCC system needs to be modified because of the requirement for a second pump and split valves [90].

Yet the fast and exponential flow scheme is accompanied by the loss of resolution, especially for late eluting compounds. Likewise the biphasic nature of CCC eluate may be difficult to handle by commonly used UV/Vis detectors. To overcome this drawback a third solvent can be introduced after the coil to generate a monophasic mixture [124].

Practically the co-current mode has been applied for the (CPC-based) determination of octanol-water partition constants of nonpolar compounds [122] and for the separation of synthetic peptides, fungal and plant extracts, and lipid classes from vegetable oils [90, 125–128].

10.3.1.4 Gradient Elution in CCC

While gradients of mobile phase are commonly used in HPLC for multicomponent analysis, this method is less frequently used in CCC (Fig. 10.12F). With increasing solvent strength, slow-moving analytes are accelerated, and run times are shortened. Due to the link between both phases in CCC, changes in the mobile phase composition also change the equilibrium between stationary and mobile phase. These changes are difficult to predict, but it is also possible to use gradients in CCC. For this purpose the solvent strength of the mobile phase can be increased either stepwise (i.e., by changing the conditions in distinct intervals) or linearly (continuously) throughout the run [129–132]. Gradient elution is typically applied to reduce CCC run times by the faster elution of compounds with high P values. This can be done by changing the mobile phase composition (or, e.g., the pH or salt concentration [133–135]). Another possibility for accelerated elution is a stepwise increase of the mobile phase flow rate [136]. However, increasing the flow rate often results in a severe loss of stationary phase (phase bleeding) and therefore impairs separations. An interesting method to circumvent these drawbacks was presented by Du et al. [137] who altered the mobile phase composition and the flow rate at the same time. As both effects counteract each other, the S_f value was quite stable throughout the whole run [137].

10.3.1.5 Triphasic Solvent Systems

Solvent combinations not necessarily need to be mono- or biphasic but can also be triphasic. For instance, *n*-hexane/methyl acetate/acetonitrile/water in the volumetric ratio 2:2:3:2 v/v/v/v is a triphasic system that has been used in CCC (Fig. 10.12G) [138, 139].

Triphasic solvent systems can be used in CCC in two ways. Either all three phases are in the coil at the same time [138–141] or the mobile phase is substituted during the run [142]. In the first, more common, case, all three phases are in the coil with two being kept stationary by the centrifugal field. For instance, when the upper phase is used as mobile phase, the lower phase and the intermittent phase are used as the stationary phase at the same time. In this case, nonpolar analytes will elute very fast (due to their preferential partitioning into the upper phase). After nonpolar analytes were eluted (due to their preferential partitioning into the upper phase), the intermediate phase that was initially stationary is pumped through the CCC coil. This setup allows the elution of medium polar compounds. Finally, polar compounds that are still in the coil can be collected by extruding the system with lower phase (elution-extrusion, Section 10.3.1.1) [140, 141]. This method has been used for samples with analytes covering a broad range of polarity. Exemplarily, Shibusawa and Ito [138] separated a mixture of water- and fat-soluble vitamins, while Yanagida et al. [140] and Liu et al. [141] used this method for the separation of a variety of plant-based analytes.

10.3.2 Modes that improve the separation of compounds with similar *P* values

10.3.2.1 Recycling Mode

Recycling is an established method in chromatography that is used to improve the separation of insufficiently resolved analytes. Du et al. [143] introduced the recycling mode in CCC (Fig. 10.12H). In recycling mode the effluent from the coil is reintroduced to the coil entrance and chromatographed again. By two cycles the coil length is doubled, in the case of three cycles tripled a.s.o. In agreement with theory, each cycle n increases the resolution R_s by the square root of the cycle number n [143] (Eq. 10.12):

$$R_n = R_s^* \sqrt{n} \tag{10.12}$$

Connection of exit and entrance of the coil generates a closed loop in which analytes are circulated and separated in multiple cycles. Ideally a nondestructive detector (e.g., UV/Vis) is implemented in the loop to monitor the separation online. In practice the number of cycles usable in recycling mode is limited by the intrinsic increase of the peak width [144–146]. The best effect is obtained when only two compounds are separated because fast eluting compounds may overtake slowly

moving analytes and coelutions will be the consequence. In the case of complex mixtures, the recycling mode can only be safely used for the separation of the last eluting compounds (when all other compounds are eluted from the coil). A practically useful application is the separation of racemates in a chiral solvent system [145, 147].

10.3.2.2 Multiple Dual Mode (MDM)

The resolution of analytes with similar P values can also be improved by repeated application of the dual mode in one run. The technique of MDM was proposed by Delaney et al. (Fig. 10.12I) [148]. When the role of mobile and stationary phase is switched several times consecutively, analytes are moved forward and back within the coil. This action also leads to an artificial increase of "coil length" and increases the number of liquid-liquid partition steps. Hence the approach is similar to CCC in recycling mode (Section 10.3.2.1). However, the resolution should be worse due to the adverse S_f value that has to be taken into account in one of the modes (Section 10.1.3). Interestingly, one excellent improvement is that sample can be introduced between two coils [149, 150]. With this setup, compounds can be eluted toward both ends of the CCC and also removed from different ends. This eventually leads to a process where sample is continuously introduced and separated [151, 152].

10.3.2.3 Two-Dimensional Heart-Cut CCC

Similar to the recycling mode, heart-cut two-dimensional (heart-cut 2D) chromatography is well established in GC and HPLC. In heart-cut 2D CCC a freely selectable distinct part of the chromatogram (first dimension) is transferred to the second dimension with the goal to separate (partly) coeluting compounds. In the case of CCC, heart-cut 2D CCC requires the combination of two CCC centrifuges [153] or the opportunity to have an exit/entrance between coils (Fig. 10.12J) [154]. In the latter case, multiple six-port selection valves and T-pieces were installed to yield a 2D CCC system. Usually, heart-cut 2D methods benefit from the use of different selectivity in both phases. Ideally the separation modes in both dimensions should be orthogonal, that is, as different as possible [155–158]. However, as the stationary phase and the mobile phase heavily affect each other in CCC, the compatibility of the solvent system used in both dimensions is the key limiting factor of 2D CCC separations [154]. Due to the rather complicated setup and the limitations in solvent systems, 2D CCC was barely used. However, Wu et al. introduced a number of CCC setups with multiple dimensions [159–161]. Only recently, Müller et al. showed that heart-cut 2D CCC results in a better resolution when compounds are

only partly transferred from the first to the second dimension [162]. This approach may also be useful for other applications.

10.4 pH-Zone Refining CCC

Another mode that utilizes the liquid nature of both phases is called pH zone–refining CCC and was discovered accidently by Ito et al. [163]. During the separation of acidic compounds, they noted that analytes were mainly eluted according to their pK_a values instead of the P value [163]. The mode is used as follows: in *head*-to-*tail* mode (the lower, aqueous phase is mobile), an acid (referred to as retainer acid) is added to the stationary phase, prior to the separation (Fig. 10.12K). Contrary to classic CCC separations, sample solution is then added to the nonequilibrated system (no rotation until this point). Then, rotation is started, and mobile phase is pumped into the system. At this point the acid in the stationary phase forms a sharp border that moves slowly in the same direction as the mobile phase but at lower speed. Analytes can cross this border during their transport in mobile phase [3]. Due to the lower pH value in the stationary phase, acidic compounds that cross the border are protonated and immediately partition into the organic stationary phase in which they are retained (no movement) [3]. However, when passed by the retainer acid border, the protonated analytes are released again into the zone of higher pH values (i.e., behind the trailing border of the retainer acid) where they are deprotonated again and therefore partition into the mobile phase. This cycle is repeated manifold and results in extremely sharp peaks as the cycle of protonation and deprotonation acts against peak broadening [164]. Moreover, analytes are strictly ordered by their pK_a value, building a "train" with the retainer acid being the locomotive, as the previous peak always functions as retainer acid for the next peak [165]. The most important advantage of pH zone-refining CCC is the increased sample loading capacity that can be $>$10 times higher than in classic CCC separations [166]. Practical applications of pH-zone refining CCC were described for several ionizable compounds in the polar range (organic acids [167], alkaloids [168–170], amino acids [171], and peptides [172, 173]) and also in the nonpolar range (fatty acids [174]).

10.5 Concluding Remarks and Recommended Reading

Some 50 years after its invention, CCC has become a highly valued method for the enrichment and purification of natural compounds. Basics and theory have been developed simultaneously, and new users can draw on hundreds of publications when they start investigating a new problem. Several key articles that might be helpful starting points are listed in Table 10.5.

TABLE 10.5 Recommended Reading for General and Specific Topics in Countercurrent Chromatography

Topic	Section in This Chapter	Literature
General	10.1–10.3	[3]
Instrumentation	10.1–10.3	[15, 108]
CCC for natural product separation	10.1–10.3	[32]
CCC in analytical chemistry	10.1–10.3	[175]
Solvent systems	10.2	[4, 79]
Elution modes	10.3	[144, 176, 177]
Elution-extrusion/back extrusion	10.3.1	[109, 178]
Cocurrent mode	10.3.1	[124]
Dual mode	10.3.1	[119]
Recycling mode	10.3.2	[143]
Two-dimensional CCC	10.3.2	[154]
pH-zone refining	10.4	[164]

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Chapter 11

Soxhlet Extraction

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Chapter Outline

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11.1 Introduction

The steps that mediate between the original sample and the solution ready to be inserted either in the detector or in high-resolution separation equipment that precedes the detector are collectively known as sample preparation. The type of sample fraction that results after each step is different from the original sample and also different between successive steps. Therefore, different types require different names, for which the fraction of interest should also have different names depending on the type of sample preparation; however, this is rarely the case and the word "sample" is usually maintained throughout. Fig. 11.1 shows some of the generic designations for the remaining fraction of a sample along with the analytical process. For example, the term "laboratory sample" is used to refer to "the sample taken or formed from the laboratory sample by a process that involves homogenization using a physical or mechanical treatment such as grinding, drilling, milling, or sieving." It is clear that the initial sample only requires some physical change to reach this stage; therefore, these steps should be designated as "sample pretreatment." On the other hand, the "test sample" is the final product of sampling, which "is obtained by subsampling the test sample to provide a form appropriate for being subjected to sample preparation." Sample preparation produces the sample fraction that determines at least one quality characteristic suitable for analysis. From the latter definition it follows that the solution resulting from solid-liquid leaching (also known as lixiviation) is given the name leachate or lixiviate; elution from a sorbent produces the eluate; liquid-liquid extraction produces an extract, and so on. Any one of these solutions, ready for insertion in the detector or high-resolution device, is called the "analytical sample." This generic name is shortened by most analytical chemists, who use the word "sample" to describe any solution from any step, after which a smaller part of the sample is contained in the solution. A great deal of misunderstanding in this context could be avoided by using the correct, unequivocal name for the solution provided by each specific treatment [1].

Obtaining the analytical sample from the bulk sample involves different degrees of complexity depending on the nature of the latter, and is particularly difficult when dealing with solid samples. For centuries a major goal of analytical chemists has been to overcome the problems associated with sample preparation by developing techniques of variable complexity, adapting them to target samples and giving place to different methods. The result of improving existing methods or developing new methods depends on the matrix-analyte(s) binomial, the number of samples to be

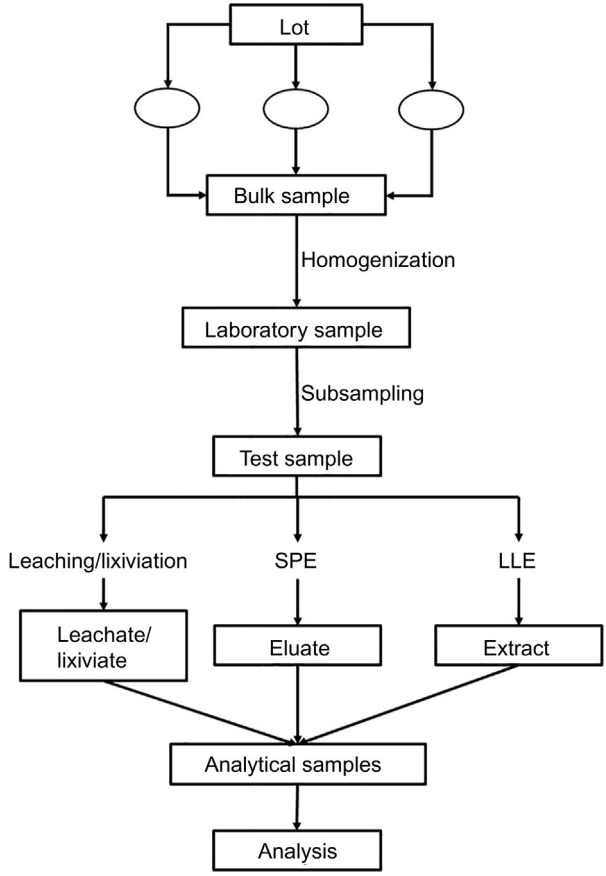

Fig. 11.1 Schematic diagram of the overall analytical process and associated terminology. *LLE,* Liquid-liquid extraction; *SPE,* solid-phase extraction.

prepared, and the contaminant nature of the reagents involved. Therefore, the primary goals in developing new and improving existing sample preparation techniques are: (1) automation to avoid or minimize human intervention; (2) acceleration to enable processing of large numbers of samples in as short a time as possible; and (3) solvent elimination, or, if impossible, use of reduced volumes of less toxic solvents. Fulfillment of this last goal leads to two key trends in analytical chemistry: green chemistry [2] and miniaturization [3].

The next step to obtaining an analytical sample is analysis, by introducing it into the detector or, more commonly, into high-resolution separation equipment that integrates with the detector. This integration is the reason why a number of authors attribute analysis to the separation system; thus the terms "analysis by gas

chromatography" or "analysis by liquid chromatography" are frequently used, even without referring to a particular detector.

This chapter discusses and compares the principles, evolution, current status, and capacities of Soxhlet extraction (SE) based on both laboratory designs and commercial equipment. Examples of applications of the conventional Soxhlet extractor or key devices based on the Soxhlet principle are given.

11.2 Performance of the Soxhlet Extractor: Positive and Negative Features

SE has been the standard technique for over a century and the methods based on it are the primary references against which performance of new leaching methods are measured. The advantages and shortcomings of SE have been used as starting points for the development of a variety of modifications intended to alleviate or suppress the latter, while keeping or even improving the former. Most of the modifications reported over the last few decades have been aimed at bringing Soxhlet closer to that of more recent techniques for solid sample preparation by shortening leaching times, using auxiliary energies, and automating the extraction assembly.

11.2.1 Description of SE

In the conventional implementation of SE, the sample is placed in a thimble that is gradually filled with condensed fresh extractant (the term used to refer to the solvent used for extraction) from a distillation flask, as shown in Fig. 11.2. When the liquid reaches the overflow level, a siphon aspirates it from the thimble and unloads it back into the distillation flask, thus carrying the extracted analytes into the bulk liquid. The operation is repeated until complete extraction is achieved. This performance makes Soxhlet a hybrid continuous-discontinuous technique. Inasmuch as the extractant acts stepwise, the assembly can be considered as a batch system; however, since the extractant is recirculated through the sample, the system also has a continuous characteristic.

11.2.2 Positive and negative aspects of SE

The most outstanding advantages of conventional SE are: (1) the sample is repeatedly brought into contact with fresh portions of the extractant, thereby helping to displace the mass transfer equilibrium; (2) the temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent; (3) no filtration is required after the leaching step; and (4) sample throughput can be increased by simultaneous parallel extraction because the basic equipment is inexpensive.

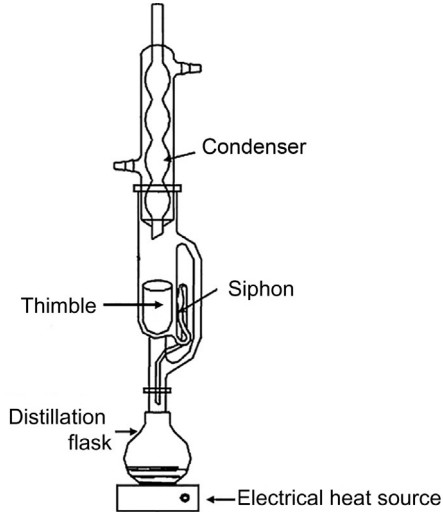

Fig. 11.2 Conventional Soxhlet extractor.

The most serious drawbacks of SE as compared to other conventional techniques for solid sample preparation are: (1) the long time required for extraction and the large amount of extractant waste, which is not only expensive to dispose of, but which can itself cause additional environmental problems; (2) samples are usually extracted at the boiling point of the extractant for a long period of time and the possibility of thermal decomposition of thermolabile compounds cannot be ignored; (3) the conventional Soxhlet extractor is unable to provide agitation, which would accelerate the step; (4) because of the large amount of solvent used, an evaporation/concentration step after extraction is mandatory; and (5) the technique is restricted to solvent selectivity and is not easily automated.

11.3 Minor Improvements to the Soxhlet Extractor

The majority of simple modifications from the original Soxhlet extractor [4] consisted of minor alterations aimed at modifying basic units such as the thimble, siphon, condenser, etc., or applying them to a particular type of sample (liquid, thermolabile, etc.). These modifications slightly improved the features, application field, and/or results of the methods thus developed.

The units that constitute a Soxhlet extractor have been modified in different ways. Thus, changes to the thimble have been aimed at: (1) simultaneous extractions, based on a glass cylinder with a stainless-steel wire platform with eight porous plastic cartridges; (2) room-temperature extractions for thermolabile compounds, by location

of the thimble separated sufficiently from the extractant flask to avoid heating [5]; (3) no turbulence in the sample zone during siphoning, using a glass support into the thimble-holder where a small weighing bottle is mounted [6]; and (4) design of a wire-meshed cylindrical holder to facilitate extractant movement by hydrostatic pressure during oil extraction from seeds [7].

Changes to the Soxhlet siphon have involved: (1) location of a sintered-glass disc at the bottom of the extraction chamber and an outlet with a polytetrafluoroethylene stopcock below the disc, thus controlling the flow of the extractant to maintain a constant level above the solid subjected to leaching, and avoiding dropping of the extractant from the thimble by closing it during flask exchange [8]; (2) conversion of the siphon tube into a constant-level device by leading a tube from its upper bend back into the extraction zone and then to the atmosphere [9]; (3) in situ evaporation of the extractant after extraction, by inserting a stopcock in the siphon tube [10]; and (4) siphon removal and use of either a sprinkler device or a cylindrical tube with a serrated bottom end (for extractants lighter or heavier, respectively, than water) for extraction of components from biological fluids [11].

Also, the geometry and performance of the condenser have been modified with the aim of: (1) maximizing the boiling rate and the extractant temperature; (2) increasing safety by minimizing or avoiding losses of the extractant by bumping when superheated [12]; (3) improving the efficiency by inserting a stirrer into the thimble sealed in the condenser [13]; and (4) duplicating the sidearm bypass and the distillation flask by an inverted Y-shaped joint to speed up the process [14].

11.4 Major Improvements to the Soxhlet Extractor

A number of major improvements to the conventional Soxhlet extractor have been developed to circumvent its shortcomings while preserving its advantages. Among the achieved improvements, some of them have evolved into more depurated prototypes, but others have not surpassed a first design. One example of the latter is the high-pressure Soxhlet extractor that achieved its working condition by placing the extractor in a cylindrical stainless-steel autoclave [15] or by using either commercial or laboratory-made supercritical fluid Soxhlet extractors [16], in which the extractants did not reach the supercritical condition. Applications of these devices to the extraction of polychlorinated biphenyls (PCBs) from different foods using CO_2 at 1000–1500 psi [17] did not significantly improve the process, but added an extra level of complexity and reduced the robustness of the extractors.

Higher effectiveness than by the conventional Soxhlet extractor was achieved by applying auxiliary energies such as ultrasound (US) or microwaves (MWs).

11.4.1 Ultrasound-assisted soxhlet extractors

The first ultrasound-assisted Soxhlet extractor was designed and constructed by the authors' team in 2004 [18] using conventional Soxhlet glassware and accommodating the extraction zone in a thermostatic bath through which US is applied by means of an ultrasonic probe, as shown in Fig. 11.3A. The new device was applied to the extraction of total fat from oil seeds such as sunflower, rape, or soybean, thus demonstrating that application of US to the sample cartridge provides efficiencies similar to, or even better than, those obtained by conventional Soxhlet leaching (official ISO method). This decreases the number of Soxhlet cycles needed in conventional procedures to less than half. In short, the most important effect of US application is

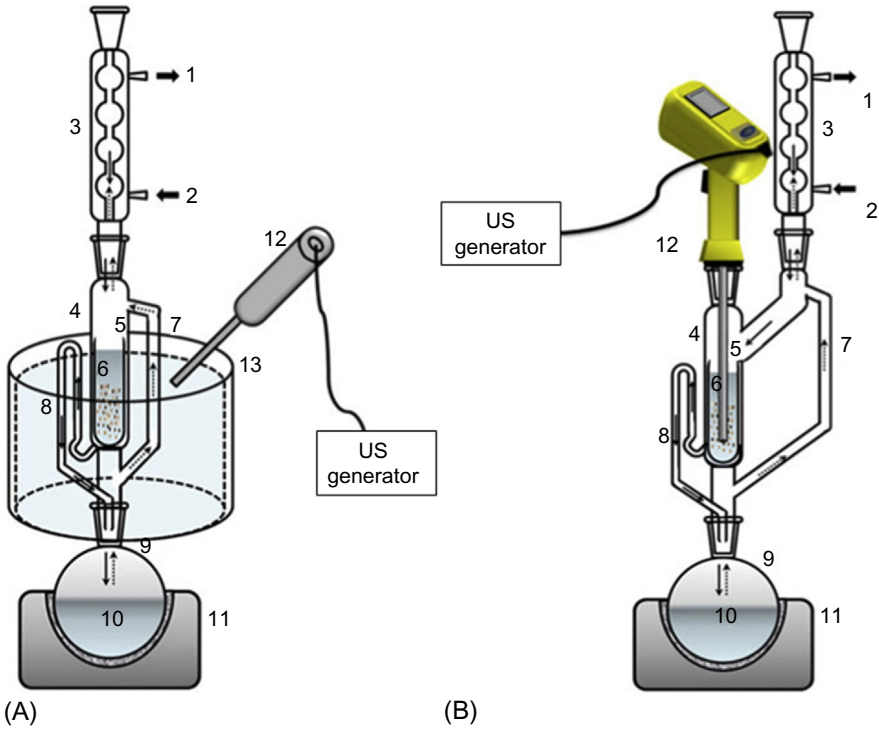

Fig. 11.3 Ultrasound-assisted Soxhlet extractors. (A) With the probe into a thermostated bath surrounding the extraction zone. (B) With the probe into the thimble (Sono-Soxhlet). 1: water out; 2: water in; 3: condenser; 4: extraction chamber; 5: thimble; 6: sample; 7: distillation arm; 8: siphon; 9: distillation flask; 10: extractant; 11: electrical heat source; 12: ultrasonic probe; 13: water bath. *(Reproduced from Ref. Djenni Z, Pingret D, Mason TJ, Chemat F. Sono-Soxhlet: in situ ultrasound-assisted extraction of food products. Food Anal Methods 2013;6(4):1229–33 with permission from Springer.)*

decompaction, which avoids typical steps of grinding several times between Soxhlet cycles to diminish the increased compactness produced by the dropping extractant. Despite the reported oxidative effect of US under drastic conditions [19], the mild conditions used in this extractor do not degrade the extracted oil.

In 2013, the Chemat team developed a new US-assisted Soxhlet extractor called the Sono-Soxhlet in which the change with respect to the previous design was to insert the probe into the thimble, as shown in Fig. 11.3B [20]. Application of the new device to extract oil from a sample (olive drupes) different from that used in the first design (seeds of sunflower, rape, or soybean) hinders comparison of their effectiveness. Nevertheless, it must be taken into account that the availability of US to degrade oils increases when the ultrasonic probe is immersed in the sample-extractant system [19], as does, in the authors' opinion, the "slight rancid/metallic odor" of the oils extracted by the new design (despite the high antioxidant content of olive drupes). The name Sono-Soxhlet does not seem the most appropriate for the new extractor because it does not work in the sound frequency zone, only in the US frequency zone. This is a very common error when working with US. To apply the name "extraction reactor" to thimble or gas chromatography (GC) analysis of the separation of fat components by GC plus the use of a flame ionization detector (FID) or a mass spectrometry (MS) detector should also be avoided [20].

11.4.2 Microwave-assisted soxhlet extractors

SE using MWs has provided the greatest improvements to the extraction process, the highest number of prototypes, and a number of commercial extractors based on the application of this type of energy.

The first approach, and the most similar to conventional Soxhlet, was designed by the authors' team in 1999, constructed by Prolabo, and called focused microwave-assisted Soxhlet extractor (FMASE) [21]. It consisted of a conventional Soxhlet extractor, but with the glassware slightly modified, and with the thimble zone placed in the MW cavity of a specially designed focused MW oven (Fig. 11.4). The performance of FMASE, similar to that of its conventional counterpart, made it a suitable alternative for almost all applications developed in a conventional Soxhlet without any changes, except the time required for quantitative extraction, which was drastically shortened. FMASE maintained the advantages of conventional SE while overcoming limitations such as the long extraction time, nonquantitative extraction of strongly retained analytes, and unsuitability for automation. Extractant distillation in FMASE was achieved by electrical heating, which is independent of extractant polarity, and recycling saves 75%–85% of total extractant volume. The main drawback of this extractor was its difficulty using water as extractant, because both thermal insulation and shortening of the original distillation device were mandatory for reception of water vapor on the thimble. After these changes, the new prototype showed its usefulness

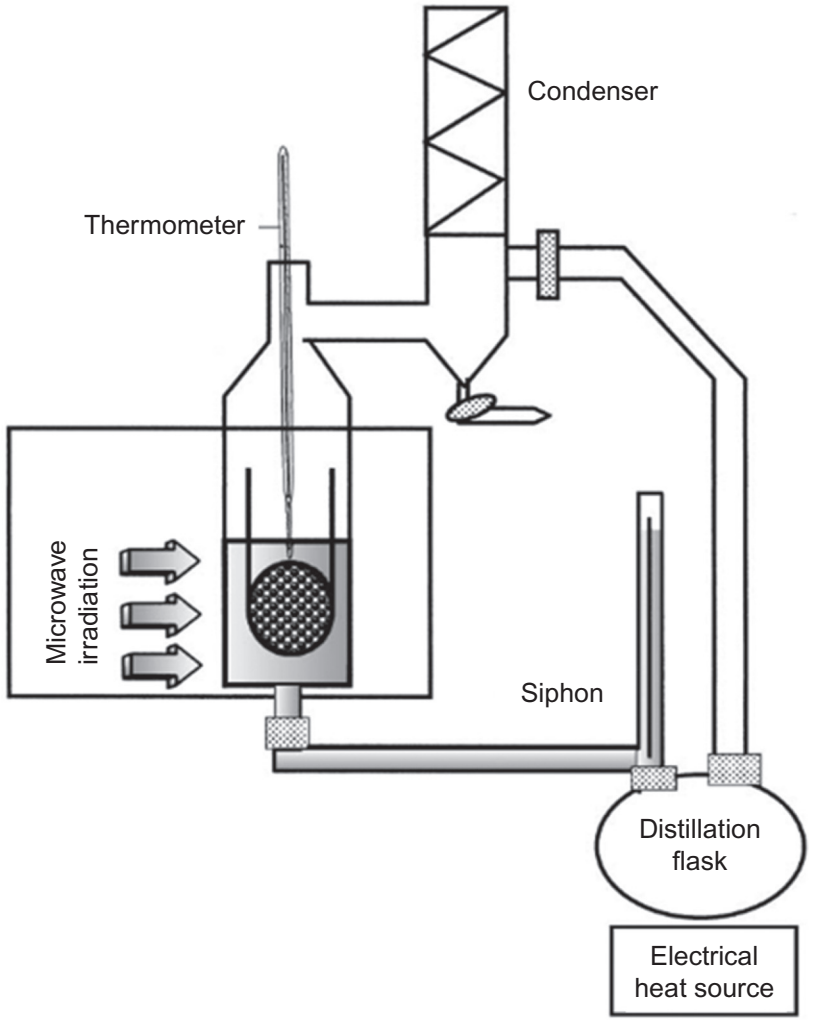

Fig. 11.4 Scheme of the focused microwave-assisted Soxhlet extraction device. *(Reproduced from Ref. García-Ayuso LE, Luque de Castro MD. A multivariate study of the performance of a microwave-assisted Soxhlet extractor for olive seeds. Anal Chim Acta 1999;382(3):309–16 with permission from Elsevier.)*

when water was used as extractant [22, 23]. Other useful changes to the original design, and couplings to subsequent steps of the analytical process, allowed full automation of FMASE [24], coupling to a fluorimetric detector for matrix-independent removal of fluorescent compounds [25] or to preconcentration-derivatization-detection steps [26], and dual simultaneous extraction [27].

In 2007 the Chemat team developed an MW-assisted extractor that was deemed similar to a Soxhlet extractor (Fig. 11.5), but that differed markedly in its operation [28], in aspects such as: (1) the sample is never brought into contact with fresh extractant; (2) the extract is not siphoned; (3) the extractant is heated by MWs, which is not

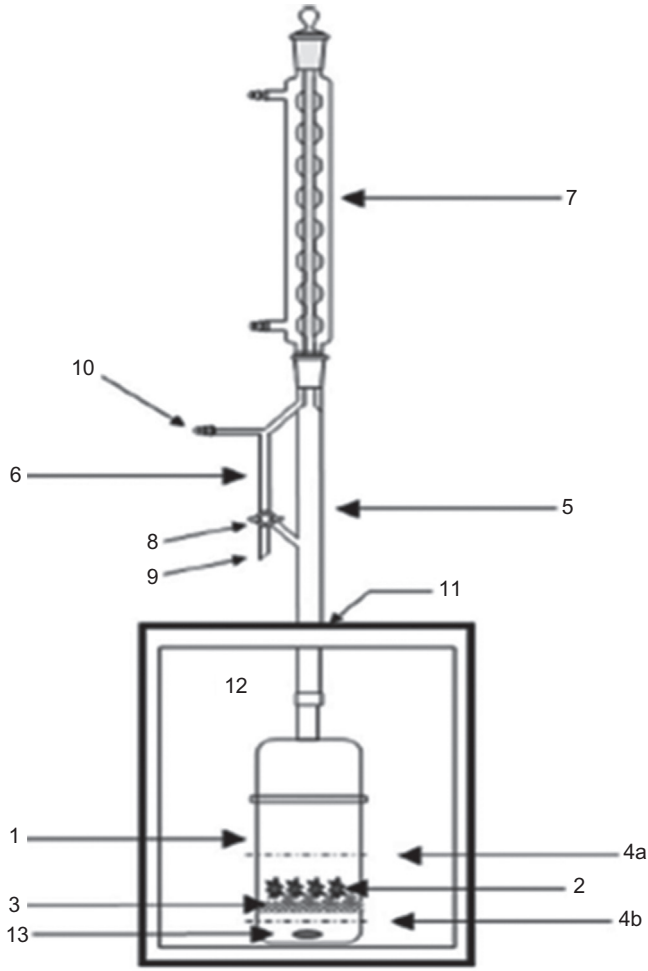

Fig. 11.5 Microwave-integrated extraction Soxhlet developed by Virot et al. 1: Extraction vessel; 2: sample; 3: support; 4a: *n*-hexane level containing the sample; 4b: *n*-hexane level below the sample; 5: extraction tube; 6: side arm; 7: condenser; 8: three-way valve; 9: side arm (open for *n*-hexane collection); 10: side arm (open to pull a vacuum in the system); 11: opening on upper surface of the microwave oven; 12: microwave oven; 13: Weflon magnetic stirrer. *(From Ref. Virot M, Tomao V, Colnagui G, Visinoni F, Chemat F. New microwave- integrated Soxhlet extraction. An advantageous tool for the extraction of lipids from food products J Chromatogr A 2007;1174(1–2):138–44 with permission from Elsevier.)*

the best option in dealing with nonpolar extractants; and (4) a filtration step is required because the sample is not held in a thimble but dispersed in the extractant. Low polar and nonpolar extractants are heated to their boiling points by heat diffusion from a Weflon magnetic bar that absorbs MW radiation. In this way, solvent vapors penetrate through the sample and are liquefied on arrival at the condenser; they then drop down onto the sample by switching a three-way valve. Therefore, the performance of this design does not rely on the Soxhlet principle that exploits the contact between the sample and fresh extractant in each leaching cycle to displace the partitioning equilibrium to complete extraction. Despite the name of the Chemat device (microwave-integrated Soxhlet—MIS), it does not integrate SE and MWs.

11.5 Commercial Extractors Based on the Soxhlet Principles

Extractors based on the Soxhlet principles but circumventing to a variable degree the shortcomings of the conventional technique while preserving its advantages have been commercialized. The old commercial models have been gradually improved giving way to others adapted to the present necessities of the users. Classification of Soxhlet-based commercial devices can be established by the heating source involved, which can be electrical or MW based.

It must be noted that most of the present commercial extractors, which vendors sometimes claim to be based on Soxhlet principles, do not provide information in their manuals about a number of scientific aspects that are of interest to the user. The manuals abound with features of the given extractor: versatility, automation, size, weight, power, number of simultaneously processed samples, selection of the extraction temperature, and handling facility, most often shown in video format. The principles behind heating, the way in which heat acts in the sample or in the extractant, or in both, seem to be unimportant for users.

11.5.1 Commercial soxhlet extractors with electrical heating

The operational procedure of these commercial extractors, based on the Randall principle, consists of three steps: leaching, rinsing, and evaporation of the extractant for its recovery. These steps are developed as follows:

(i) In the leaching step the thimble containing the sample is immersed in the boiling extractant until the transfer equilibrium is established, while the extractant vapor is refluxed in the condenser and returned to the boiling extractant passing through the sample. The key for accelerating this step is the close contact between the boiling extractant and the solid sample.

(ii) Rinsing takes place when the thimble is lifted above the boiling solvent and remains suspended for an interval to allow complete displacement of the extraction equilibrium until the residual traces of extractable compounds are flushed out of the sample and fall into the extractant reservoir. This step resembles performance of the original Soxhlet technique; it involves mechanical complexity of the extractor for lifting the thimble, which is indispensable for displacing the solid-extractant partitioning equilibrium to completion.

(iii) Subsequent evaporation of the extractant allows both recovery and concentration of the extract.

A number of firms (such as Tecator, Foss, and Büchi Labortechnik, among others) commercialize electrically heated extractors used to develop thoroughly tested methods available as Application Notes and widely applied in the agricultural, food, and industrial areas. Most officially endorsed methods are based on these extractors, which are also compared with others based on different principles, such as accelerated solvent extraction (ASE) and MW-assisted extraction (MAE).

One of the present extractors based on the three above-expressed steps is the fully unattended automated Soxtec 8000 from Foss, which adds a final step for auto shutting down the Hydrocap filter that contains the rest of the solid sample, which prepares the system for a new batch of samples, the number of which can vary from 1 to 12. The extractor is endowed with a wide menu with programmed addition of reagents, if required.

Commercial extractors more similar to the conventional Soxhlet extractor—both in the glassware system and performance—but working in an automated fashion are the Büchi B-811 and B-811 LSV systems. Basically, the extractor consists of the parts described in Fig. 11.6A that allow development of the three individual steps of extraction, rinsing, and drying. The two heating zones—lower and upper—and the glass valve allow four methods to be applied: Soxhlet standard (by activating only the lower heating zone, Fig. 11.6A), Soxhlet warm (the upper heating zone is activated when sensor five detects the level of condensed extractant, which, after extracting, is completely unloaded to the extractant beaker, Fig. 11.6B), hot extraction (both heating zones work, but the extract is only partially unloaded to the beaker, Fig. 11.6C), and continuous flow (only heating zone one works, and the condensed extractant washes down the condensation tube through the sample into the beaker by keeping open the glass valve and deactivating the optical sensor, Fig. 11.6D).

11.5.2 Commercial soxhlet extractors with microwave heating

Commercial Soxhlet extractors assisted by MW energy differ from other types of extractors also based on MW assistance. The main differences are as follows: (1) the extraction vessel is open, thus working under normal pressure; (2) MW

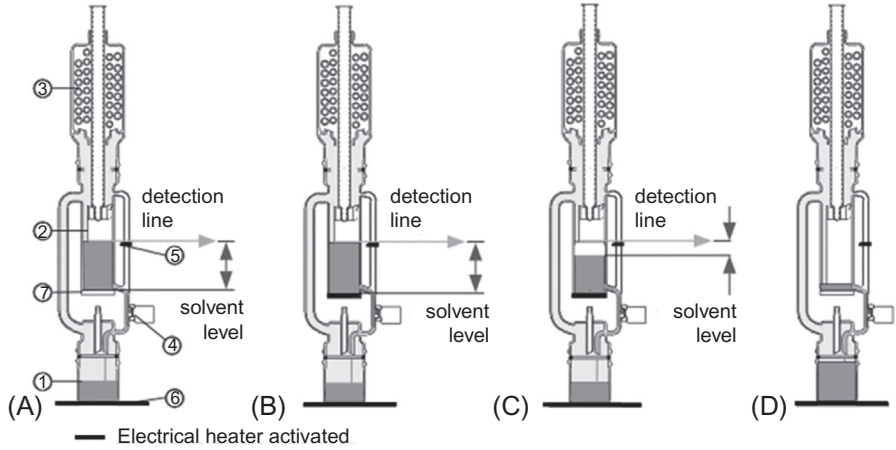

Fig. 11.6 Scheme of the extraction system B-811 from Büchi Extraction Systems. *(Adapted with permission from Büchi Extraction Systems. (A) Soxhlet standard. 1: distillation flask; 2: thimble or glass sample tube; 3: condenser; 4. glass valve; 5: optical sensor; 6: lower heating level; 7: upper heating level. (B) Soxhlet warm. (C) Hot extraction. (D) Continuous flow. For details, see text.)*

irradiation is focused on the sample; (3) extraction is mainly performed as in the conventional technique, especially the permanent sample-fresh extractant contact; and (4) no subsequent filtration is required.

MW-assisted Soxhlet extractors were largely marketed by Prolabo until its focused-MW section was acquired by CEM. One of the most used extractors commercialized by Prolabo was the Soxwave-100, the principle behind which is similar to Kumagawa extraction and its operational procedure is similar to the Soxtec System HT [29]. As in the Randall approach, the overall extraction involves three steps: (1) the thimble containing the solid sample is immersed into the boiling extractant; (2) the sample is lifted above the extractant and the condensate is dropped onto the thimble; and (3) extractant recovery with extract concentration is accomplished.

The fact that the Soxwave-100 and its counterparts use a single heating source and focused MW, acting on both sample and extractant, makes the dielectric constant of the latter of crucial importance, with decreasing efficiency from polar to low-polar and nonpolar extractants. Another drawback of the existence of a single MW-based heating source is that the amount of energy required by the extractant to boil differs from that needed to remove the analytes from the sample, thus making it mandatory to adopt a compromise in this respect. From the acquisition of the focused-MW section of Prolabo, CEM does not take commercial advantage of the use of this energy.

11.6 Applications of SE

The number of official methods based on conventional SE is impressive. Most of them deal with fat extraction from different matrices, and less frequently with other more polar compounds. An intermediate solution to save time has been adopted by users who require continuous use of official methods based on SE: they use a faster extraction method and compare, from time to time, the results thus obtained with those from the official Soxhlet method. Automated Soxhlet methods are gradually being accepted as official, as is the case with the US EPA Method 3541 that uses an automated SE system, which can be Soxtec or equivalent equipment. Alternatives approved by the AOAC are based on filter bag technology as proposed by Ankom Technology Inc.

The analytical literature is abundant in the use of SE with different aims. One of the most frequent is comparison of the merits of new methods, which usually surpass those of conventional Soxhlet. This comparison is the subject of Section 11.7.

Most of the Soxhlet methods recently developed deal with fat extraction, usually from matrices such as algal biomass [30], or *Nannochloropsis* microalgae [31], rambutan (*Nephelium lappaceum* L.) kernel [32], kokum (*Garcinia indica*) kernel to obtain cocoa butter [33], palm (*Phoenix dactylifera*) seed [34], castor (*Ricinus communis* L.) [35], *Moringa oleifera* Lam. seeds [36], or *Pistacia atlantica kurdica* [37].

A single compound is sometimes the extraction target, as in the case of the extraction of γ-oryzanol from rice bran oil soapstock, a compound with cosmetics applications, and very abundant in this by-product from rice oil production [38]. Extraction of polar compounds in fat-rich raw materials requires a first extraction of the fat; then, the defatted residue is subjected to the action of polar extractants. This is the case with flavonoids from *Chenopodium album* aerial parts, compounds with healthy properties, which require successive extractions with ethyl acetate, acetone, and methanol [39]. Also, sequential SE with petroleum ether, toluene, ethyl acetate, and acetone was required to evaluate the antioxidant and antimicrobial properties of *Manilkara zapota* L. leaves [40]. A special SE method was required to extract salicylic acid from a molecular-imprinted polymer used for selective separation of this acid from human urine and blood plasma [41].

A number of studies have been aimed at optimizing the variables influencing the extraction process (with special emphasis on the type of extractant or extractant mixture, which determines the temperature in the distillation flask), but also taking into account that the method is as green as possible. Thus, mixed-polarity azeotropic extractants have been studied to achieve efficient removal of lipids from *Nannochloropsis* microalgae, finding nonhalogenated extractants as the best and greener alternative [42]. Ethanol, petroleum ether, and *n*-hexane were studied for extraction of castor oil from different particle sizes subjected to different extraction times [35]. Extraction yield, fatty acids (FAs) composition of the

extracts, and physicochemical properties of the oil (such as refractive index, unsaponifiable matter, volatile matter, free fatty acid, phospholipids, peroxide value, iodine value, saponification value, and acid value) were characterized, and the significance of each of the variables was tested by analysis of variance. No clear conclusions were obtained from the study [35]. Thirteen extractants spanning a range of polarity and solubility, increased by their binary mixtures, were used to optimize lipid extraction from algal biomass. Analysis of the extracts by GC-FID showed that the extractants ethanol, chloroform, and hexane were the most efficient [30]. No information was given regarding the nature of the most abundant lipids as a function of extractant polarity/solubility.

Alternatives to traditional contaminant extractants for lipids, mainly *n*-hexane, have been reported, as is the case with terpenes (D-limonene, α-pinene, *p*-cymene) obtained from renewable feedstocks [43]. Yields of crude extracts were compared by gravimetric determination and the Bligh and Dyer method [44], and by individual determination of the target lipids by GC-MS; no significant differences were found among extractants.

The cleanest extractant, water, has been reported for SE of dissolved organic matter (DOM) in marine sediments, and the extracts thus obtained were compared with those provided by conventional interstitial water sampling methods such as Rhizon sampling. The molecular composition of the solutions from both methods was analyzed by Fourier transform ion-cyclotron resonance mass spectrometry after a solid-phase extraction step [45]. The aqueous SE of sediments provided access to a larger and more diverse DOM pool than conventional interstitial water sampling methods.

11.7 Comparison of Soxhlet With Other Extraction Methods

Comparison of extraction methods is a common practice in research on sample preparation, which can be implemented with different aims: (1) to select among conventional, well-established methods the most appropriate for a given sample-analyte(s) binomial taking into account variables such as temperature, type of extractant, sample-extractant ratio, extraction time, number of samples, etc.; (2) to emphasize the advantages of a new method, usually assisted by some type of accelerating energy (namely, MW, US, high pressure/high temperature), for which it is compared most often with the corresponding Soxhlet method; and (3) to decide among new methods based on different auxiliary energies, then compared with Soxhlet.

The rigor in the comparison closely depends on the analytical method chosen to determine the target parameter(s) in the extracts. Thus, in dealing with fat extraction, a gravimetric method used for comparison of the amount of extracted fat will not

provide information on modifications in FAs induced by the extraction conditions. When extraction of given families of compounds is the aim, the use of a method to determine the total global content—for example, the Folin-Ciocalteu (F-C) method for total phenols [46]—does not provide information on the individual compounds that could be degraded during extraction, but the degradation product contributes to the global response. The best way to obtain a confident comparison of the extracts obtained by the methods involved in the comparison is chromatographic separation and identification of each component by MS. Examples of key comparisons are given in Table 11.1 and some of them are discussed next. While the technique on which the method is based appears in column 3, the characteristics of the target method are given in subsequent columns.

11.8 Comparison of Conventional Methods

The contents of FAs in microalgae obtained by SE, by in situ transesterification (ISTE), and by gravimetry were compared [31]. The results confirmed the usefulness of ISTE, but mainly the limitations of gravimetric methods and the need for a more resolutive analysis of both FAs and lipid classes.

An example of the scant, convincing, and sometimes contradictory results provided by global methods of analysis is the extraction of compounds from horseradish roots using different pure and in-mixture extractants, and with subsequent global analysis (F-C method) or scavenging activity (DPPH·) monitoring [47]. The extracts obtained by the conventional stirring method at ambient temperature and by the Soxhlet method provided a higher total phenols content (TPC) in the latter, but the scavenging activity was similar. The conclusion that the Soxhlet method extracts more noneffective antioxidant compounds was not demonstrated by the authors.

Global determinations, such as that for TPC by the F-C method, radical scavenging activity (by the ABTS and DPPH methods), and browned compounds (monitoring absorbance at 420 nm), were used to study the antioxidant capacity of defatted spent coffee extracts obtained by Soxhlet for 1 h, by automated Soxhlet for 165 min, with an in-suspension sample at 80°C for 10 min, or with a filter coffeemaker for 6 min at 90°C. The extractants were pure water, four different water-ethanol mixtures, and two different water-methanol mixtures. Despite the poor analytical information, in the authors' opinion the obtained results allowed them to select the spent coffee extracts powder with the highest antioxidant capacity for use as an ingredient or additive in the food industry with potential preservation and functional properties [48].

TABLE 11.1 Comparison of Other Extraction Methods With Their Soxhlet Counterpart

Matrix	Target Extracted Compound(s)	Other Applied Technique(s)[a]	Extraction Time (h)		Extractant Tested		Extractant Volume (mL)		Monitoring[c]	References
			Other Methods[b]	Soxhlet Method	Other Methods[b]	Soxhlet Method	Other Methods[b]	Soxhlet Method		
Microalgae	FAs	ISTE	1	1.5 (Soxtec)	FAMEs formation solution	$CHCl_3$/EtOH			Gravimetric	[31]
Horseradish roots (*Armoracia rusticana*)	Phenols	CE	1	2	*n*-Hexane, ethyl acetate, diethyl ether, IPA, acetone, EtOH, EtOH:H_2O:acetic acid, EtOH:H_2O		50	170	F-C/DPPH	[47]
Spent coffee	Phenols/ Antioxidant compounds/ Browned compounds	CE Filter coffeemaker	0.16 0.1	3	H_2O, H_2O:EtOH, EtOH, H_2O:MeOH, MeOH		400 400	400	F-C/ABTS/ DPPH/UV-Vis spectrometry	[48]
Licorice root	Phenols	MAE	1.5	6	60% EtOH	80% EtOH	20	250	F-C	[49]
Agaricus bisporus L.	Ergosterol	USAE	0.25	4	*n*-Hexane, EtOH, limonene	*n*-Hexane, EtOH, limonene	100	150	HPLC-UV	[50]
Yellow passion fruit seeds (*Passiflora edulis f. flavicarpa*)	Fatty acids	USAE Stirring	1 8	24	Acetone, ethanol, IPA, *n*-hexane	Acetone, EtOH, IPA, *n*-hexane	1/4 (*w*/*v*) 1/4 (w/v)	100	GC-FID	[51]

Continued

TABLE 11.1 Comparison of Other Extraction Methods With Their Soxhlet Counterpart—cont'd

Matrix	Target Extracted Compound(s)	Other Applied Technique(s)[a]	Extraction Time (h)		Extractant Tested		Extractant Volume (mL)		Monitoring[c]	References
			Other Methods[b]	Soxhlet Method	Other Methods[b]	Soxhlet Method	Other Methods[b]	Soxhlet Method		
Waste lemon peels	D-Limonene	HPTE	0.5	4	*n*-Hexane		25	100	GC-FID	[52]
Kiwi seed	FAs	USAE MAE SFE MIS	0.5 0.3 1 0.21	4	*n*-Hexane *n*-Hexane Supercritical CO_2 *n*-Hexane	*n*-Hexane	400 50 – 300	300	GC-MS	[53]
Lonicera macranthoides	Volatile fraction	USAE MAE HD ME	0.5 0.16 6 24	6	Ethyl acetate Ethyl acetate H_2O Ethyl acetate	Ethyl acetate	30 30 200 30	200	GC-MS	[54]
Portulaca oleracea L.	Flavonoids	MAE USAE CRE ME	0.15 1 2.5 48	4.5	EtOH:H_2O	EtOH:H_2O	25 25 25 25	80	UV-Vis spectrometry	[55]
Spearmint (*Mentha spicata* L.) leaves	Flavonoids	SFE	1	6	Supercritical CO_2	MeOH, EtOH, EtOH:H_2O, petroleum ether	–	150	HPLC-UV	[56]
Soil, fish	PCBPs, PBDEs	ASE MAE	0.25 0.15	24	*n*-Hexane: acetone	*n*-Hexane: acetone	100 300	150	HRGC/ HRMS	[57]

Surface sediments	Polycyclic musks	USAE	0.08	24	*n*-Hexane: CH_2Cl_2	*n*-Hexane	15	300	GC-MS	[58]
		SDSE	5		H_2O + *n*-hexane		550			
		MAE	0.08		95% acetone + *n*-hexane		20			
Grape (*Vitis vinifera* L.) seeds	FAs	USAE	0.5	6	*n*-Hexane	*n*-Hexane	200	300	GC-FID	[59]
Sewage sludge, soils, sediments	*N*-nitrosamines, aromatic amines	USAE MAE	1 0.05	18	MeOH	MeOH	6 6	100	GC-FID	[60]
	PAHs	USAE	0.5	36	Acetone:*n*-hexane	Acetone:*n*-hexane	20	160	GC-MS	[61]
Biochar, biochar-amended soil		Reflux	4		Toluene; CH_2Cl_2; acetone:*n*-hexane		80			
Sesame (*Sesamum indicum* L.)	FAs/Antioxidant compounds	SFE	3.5	8	Supercritical CO_2	Petroleum ether, EtOH	–	100	GC-MS	[62]
		SQE	3		Ethyl ether + EtOH + H_2O		200			
Krill meal	Total lipid content, PLs, FAs, sterols, astaxanthin, vitamin A, tocopherols	CE	2	8	EtOH, IPA, acetone, ethyl acetate, isohexane, *n*-hexane	Petroleum ether	120	100	Gravimetric/ GC-MS/ HPLC-ELSD/ HPLC-UV	[63]
		Folch	–		CH_2Cl_2:MeOH, H_2O		200			
		SBFE	1		Superheated butane		–			

Continued

TABLE 11.1 Comparison of Other Extraction Methods With Their Soxhlet Counterpart—cont'd

Matrix	Target Extracted Compound(s)	Other Applied Technique(s)[a]	Extraction Time (h)		Extractant Tested		Extractant Volume (mL)		Monitoring[c]	References
			Other Methods[b]	Soxhlet Method	Other Methods[b]	Soxhlet Method	Other Methods[b]	Soxhlet Method		
Insects	FAs, triacylglycerols, lipid classes	Folch	2.5	6	CH_2Cl_2:MeOH, H_2O	Petroleum ether	225	30	GC-FID/TLC	[64]
		USAE	0.7		H_2O		600			
Mango peels (*Mangifera indica* L.)	Phenols	SBFE	1	1.5	Superheated H_2O	EtOH	–	40	F-C	[65]
Fish	Total lipid content	Bligh & Dyer	0.1	4	MeOH + $CHCl_3$ + H_2O	*n*-Hexane	48	30	Gravimetric	[66]
		Modified Bligh & Dyer	0.1		H_2O:IPA:*n*-hexane		48			
		Folch	0.08		$CHCl_3$:MeOH, H_2O		105			
		Modified Folch	0.08		Ethyl acetate: ethyl alcohol, H_2O		105			
		Hara & Radin	0.01		*n*-Hexane:IPA		18			
		Roese-Gottlieb	–		Diethyl ether, petroleum ether		34			
		MAE	0.5		Petroleum ether:acetone		30			

Antarctic krill (*Euphausia superba*)	FAs, PLs, and TAGs	CE	–	18	Acetone:EtOH, acetone, ethanol	Petroleum ether	18	100	GC-FID/TLC [67]
		Folch	0.08		$CHCl_3$:MeOH (2:1) + H_2O		80		
Moringa oleifera seeds	FAs	Pilot-scale SFE	2.5	8	Supercritical CO_2	*n*-Hexane	–	250	GC-FID/RSC [68]
Olive leaves	Oleuropein	SFE	2	24	Supercritical CO_2	*n*-Hexane, water, EtOH, MeOH, MeOH:*n*-hexane	–	250	LC-MS/MS [69]

ABTS, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; *ASE*, accelerated solvent extraction; *CE*, conventional extraction; *CRE*, condensing reflux extraction; *DPPH*, 2,2-diphenyl-1-picrylhydrazyl; *ELSD*, evaporative light scattering detector; *EtOH*, ethanol; *FAMEs*, fatty acids methyl esters; *F-C*, Folin-Ciocalteu; *FID*, flame ionization detector; *FAs*, fatty acids; *GC*, gas chromatography; *HD*, hydrodistillation; *HPLC*, high-performance liquid chromatography; *HPTE*, high-pressure/high-temperature extraction; *HRGC/HRMS*, high-resolution gas chromatography coupled with high-resolution mass spectrometry; *IPA*, 2-isopropanol; *MAE*, microwave-assisted extraction; *ME*, marinated extraction; *MeOH*, methanol; *MIS*, microwave-integrated Soxhlet; *MS*, mass spectrometry; *PAHs*, polycyclic aromatic hydrocarbons; *PBDEs*, polybrominated diphenyl ethers; *PCBPs*, polychlorinated biphenyls; *PLs*, phospholipids; *SBFE*, subcritical fluid extraction; *SDSE*, simultaneous distillation-solvent extraction; *SFE*, supercritical fluid extraction; *SQE*, sequential extraction; *TAGs*, triacylglycerols; *TD-NMR*, time-domain nuclear magnetic resonance; *TLC*, thin layer chromatography; *USAE*, ultrasound-assisted extraction; *UV-Vis*, ultraviolet-visible.

[a] Other techniques compared with Soxhlet.

[b] Methods compared with Soxhlet Method.

[c] Analytical measurement monitored during the comparison.

11.8.1 Comparison of methods assisted by high-energy sources versus SE

The most effective energies used to improve, accelerate, or automate leaching are MW, US, or high pressure/high temperature.

11.8.1.1 MAE Versus the SE Method

MAE-based methods have been compared with their Soxhlet counterparts, either by global or individual analysis of the extracts. Thus, the use of a domestic MW oven to favor the extraction of phenols from licorice root can clearly provide more drastic conditions than Soxhlet, therefore resulting in higher extraction yield (47.47 mg/g and 16.38% vs. 41.709 mg/g and 14.49%, respectively), but mainly in dramatic shortening of the extraction time (5–6 min vs. 6 h) after exhaustive multivariate optimization of both types of extraction. The F-C and DHHP· methods used for analysis did not provide information on potential degradation caused by MW energy [49].

11.8.1.2 USAE Versus the SE Method

Two very different tools have been mostly used to implement ultrasound-assisted extraction (USAE): (1) commercial ultrasonic cleaning baths (with nonuniformity in US transmission and a decline in power over time), and (2) ultrasonic probes that provide more reproducible US conditions and allow selection of the power and duty cycle. US-assisted reactors are very common in organic synthesis [1]. In general, analytical US users do not provide information on the characteristics of the US tools involved in their research.

As commented before, it must be emphasized that US is not the best type of energy to accelerate fat removal because of the oxidative power of this energy. Such is the case with USAE of mycosterols (particularly ergosterol) from *Agaricus bisporus* L., optimized by surface methodology and compared with SE with *n*-hexane [50]. Despite the fact that USAE provides a yield of 671.5 mg ergosterol/100 g of dry sample in 15 min, similar to that provided by SE in 4 h, because the analyses were performed by high-performance liquid chromatography with an ultraviolet (UV) detector does not ensure total absence of degradation.

The growing trend for green chemistry leads to green extractants and their comparison with different extraction techniques. Thus, the extraction of passion fruit oils with acetone, ethanol, or isopropanol using US-assisted, stirring, and Soxhlet methods has been reported. The use of a cleaning US bath of unknown power seemed to be compensated for by the exhaustive analysis of the extracts: oil color at room temperature by visual inspection, oil density determined by the AOAC 985.19 method, refractive index at room temperature, and chemical indices determined

by official AOAC methods of analysis (1990), including the acid value (AOAC 969.17), peroxide value (AOAC 965.33), saponification number (AOAC 920.160), nonsaponification matter (AOAC 933.08), iodine value (AOAC 993.20), insoluble impurities (AOCS Ca 3a-46), and moist and volatile matter (AOAC 926.12); all data are supplied in triplicate, but they do not provide information on potential degradation in small proportions [51].

11.8.1.3 High-Pressure/High-Temperature Extraction as Compared with SE

Another current trend is valorization of low-valued residues or even waste. This is the case with exploitation of citrus peels from the juice industry to extract D-limonene as the major constituent in lemon essential oil. After exhaustive multivariate optimization of extraction using a commercial high-pressure/high-temperature extraction device, comparison with conventional SE showed better performance of the former in terms of energy saving (0.6 kW h vs. 2.5 kW h), extraction time (30 min vs. 4 h), and product yield (2.97% vs. 0.95%). Analysis of the extracts by GC with no information on the detector hinders discussion on potential degradation of D-limonene under the extraction temperature (150°C) [52].

11.8.1.4 Comparison of Several Extraction Methods and SE

Comparison of several extraction methods varies from finding the method that produces less degradation of the target compounds [53] to demonstrating the well-known properties of a given method [54].

The low oxidative stability of kiwi seed oil makes it mandatory to search for an appropriate extraction method, adequate packaging, and storage. The desire to achieve higher extraction yields with shorter extraction times and lower energy consumption with the best fatty acid profile has led to develop this study [53].

Several extraction methods based on four nonconventional techniques (USAE, MAE in closed vessel, MIS with *n*-hexane as extractant, and supercritical fluid extraction (SFE) using CO_2) were compared with SE with *n*-hexane. A sensory evaluation test showed the presence of off-flavors in the extracts obtained by Soxhlet or US assistance, which is an indicator of partial degradation, in this case caused by the formation of (*Z*)-hept-2-enal and (2*E*,4*E*)-deca-2,4-dienal. Table 11.2 lists the values of extraction time, temperature, etc.

The comparative extraction of flavonoids from a Chinese medicinal plant was addressed by five methods based on MAE, USAE, reflux extraction, SE, and marinated extraction [55]. The results showed that the MAE method was the most suitable because of its high yield and short extraction time. Nevertheless, quantitation by UV-Vis spectrophotometry with the help of the chromogenic system $NaNO_2$-Al

TABLE 11.2 Extracted Time, Temperature, Yield, and Sensory Evaluation of Extracts From Kiwi Seeds

	SE	MAE	SFE	USAE	MIS
Oil content (w% ± SD)	28.3 ± 1.0	27.8 ± 1.0	26.8 ± 0.5	28.9 ± 1.0	28.0 ± 1.0
Time	8 h	20 min	2.5 h	30 min	30 min
Temperature	69°C[a]	80°C	40°C	50°C	69°C[a]
Color	Pale yellow	Yellow	Pale yellow	Yellow	Pale yellow
Olfactive note[b]	1	2	3	4	5

MAE, Microwave-assisted extraction; *MIS*, microwave-integrated Soxhlet; *SD*, standard deviation; *SE*, Soxhlet extraction; *SFE*, supercritical fluid extraction; *USAE*, ultrasound-assisted extraction.
[a] *n*-Hexane boiling point.
[b] 5-Point hedonic scale, where 5 means absence of off-flavors and 1 corresponds to the greatest detectable presence of off-flavors.
Reproduced from Ref. Cravotto G, Bicchi C, Mantegna S, Binello A, Tomao V, Chemat F. Extraction of kiwi seed oil: Soxhlet versus four different non-conventional techniques. Nat Prod Res 2011;25(10):974–81 with permission from Elsevier.

$(NO_3)_3$-NaOH reduces significantly the validity of the results found in the research. In fact, when SFE with CO_2 as extractant was compared with SE with ethanol, methanol, or petroleum ether, also for the extraction of flavonoids, in this case from spearmint with analysis of the extracts by HPLC separation and UV detection, more bioactive components were extracted by the former (seven vs. five bioactive flavonoids, respectively) [56].

The omnipresence of persistent organic pollutants such polycyclic aromatic hydrocarbons, PCBs, or polybrominated diphenyl ethers (PBDEs) in contaminated environments has promoted the development of a huge number of methods aimed at determining them quickly; in addition, acceleration of sample preparation is a crucial step when the strong retention of these compounds in solid matrices is taken into account. An example of this is the comparison of methods based on ASE, MAE, and SE to remove PCBs and PBDEs from soil and fish using high-resolution gas chromatography coupled with high-resolution mass spectrometry for analysis [57]. The values of extraction times (SE: $\geq$24 h; ASE: $\sim$40 min; MAE: $\sim$30 min), but with temperatures not given, express the mild conditions of SE; however, the much higher pressures of the ASE method (1500 psi, MAE, $\leq$200 psi) could avoid degradation of PBDEs that occurs with the MAE method.

The determination of pharmaceutical and personal care products (PPCPs) in different environmental compartments has become a popular issue due to the emergence of PPCPs as contaminants. Comparison of methods based on MAE, USAE, SE, and simultaneous distillation-solvent extraction for removal of polycyclic musks from sediments was made through the analytical results obtained by GC-MS [58]. The authors used an ultrasonic probe for USAE, but did not supply information on US frequency, probe dimensions, or duty cycle in case US application was

discontinuous. The lack of information stops potential users from reproducing the method.

An unusual comparison was that of the method traditionally used to extract the volatile fraction in plants (hydrodistillation) with SE, MAE, USAE, and cold maceration. The authors concluded that hydrodistillation is still the best choice for pure volatile fraction without organic solvent pollution; a foreseeable conclusion taking into account that heating in open vessels or US application (used to remove gases dissolved in chromatographic phases) does not provide the appropriate conditions to keep volatile components in a liquid phase. Subsequent concentration of the extract is not a suitable step either [54].

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Chapter 12

Ultrasound and Microwave as Green Tools for Solid-Liquid Extraction

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Chapter Outline

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Abbreviations

UAE ultrasound-assisted extraction
US ultrasound
DW dry weight
MAE microwave-assisted extraction
MW microwave
MIS microwave-integrated Soxhlet
MAHD microwave-assisted hydrodistillation
HD hydrodistillation

12.1 Introduction

Conventional extraction of vegetable and microalgal biomass comprises solid-liquid techniques involving organic solvents that present various drawbacks such as toxic residues, chemical transformation of extracts, and toxic wastes. In addition, traditional extraction methods are characterized by limited extraction efficiency. For this reason the development of new processes avoiding the use of toxic solvents while increasing efficiency and sustainability is needed. This is closely related to the principles of green chemistry, whose aim is a more efficient use of energy and resources. Derived from principles of green extraction, six specific aspects related to natural product extractions were formulated, including the search for new renewable natural sources, use of alternative green solvents (mainly water), reduction of energy consumption and unit operations, and the reduction and management of extraction wastes as by-products [1]. In this context an increasing demand for natural molecules produced from a clean and green extraction process with safer solvents is observed. Green extraction of natural products is based on the design of extraction processes, reducing energy consumption and petroleum solvents while ensuring a safe extract with a high quality. This concept encompassed within the 21st century challenges aims to protect both our environment and consumers. In the meantime, green processes must enhance competition of industries and encourage them to be more ecological, economic, and innovative.

Ultrasound and microwaves, considered as green techniques, present several advantages in terms of shortening the extraction time, decreasing solvent volumes, and increasing the yield of targeted compound in comparison with conventional methods. Both techniques can be applied to microalgae biomass, which represent a promising renewable resource. Microalgae are known worldwide for their applications and are recognized as rich raw materials since they are composed of a plethora of bioactive compounds, namely, pigments (carotenoids and chlorophylls), proteins, polysaccharides, and essential fatty acids [2]. These various compounds have been used over the years in different industries, including cosmetics, animal feed, human food, and energy. Despite their high economic value, commercialization has not reached its maximum due to the high costs of conventional extraction and purification processes

typically used. In this chapter the benefits of using either ultrasound or microwaves in extraction process will be discussed. These two techniques offer different approaches: ultrasound is generally used to improve conventional solvent extraction, whereas microwaves are known for their ability to extract bioactive compounds via heating without any solvent. The first part of this chapter is dedicated to the presentation of ultrasound principles and applications to microalgae followed by instrumentation. The second part will focus on microwaves as an extraction technique.

12.2 Ultrasound

12.2.1 Principle and Theory

12.2.1.1 General Definitions

Ultrasound is a mechanical oscillating sound wave requiring an elastic medium to sustain it. Ultrasound frequencies range from 20 kHz to 10 MHz, higher than the threshold for human auditory detection, which is between 16 Hz and 20 kHz. The main physical parameters of ultrasound include power, frequency, and amplitude. The frequency used mainly depends on process considerations related to equipment. Ultrasonic power energy transmitted through the medium can be expressed as ultrasonic power (W), ultrasonic intensity (W/cm^2), or ultrasonic power density (W/cm^3 or W/mL) [3]. Calorimetric measurements to assess actual ultrasound power P ($J\ min^{-1}$) are calculated by the following Eq. (12.1):

$$P = m \times C_p \times \frac{dT}{dt} \tag{12.1}$$

where C_p is the heat capacity of the solvent at constant pressure (for water, $C_p = 4180\ J\ K^{-1}\ kg^{-1}$), m is the mass of solvent (kg), and dT/dt is temperature increase per minute ($K\ min^{-1}$).

Then the applied ultrasonic intensity (UI) is determined, using the calculated power, as shown by Eq. (12.2):

$$\mathrm{UI} = \frac{4P}{\pi D2} \tag{12.2}$$

where UI is the ultrasonic intensity (W/cm^2), P the ultrasound power (W) as calculated by Eq. (12.1), and D the internal diameter (cm) of the ultrasonic probe.

Ultrasounds can be classified into diagnostic and power ultrasound types according to their frequency and intensities:

- Diagnostic ultrasound (low power and high frequency) between 1 and 10 MHz and intensity below 1 $W\ cm^{-2}$ are mainly used in the medical field as a diagnostic or a control tool.

- Power ultrasound (high power and low frequency) from 20 kHz to 1 MHz [4] and intensity above 1 W cm^{-2} are employed to generate acoustic cavitation bubbles, leading to physical or chemical effects in the medium for sonochemistry (chemical reaction acceleration), agriculture (water dispersion), and industry (cutting and plastic welding).

12.2.1.2 Acoustic Cavitation Phenomenon

As the power ultrasound wave spreads through an elastic medium, it induces a longitudinal displacement of particles, acting as a piston on the medium surface, resulting in a succession of compression and rarefaction phases [5]. When ultrasonic power reaches a certain threshold, tiny bubbles are formed by negative pressure overcoming the attractive force of molecules in the medium during the rarefaction cycle. The critical distance between molecules (R) for water is around 10^{-8} cm, and the applied pressure is 10.1×10^5 kPa, where $Pc = 2\sigma/R$, σ being the surface tension [5].

In the case of transient cavitation, ultrasound waves induce cavitation bubbles, whose size varies with the frequency of the sound wave. Due to rectified diffusion, bubbles grow during rarefaction cycles and shrink during compression phases [6]. When bubbles reach a critical diameter, they collapse during a compression cycle, inducing a transitory release of a huge amount of energy (Fig. 12.1). The temperature and pressure reached during bubble collapse have been estimated to be up to 5000 K and 100 MPa [7]. Localized hot spots can significantly accelerate the chemical reactivity of a medium. If the acoustic cavitation bubbles are formed close to a solid surface, asymmetrical collapse generates microjets and shock waves directed toward the solid surface [8]. This results in the permeation of microalgae cells, with the release and solubilization of bioactive compounds by the medium (Fig. 12.2) due to physical forces such as mechanical agitation, microjets, shear forces, microstreaming, hot spots, and shockwaves [6].

12.2.1.3 Factors Influencing Ultrasound Assisted Extraction

Cavitation is affected by a solvent's physical properties such as viscosity, saturation vapor pressure, and surface tension [7]. Indeed, for cavitation, it is necessary to apply a negative pressure during the rarefaction cycle large enough to disrupt the solvent's natural cohesive forces and thus create a vacuum [9]. Moreover, an increase in viscosity intensifies molecular interactions and thus hinders cavitation. Similarly, a high surface tension and vapor pressure (both temperature-related) adversely affect cavitation phenomenon. In the case of pure water, a pressure of the order of 10^5 kPa is required to initiate cavitation [5].

Temperature can also impact cavitation by changing the vapor pressure, the viscosity, and the surface tension of the solvent. Moreover, when temperature is close to

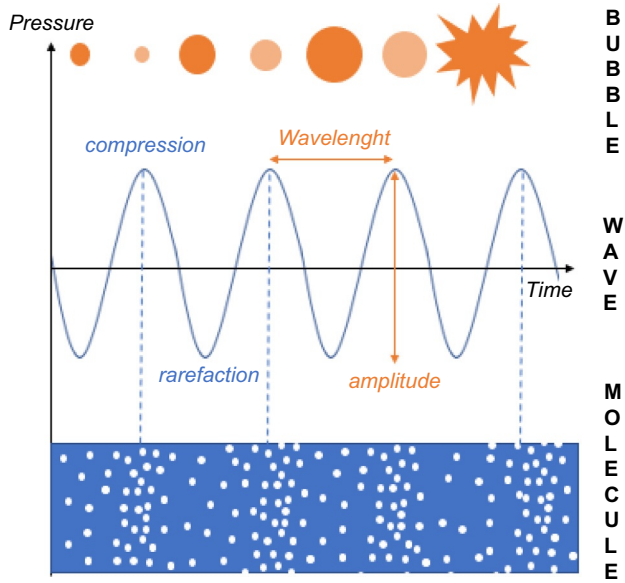

Fig. 12.1 Ultrasonic parameters related to cavitation phenomenon.

the boiling point of the solvent, cavitation bubbles are generated more easily than at low temperature; however their implosion capacity is reduced. High temperature also enhances solvent diffusion rates and mass transfer. Therefore solvent temperature must be controlled during UAE [7].

Ultrasound frequency and intensity play a key role in cavitation. For extraction the most commonly used frequencies are between 20 and 40 kHz. For cavitation bubbles to form requires a short delay during the rarefaction phase; high frequencies adversely impact cavitation because the higher the frequency, the shorter are the rarefaction phases, thus making the formation of the cavitation bubble difficult. Therefore high-frequency ultrasound is said to be nondestructive, since the frequency is too high to allow the acoustic cavitation phenomenon.

Moreover, cavitation bubbles can only be generated when the pressure applied to the medium (PL) drops below the saturation vapor pressure of the liquid (PV). The pressure applied to the medium under ultrasound results from the sum of the hydrostatic pressure and the acoustic pressure. Therefore the higher the pressure applied to the medium, the more difficult it will be to induce cavitation. To allow cavitation, ultrasonic pressure and intensity must be increased.

Acoustic cavitation bubbles are usually made of gas such as water vapor. Cavitation thus originates from gaseous occlusions in the solvent to which ultrasound is applied. The presence of dissolved gas and impurities is conducive to the cavitation phenomenon. Each impurity in the liquid or on the surface of the equipment can

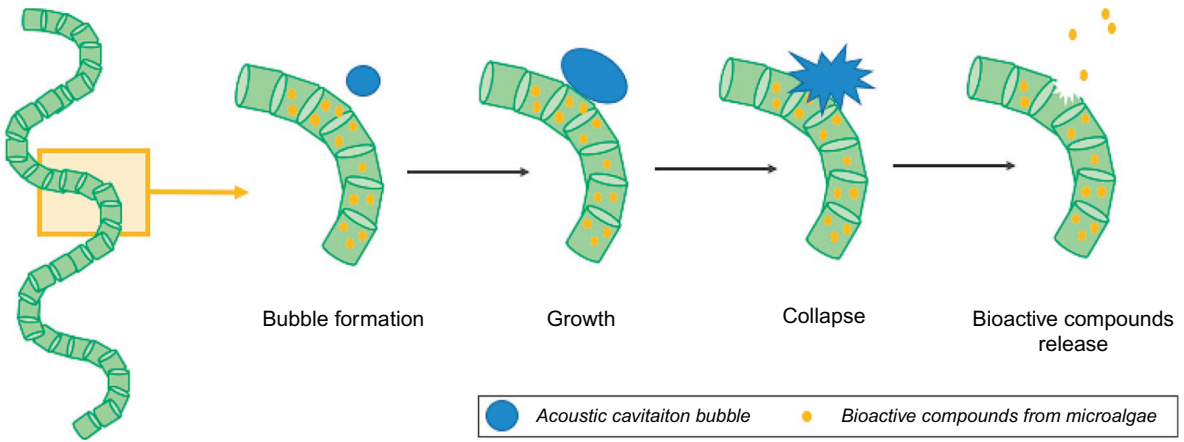

Fig. 12.2 Acoustic cavitation phenomenon and mechanism.

serve as a cavitation site for new bubbles. In fact, gases trapped in the interstices and depressions of impurities are released during the rarefaction cycles and constitute a new cavitation nucleus.

12.2.2 Protocols and applications

Microalgae are one of the most promising renewable resources that can strongly contribute to future sustainable development. Thanks to their extensive biodiversity and metabolism, microalgae can produce a wide range of interesting bioactive compounds suitable for several industrial applications. Microalgal cells contain algeenan and are notoriously difficult to lyse. Thus, to extract microalgal compounds, cell disruption is a key step. Cell disruption can be addressed by several methods including ultrasound. The application of ultrasound-assisted techniques to the extraction of target compounds from diverse microalgae is summarized in Table 12.1 [15].

In most cases, probe systems were used to extract target compounds from microalgae such as *C. vulgaris*, *P. tricornutum*, and *Nannochloropsis* [11, 12, 14]. For example, fucoxanthin UAE extraction from *P. tricornutum* was performed using 0.5 g of dried powder of *P. tricornutum* in 25 mL of ethanol at 70 kHz for 30 min at room temperature. UAE decreased the extraction time compared with conventional treatment and enabled 15.96 mg of fucoxanthin per gram of *P. tricornutum* dry weight to be isolated [11]. To target lutein, fresh *C. vulgaris* (1 g) was treated with 1.23% (v/w) Viscozyme at optimal conditions (pH 4.5, 50°C, and 2 h) and then

TABLE 12.1 Ultrasound-Assisted Extraction of Bioactive Compounds From Microalgae

Microalgae	Targeted Compound	UAE Protocol	Yield	Reference
Dunaliella salina	Total carotenoids	US 3 min, 0.105 g in 5 mL of DMF	27.7 μg/mg dw	[10]
Phaeodactylum tricornutum	Fucoxanthin	US 70 kHz, 30 min, 0.5 g in 25 mL of ethanol	15.96 mg/g dw	[11]
Chlorella vulgaris	Lutein	35 kHz, 57 W/cm^2, 5 h, 37.7°C 90% ethanol	3.16 ± 0.03 mg/g[a]	[12]
Haematococcus pluvialis	Astaxanthin	40 kHz, 200 W, 16 min, 41.1°C, liquid-to-solid ratio 20:1 (mL/g), 48.0% ethanol in ethyl acetate	27.58 mg/g	[13]
Nannochloropsis spp	Total phenolics	US probe, 24 kHz, 400 W, 15 min	33%	[14]

[a] Lutein recovery, 3.16 ± 0.03 mg/g wet *C. vulgaris*.

sonicated. Ultrasound extraction was performed in a penetration four-channel ultrasonic generator at 35 kHz and 57 W/cm^2 for 5 h. Lutein was efficiently extracted by ultrasound combined with enzymatic pretreatment, 3.16 ± 0.03 mg/g of wet *C. vulgaris* [12]. High-added-value compounds were also extracted from *Nannochloropsis* cell using US-assisted extraction with a probe system. The extraction was carried out on a 250 g microalgal suspension with at 400 W and 24 kHz for 15 min. The yield of total phenolic compounds from *Nannochloropsis* was 33% [14]. In another study, an ultrasound bath or reactor was used to disrupt the microalgae cell wall [10, 13]. To extract total carotenoids from *D. salina*, 0.105 g of lyophilized microalgae suspended in 5 mL of *N*,*N*-dimethylformamide was sonicated for 3 min. A maximum yield of 27.7 ± 1.4 μg total carotenoids per milligram of dry *D. salina* was achieved [10]. Likewise, astaxanthin was extracted from *H. pluvialis* using ultrasound. One gram of microalgal powder was mixed with 48.0% (v/v) ethanol in ethyl acetate with a liquid-to-solid ratio 20:1 (mL/g) that was extracted by ultrasound for 16 min at 40 kHz in a US bath. The maximum yield of astaxanthin by UAE was 27.58 ± 0.40 mg/g [13].

12.2.3 Laboratory and industrial scale ultrasonic devices

Ultrasonic equipment consists of an electric power generator, transducer, and emitter, which guides the ultrasonic waves into the medium. High-power ultrasound can be applied using either of two devices, an ultrasonic bath or probe. Both systems utilize a transducer as the source of ultrasound power [7]. The transducer converts electric energy into sound energy by mechanically vibrating at ultrasonic frequencies. Piezoelectric (pzt) transducers, based on a crystalline ceramic material, are typically used in ultrasonic processors and reactors [9]. This section will focus on the different types of ultrasound equipment, from the laboratory to pilot scale. Combined and industrial techniques will also be presented.

Ultrasonic baths usually operate at a frequency around 40 kHz and may be equipped with temperature control. They are inexpensive, available, and easy to use. However, compared with probe systems, the poor reproducibility and low power of ultrasound delivered to the sample are major drawbacks. Indeed, the intensity is highly attenuated by the water contained in the bath and the glassware used for the experiment [7]. Another bath system developed by REUS (France) operating at 25 kHz is mostly used for extraction applications (Fig. 12.3A). It consists of a stainless-steel reactor equipped with a double-layered mantle with water circulation to allow temperature control with cooling/heating systems. REUS developed different reactor models to support process scale-up from the laboratory to the industrial scale as shown in Fig. 12.3. Reactors of 1, 3, and 5 L can be used for process optimization at the laboratory scale (Fig. 12.3 A and B) and a 30 L reactor (Fig. 12.3C) for pilot plants. The pilot reactor has four transducers generating ultrasound

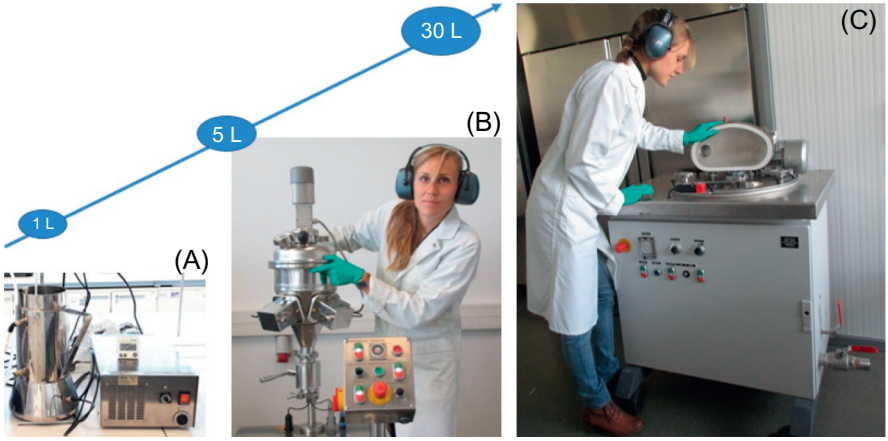

Fig. 12.3 Ultrasound devices: from laboratory (A and B) to pilot scale (C).

frequency at 25 kHz and a total power of 800 W. This apparatus was used to extract polyphenols from maritime pine sawdust waste and apple pomace [16, 17].

High-power ultrasonic probes are generally preferred for extraction. The probe system is more powerful than the bath design due to delivery of a more intense ultrasonic wave through a smaller surface (tip of the probe) directly immersed in the solution. Generally operating at 20 kHz, the probe is bounded to a transducer immersed into the reactor resulting in a direct delivery of ultrasound to the extraction media with minimal energy loss. There are several probe designs with different lengths, diameters, and tip geometries. Probe selection is made according to the application and the sample volume. As the ultrasound intensity delivered by the probe to the liquid medium generates heat, a cooling system is required for extraction. Manufacturers of high-power ultrasound equipment have also designed devices with specific operational features such as continuous flow mode with pressure control. In addition, the system can be equipped with temperature control, enabling manothermosonication, which is a technique involving the simultaneous application of pressure, temperature, and ultrasound.

Hielscher (Germany) build ultrasonic device with probe system from lab to pilot scale (Fig. 12.4). Probe size and design and reactor capacity vary according to the volume of sample to be treated.

For a higher efficiency and maximum yield, ultrasound can be combined with another extraction technique. For instance, it can be coupled with a Soxhlet extractor. Fats and oils are traditionally extracted using Soxhlet extraction, which was invented in 1879. This apparatus functions by iterative percolation of condensed vapors of a boiled solvent, generally n-hexane, in a series of permeation and solubilization processes to wash intracellular constituents out of the plant matrix. Several hours of

Fig. 12.4 Ultrasonic devices equipped with a probe: from lab to pilot scale. (A and E), lab scale probe with US chamber of 200 mL, 40 L/h. (B, C, and D), pilot ultrasound equipment with a 1 L US chamber, 1000 L/h.

Soxhlet extraction are needed to extract lipid compounds, and a rather large volume of organic solvent is needed. To accelerate the extraction, Soxhlet extraction can be combined with ultrasound (Fig. 12.5A). A few processes utilize the "sono-Soxhlet" method to reduce extraction time and improve oil recovery rate [9]. This technique was developed by Luque de Castro and Chemat's team. Ultrasound can be applied outside or inside the extraction chamber to help solid-liquid extraction and migration of metabolites from a solid matrix to solvent. Thus sono-Soxhlet combines advantages of both Soxhlet extraction (extraction repeated by a fresh solvent) and ultrasound extraction (reduction of extraction time) by enhancing mass transfer.

To extract essential oils from vegetable biomass, traditional alembic distillation is used. This technique, also called Clevenger extraction, isolates aromatic compounds by distillation and boiling of the matrix in water. It takes several hours (from 6 to 24 h) and requires a large volume of water to obtain a useful yield of essential oil. During distillation, fragrant plants exposed to boiling water or steam release their essential oils through evaporation. Steam and essential oil vapors are condensed together, collected, and separated in a vessel called the "Florentine flask." As there is a growing interest in natural products, the flavor and fragrance industry are seeking new extraction methods that are more efficient and faster than traditional methods. As a solution, a sono-Clevenger was developed (Fig. 12.5B). This is an original combination of ultrasound cavitation and Clevenger distillation at atmospheric or reduced pressure for the extraction of essential oils from plant materials.

For industrial purpose, ultrasound equipment with multiple transducers was developed to provide sufficient power to treat large-scale samples. Two main companies are involved in manufacturing ultrasonic devices for industrial-scale applications: REUS (France) and Hielscher (Germany). Hielscher developed an ultrasonic device for industrial use with a power range from 500 to 16,000 W. These are probe

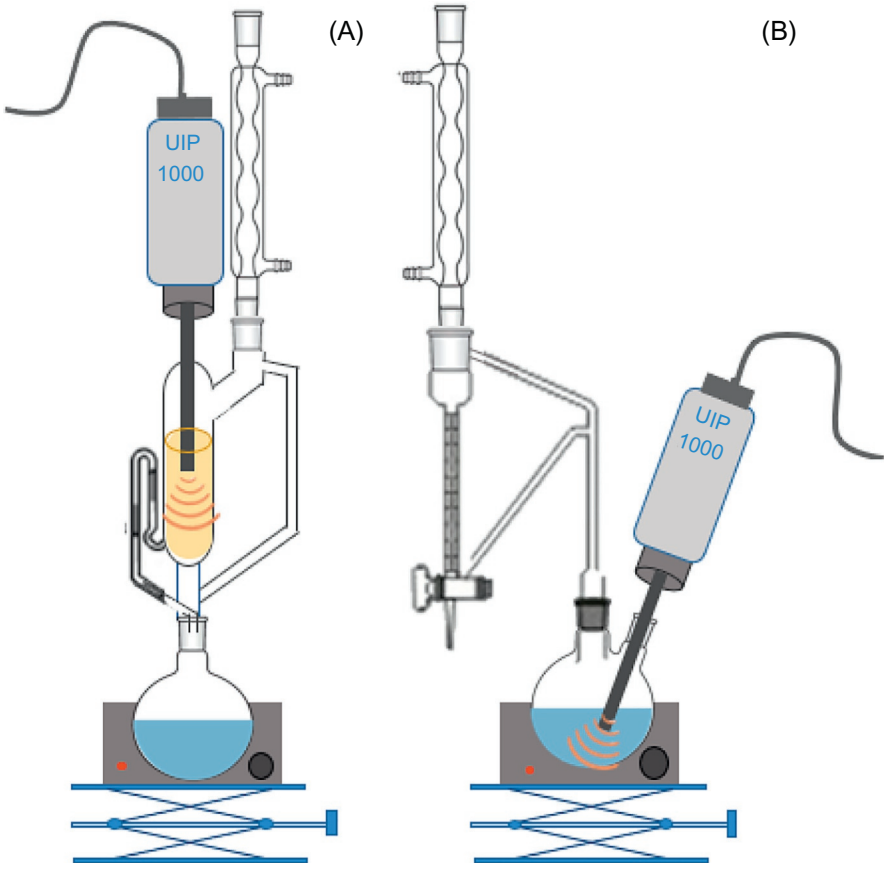

Fig. 12.5 Extraction techniques combined with ultrasound. (A) Sono-Soxhlet. (B) Sono-Clevenger.

system, which enable an increase in extraction yield and time-saving. REUS builds ultrasonic reactors combined with a pump system to facilitate mixing of the extract, tank draining, and reactor cleaning. Reactor capacity ranges from 30 to 1000 L [7].

12.3 Microwave

12.3.1 Principle and theory

12.3.1.1 General Definitions

Microwaves (MW) are nonionizing electromagnetic radiation with a frequency from 300 MHz to 300 GHz [18]. However only few frequencies are allotted for industrial,

scientific, and medical uses, such as 0.915 and 2.45 GHz [19]. In the last decade, MAE has been successfully applied to various fields of chemistry since Ganzler et al. firstly reported this technique [20]. The fundamentals of the MAE process are different from those of conventional methods, such as maceration, because extraction occurs as the result of changes in the cell structure caused by electromagnetic waves. In MAE the process acceleration and high extraction yield are the result of a synergistic combination of two transport phenomena: heat and mass gradients [21].

12.3.1.2 MW Mechanism

Microwave heating is based on two principles: ionic conduction and dipole rotation [6]. Ionic conduction refers to the induced electrophoretic migration of charge carriers such as ions and electrons under the influence of the electric field produced by microwaves. This displacement is responsible for friction between moving ions and the medium leading to heat production.

Dipoles are molecules with polarized bonds due to electronegativity difference between atoms.

In the absence of an electric field, molecules are randomly oriented by thermal agitation (Fig. 12.6). When a continuous electric field is applied to the medium, dipoles are placed uniformly in the direction of the field: this is the induced global dipole moment. For microwaves, an alternating electric field is applied to the medium. Under the effect of this field, dipoles are oriented in the direction of the field and disorient when the field is canceled during the microwave treatment at a frequency of 4.9×10^9 per second (Fig. 12.6) [21]. Dipole rotation happens when dipolar molecules attempt to align themselves with the alternating electric field. The oscillation of these dipolar species leads to collisions between themselves

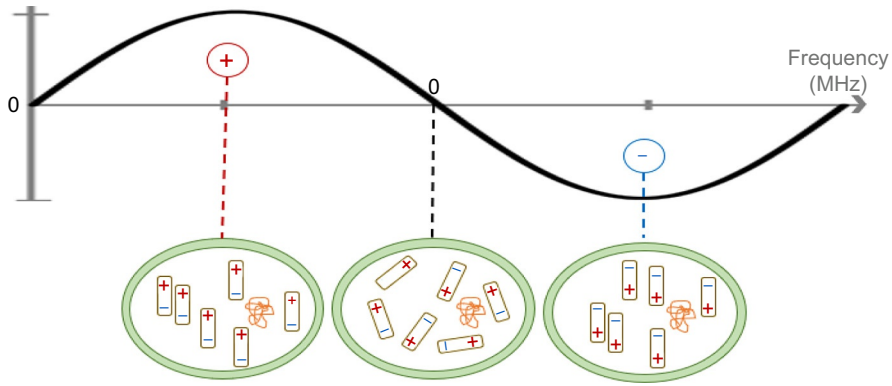

Fig. 12.6 Dipole orientation in the cell according to MW alternating electric field.

and surrounding molecules and thus creates heat. Therefore electric energy is converted into kinetic energy with heat transmitted from the inside of the system to the outside, unlike conventional heating. Heat transfer by microwave irradiation can also cause evaporation of moisture inside the cell, developing significant pressure inside the biological matrix. This pressure change can rupture cell membranes and accelerate solvent penetration and release of intracellular compounds. Contrary to conventional heating, where heat is transferred from the heating medium to the interior of the sample, heat is dissipated volumetrically inside the irradiated medium in the case of MAE. It is also possible to work directly with fresh material containing natural water, also called in situ water.

12.3.1.3 Parameters Influencing the Propagation of Microwaves

For microwave-assisted extraction, various parameters are to be considered, such as the nature of the solvent and the matrix. Indeed, MW energy is strongly dependent on the dielectric susceptibility of both the solvent and solid matrix. Owing to a high natural moisture content, microalgae are susceptible to microwave disruption. Rapid internal heating of these structures brings about effective cell rupture, which facilitates releasing the analytes into the cold solvent [22]. Most of the time, the selected solvent has a high dielectric constant, and therefore strongly absorbs microwave energy. Two solvent properties are important: solubility of the bioactive compound in the solvent and ability of the solvent to absorb microwave energy. However, for thermally labile compounds, a significant increase in temperature is undesirable. Microwave-transparent solvents are then used so that only the matrix is heated and releases the target compounds into a cold environment. The mechanism of microwave-assisted extraction typically implies the presence of intracellular water. Indeed, during microwave treatment, heating of the intracellular water causes evaporation inside the cell, initiating wall rupture, and the release and solubilization of target compounds in the solvent. The ability of a solvent to absorb microwave energy and convert it to heat depends on its dissipation factor (tan δ) [21]:

$$\tan \delta = \varepsilon' / \varepsilon'' \tag{12.3}$$

where ε' denotes the dielectric constant or relative permittivity of the solvent, reflecting its capacity to be polarized by an electric field, and ε'' is the dielectric loss factor related to the transformation efficiency of electromagnetic energy into heat. Thus the higher the dissipation factor, the greater the amount of thermal energy that will be produced. In this way, polar solvents such as water have a high dielectric loss, meaning that they strongly absorb microwaves. Conversely, nonpolar solvents such as hexane do not heat up under the effect of microwaves; they are said to be "transparent" to microwaves [23]. Moreover, to propagate properly, microwave radiation must be used in a suitable container. Like solvents, materials have different

behaviors toward microwaves according to their composition and can be classified into three categories: transparent, absorbent, and reflective [24]:

- *Transparent* materials allow microwave radiation to pass through easily with little attenuation. They are considered as low dielectric loss materials or insulating materials, such as glass (Pyrex), ceramics, and air.
- *Absorbents*, as their name implies, absorb microwaves. The absorbed electromagnetic energy is then converted to heat. These are loss dielectrics or high dielectric loss materials whose properties range from conductors to insulators.
- *Reflective* or opaque materials, typically conducting materials with free electrons such as metals, are to be avoided in microwave radiation. Indeed, they could degrade the equipment by creating an electric arc with reflected waves.

12.3.2 Protocols and theory

Some microalgal species present complex exopolysaccharide envelopes that require intense disruption techniques such as microwave treatment. The main advantages of MAE are minimal energy and solvent consumption, shorter operation times, good selectivity, high extraction yields, and good reproducibility. MAE causes the compounds of interest to desorb from the microalgal matrix because free water molecules inside the cells are heated. By this, localized heating and expansion occur, during which microalgal cell walls are ruptured, allowing the extracted molecules to flow toward the solvent. Several targeted compounds extracted from microalgae using microwaves are presented in Table 12.2.

MAE of *C. closterium* allowed total extraction of fucoxanthin in 3–5 min. Fifty milligram of freeze-dried cells suspended in 30 mL of acetone were irradiated at 50 W and 56°C with magnetic stirring. The yield of fucoxanthin was 4.24 ± 0.09 μg per milligram of dry microalga [25]. For the extraction of astaxanthin from *H. pluvialis*, MAE was conducted with 0.1 g of microalgae in 10 mL of acetone. The highest astaxanthin recovery (74.4%) was obtained with 60% of 1200 W power at 75°C for 5 min [26]. In comparison with other extraction techniques (maceration and Soxhlet), MAE was the most efficient. To extract total carotenoids from *A. platensis*, two extraction techniques were tested: MAE and SFE. MAE extracted the tocopherols and carotenoids (629 ± 0.13 μg/g expressed as equivalent of β-carotene). Procedural details were as follows: methanol/ethyl acetate/light petroleum (1:1:1 v/v) at 400 W power, 1 bar, and 15 min extraction time with a 0.06 ratio of biomass to solvent (w/*v*). The extraction vessel was made of PFA Teflon [27]. Likewise, total lipids were extracted from *N. oculata* using microwave-assisted extraction. A total of 0.5 g of dried biomass was mixed with distilled water (50% w/w), 1.5 mL of a chloroform/methanol mixture (2:1 v/v) was added, and microwave was extracted for 1 min at 300 W and 80°C. MAE achieved a significantly higher

TABLE 12.2 Microwave-Assisted Extraction of Bioactive Compounds From Microalgae

Microalgae	Targeted Compound	MAE Protocol	Yield	Reference
Cylindrotheca closterium	Fucoxanthin	50 W, 3–5 min, 56°C, 50 mg microalgae in 30 ml acetone	4.24 ± 0.09 μg/mg dw	[25]
Haematococcus pluvialis	Astaxanthin	720 W, 5 min, 75°C, 0.1 g, 10 mL acetone	74 ± 4% (recovery[a])	[26]
Arthrospira platensis	Total carotenoids	400 W, 15 min, 50°C, 0.06 biomass/solvent (w/v), methanol/ethyl acetate/light petroleum (1:1:1 v/v)	629 ± 0.13 μg/g[b]	[27]
Nannochloropsis oculata	Total lipids	300 W, 1 min, 80°C, chloroform/methanol mixture (2:1 v/v)	33.6 ± 2.6%	[28]
Phaeodactylum tricornutum	Fucoxanthin	850 W, 2.45 GHz, 2 min, 30°C, 0.5 g algae, 10 mL ethanol	4.59 mg/g[c]	[29]

[a] Astaxanthin recovery (%) calculated as follows: $\frac{\text{astaxanthin extracted}}{\text{total astaxanthin in the microalga}}$.
[b] Quantified as equivalents of β-carotene in mg/g.
[c] Yield expressed as milligram of fucoxanthin per gram of microalgae.

yield (33.6 ± 2.6%) with only 1 min of extraction time compared with conventional ultrasonic extraction (20 min, 25°C, and 28.51 ± 0.8% yield) from wet *N. oculata* biomass [28]. In another study, fucoxanthin was extracted from *P. tricornutum* by MAE at 850 W and 2455 MHz. Fixed parameters were sample weight (0.5 g), solvent volume (10 mL of 100% ethanol), and stirrer speed (1000 rpm). The fucoxanthin recovery rate was 32.26% (4.59 mg of fucoxanthin per gram of microalga) [29].

12.3.3 Microwave devices for laboratory and industrial scale extraction

The microwave oven consists of three main elements: the magnetron that generates the MW frequency, the waveguide, and the cavity (Faraday cage). The magnetron generates electrons that are guided by the continuous electric field via a cylindrical metal tube (the waveguide) to the microwave cavity. Microwave systems for the extraction of bioactive compounds are of two different types depending on the way the microwave energy is applied to the sample: (1) multimode systems, in which the microwave radiation is randomly dispersed in a cavity enabling it to irradiate the sample evenly, and (2) single mode, in which the microwave radiation is

focused on a restricted zone where the sample is more intensely irradiated than in the case of multimode systems [6]. MAE can be performed in open or closed extraction vessels. Open vessels are used for low-temperature extraction at atmospheric pressure, whereas closed vessels are suitable for high temperature extractions [18].

Microwave equipment is available from a laboratory to a pilot plant scale (Fig. 12.7). The pilot plant apparatus is a multimode microwave reactor, with four magnetrons (2450 MHz) and a maximum total power of 6000 W delivered in 500 W increments [30]. The stainless-steel microwave cavity has a capacity of 150 L and possesses a removable, rotating PTFE drum allowing up to 75 L of plant material to be processed. To ensure a homogeneous microwave distribution of the material inside the drum, the apparatus is fitted with a rotation system. The drum circumference, entirely perforated, allows vapor and liquid to circulate. The device is controlled by an industrial touch screen control terminal and possesses sensors placed on waveguides to measure microwave absorption to adjust the delivered power if absorption is too low. Temperature is monitored by a temperature detector (PT-100) inserted into the cavity.

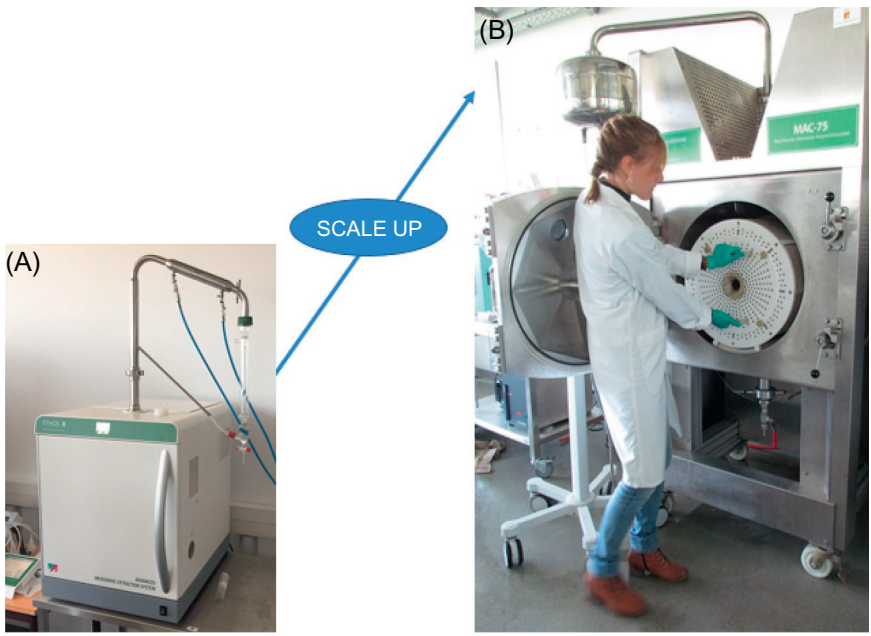

Fig. 12.7 Microwave equipment from lab to pilot scale: Ethos X apparatus (A) for laboratory experiments, Mac 75 (B) to treat sample till 75 kg.

To improve efficiency, microwave energy can be combined with other extraction methods. For example, to extract fatty acids, a microwave-integrated Soxhlet (MIS) apparatus was developed (Fig. 12.8A). This process involves the use of a polytetrafluoroethylene/graphite material that allows the diffusion of heat created by microwave absorption. It is particularly useful for solvents transparent to microwave irradiation, such as hexane. MIS extraction preserves the advantages of conventional Soxhlet extraction (matrix depletion) while overcoming restrictions such as the long extraction time and nonquantitative extraction of strongly retained solutes due to the enhanced cleavage of solute-matrix bonds by microwave energy [31]. This process ensures complete, rapid, and accurate extraction of both dry and wet samples, without an extra heat source. Microwave irradiation accelerates the extraction process without inducing noticeable changes in oil composition of the extract [21]. Moreover, no evaporation step is needed since it concentrates the final extract.

Another combined technique used to extract volatile compounds is microwave-assisted hydrodistillation (MAHD) (Fig. 12.8B). The hydrodistillation (HD) apparatus is placed inside a microwave oven with a side orifice through which an external cooler joins the vessel containing the plant material and water, inside the oven. Microwave heating is responsible for water boiling and reflux. Essential oil is then

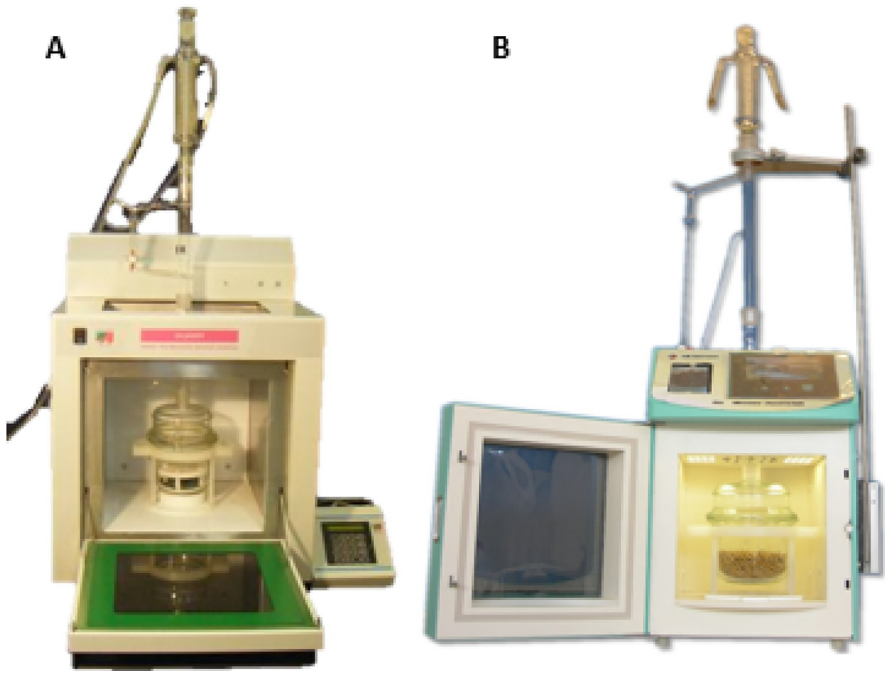

Fig. 12.8 Microwave combined with Soxhlet (A) and Clevenger (B).

decanted from the condensate. This method was reported to be more efficient for the extraction of essential oils from *Thymus vulgaris* than a Clevenger-type apparatus (Fig. 12.8B) [32]. The extraction time was reduced by one-half compared with the conventional process and microscopic analysis of the thyme material after extraction MAHD and HD indicated that microwaves enhanced rupture of the glandular walls leading to a higher extraction yield. Moreover, MAHD is eco-friendly since it requires less water and time than conventional hydrodistillation.

At an industrial scale, some installations can extract up to 100 kg of fresh material. For instance, Huayuan technology produces microwave extraction equipment with capacities of 50–500 L [21]. SAIREM also offers microwave extractors for industrial processes such as microwave-assisted continuous flow reactors equipped with auger-type stirring systems to ensure good homogeneity of heating and product transfer. The reactor can treat up to 200 kg of biomass per hour with a power of 75 kW.

12.4 Conclusion and Perspectives

Ultrasound and microwave-assisted extraction offer several advantages such as efficient cell wall disruption. In numerous articles, these techniques were utilized for the extraction of target bioactive compounds from microalgae, for example, carotenoids (fucoxanthin and astaxanthin), lipids, and phenolics. Besides these techniques are considered green and eco-friendly. Indeed, compared with conventional methods, such as maceration or hydrodistillation, they require less solvent and time and lead to a higher extraction yield and an extract of equivalent or even better quality. Moreover, it is also possible to work with green solvent such as water or without any solvent from fresh material in the case of microwave-assisted extraction. These two techniques are considered "mature" technologies since they are already used in industry, particularly the food industry, for various purposes. It is also easy to find ultrasound and microwave generators from the laboratory to pilot plant scale.

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Chapter 13

Pressurized Liquid Extraction

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Chapter Outline

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13.1 Introduction

Pressurized liquid extraction (PLE) is regarded as an advanced extraction technique, due to the advantages it has over traditional extraction processes. PLE employs solvent extraction at high temperatures and pressures, always below their respective critical points, so that the solvent is maintained in the liquid state during the whole extraction procedure. This technique is also referred to as accelerated solvent extraction (ASE), pressurized fluid extraction (PFE), pressurized hot solvent extraction (PHSE), high-pressure solvent extraction (HPSE), high-pressure high-temperature solvent extraction (HPHTSE), and subcritical solvent extraction (SSE).

As a result of utilizing these particular conditions of pressure and temperature, a change in the solvent physicochemical properties occurs. For instance, mass transfer rates are enhanced, while at the same time, solvent surface tension and viscosity are decreased and solubility of analytes is increased. This allows the solvent to penetrate easier and deeper into the solid matrix being extracted. As a consequence, significantly higher extraction yields are obtained compared with conventional extractions. Therefore PLE results not only in faster extraction processes but also in lower solvent consumption for the sample preparation of solids. In addition, most of the instruments used for PLE are automated, allowing the development of less-labor intensive methods and improving reproducibility.

The possibility of choosing a wide range of solvents makes PLE a versatile technique. A solvent must be selected by considering the nature of the compounds to be extracted. The use of generally recognized as safe (GRAS) solvents, such as ethanol, ethyl acetate, ethyl lactate, or D-limonene, is preferred as environmentally friendly solvents. Among them, the greenest possibility involves the use of water. When water is employed as extraction solvent, this technique is commonly called pressurized hot-water extraction (PHWE), but terms such as hot-water extraction (HWE), subcritical water extraction (SWE), and high-temperature water extraction (HTWE) are also used. As it will be described later, water at elevated temperatures and pressures could be an alternative to the use of ethanol or acetone to extract medium- or low-polarity compounds [1].

The parameters that should be carefully considered in a PLE method development are described and discussed in Section 13.2; however, it is worth to mention that extraction temperature is by far the most important factor. In theory, the higher the temperature, the greater the yield. This statement has to be closely examined, because it is widely known that some bioactive compounds, commonly extracted by PLE, are thermolabile and high temperatures might have a negative effect on their bioactivity. On the other hand, several reports have demonstrated that the influence of extraction pressure is null beyond the point at which the solvent is maintained in the liquid state [2–4]. Extraction time should be minimal but

sufficient for adequate mass transfer. In this regard the most critical parameter is the type of extraction (dynamic mode, static mode, or a combination of both). In the dynamic mode, the solvent continuously flows through the sample, while in the static mode, sample and solvent are maintained for a specific time at constant temperature and pressure. In the latter, longer extraction times favor thermal degradation, once the solvent solution is saturated with analytes extracted from the sample and resulting in slower extraction procedures. Therefore, optimization of different factors influencing the outcome of the extraction process for every sample type is mandatory. A full factorial experimental design 3^2, three levels and two factors, extraction temperature and solvent composition [5, 6], can be useful to optimize the extraction conditions.

The importance given to developing faster, less toxic, and more environmentally friendly extraction methods has made PLE popular, above all in pharmaceutical and food industries. Different PLE applications have been developed for the extraction of contaminants from different food [7]; for organic pollutants from a variety of solid and semisolid environmental samples, such as soil matrices, sediments, and sewage sludge [8]; and for the extraction of bioactive compounds from natural matrices [2]. Regarding natural products, plants are, by far, the most frequently studied samples [3]. However, different reviews have described the extraction of bioactive compounds from seaweed, microalgae, and other food by-products [9, 10]. PLE is mostly used to extract antioxidants (e.g., phenolic compounds and carotenoids) and other bioactive compounds with antiinflammatory, antimicrobial, and antiviral properties.

The basic principles of PLE and each operational mode (static or dynamic), together with the basic instrumentation elements, are discussed in detail in this chapter. In Section 13.4 the applicability of PLE is evaluated with special emphasis on contaminant compounds and on the diversity of matrix components that can be extracted.

13.2 Principles of PLE—Parameters Affecting Performance

Pressurized liquid extraction employs the use of solvents at high temperatures, above its boiling point and below its critical point, under enough pressure to maintain them in a liquid state. In this section, the essential criteria for the selection of appropriate operational parameters will be provided from a theoretical point of view. Additionally, the fundamental principles of PLE for solid samples are described. Due to the limitations of commercial equipment, the only possibility to deal with liquid samples is by transforming them into solids, for example, by adding an ab/adsorbent.

The process of extracting analytes from semisolid and solid samples can be described by the following five steps [11]:

(1) Moistening the sample (analytes to be extracted and matrix) with extraction solvent
(2) Desorption of compounds from the matrix (including or not the breakdown of chemical bonds)
(3) Solvation of the compounds in the extraction solvent
(4) Dispersion of the compounds out of the matrix
(5) Diffusion through the nearest solvent layer around the matrix to finally reach the bulk solvent

Extraction efficiency depends on both kinetic and thermodynamic parameters. Therefore, extraction effectiveness is influenced by three interrelated aspects: matrix effect, mass transfer, and solubility. These different features in PLE are limited by different considerations, such as the selection of flow rate, pressure, temperature, and time used in the extraction.

13.2.1 Temperature

Temperature is one of the most important parameter to be optimized in an extraction using PLE, because the physicochemical properties of the solvents are modified by the increase of temperature, which influence the efficiency of all steps described earlier [4]. For instance, under subcritical solvent conditions, the relative static permittivity of certain solvents (also referred to as dielectric constant, κ), especially water, decreases by increasing temperature, which implies that the polarizability of the solvent can be adjusted by altering its temperature. This effect is particularly drastic for water; pure water at room pressure and temperature has a κ around 79, while at 200°C and a pressure of 1.5 MPa (necessary to maintain the liquid state), κ drops to 35, a significant reduction [12]. This means that κ_{water} under these conditions is similar to $\kappa_{methanol}$ at room temperature [13]. Moreover, liquid water at very high temperatures is a solvent of low density and polarizability/polarity. In addition, temperature affects mass transfer properties by modifying the solvent surface tension, diffusivity, and viscosity. In this sense, surface tension and viscosity decrease, while diffusivity increases by increasing solvent temperature. All these changes of solvent properties obtained by an increase in temperature enable faster mass transfer and improve wetting of the sample. Moreover, desorption of analyte from the matrix to the solvent is promoted by high temperatures since the intermolecular interactions that bind the analyte to the matrix are reduced. This, in turn, may lead to a more complete extraction and a faster extraction processes.

On the other hand, it should be considered that although an increase in temperature may increase the solubility of the analyte, it may also increase the solubility of

other compounds (desired or not) in the matrix, thus converting PLE to a less selective extraction. The use of high temperatures must be carefully evaluated when the target compounds are thermolabile since degradation of the analyte can occur during the extraction process.

Likewise, certain chemical reactions in the sample matrix can occur at higher temperatures. These reactions may form toxic or unwanted compounds. The extent of chemical reactions and degradation of thermolabile compounds can be decreased using lower residence times by performing, for instance, an extraction in the continuous-flow mode, as will be described later.

Therefore, it is important to find the optimal extraction temperature in PLE for the extraction of analytes of interest so as to take full advantage of enhanced solubility and improved mass transfer, but still minimizing the undesirable drawbacks of selectivity loss, degradation, and chemical reactions described earlier.

13.2.2 Pressure

Pressure itself has a limited impact on the solvent characteristics as long as the solvent is maintained in the liquid state [14] or in PLE extraction efficiency [15]. In addition, an elevated pressure might help to wet the sample matrix, resulting in improved extraction efficiency [16]. Thus, a pressure of 5–15 MPa is typically employed unless the solvent saturation pressure is used.

13.2.3 Flow rate and extraction time

Extraction time in PLE is defined as the time during which the solvent is in contact with the matrix at the desired pressure, temperature, and flow. The extraction time needed to fully extract a particular matrix will depend on the matrix, type of compound, and extraction mode (static or dynamic), being the latter the most critical parameter. PLE can be performed either in a continuous flow or static mode. Different instrument designs are required for each mode.

In the static mode the extraction solvent is not continuously replaced, but if more than one extraction cycle is employed, the solvent is partly or completely replaced after some time [17]. An equilibrium between the compounds still linked to the matrix, and the liquid phase in which analytes are already solubilized could be established in the static extraction mode. Thus the efficiency of the extraction procedure will not increase beyond this point, and the degradation of some compounds and undesirable chemical reactions might happen more easily. That is why a careful optimization of the static extraction time is important.

In a continuous-flow extraction mode, there is a constant flow of the extraction solvent through the extraction cell [17], and this mode is more favorable, theoretically, for the complete extraction of the matrix because equilibrium is avoided.

Another parameter to study in a continuous-flow mode is the flow rate. The extraction process is either solubility controlled, desorption controlled, or a combination of both (solubility controlled initially and then desorption controlled). This depends on where the analytes are located in the matrix and how they interact with the matrix [18]. If the extraction is solubility controlled, higher flow rates will improve the extraction yield per unit time, whereas if it is desorption controlled, the extraction yield per unit time will be independent of the flow rate. An appropriate flow rate should allow a short contact time between the sample and the solvent, allowing the solubilization of the analytes of interest. An interesting way to reduce chemical reactions and the degradation of compounds of interest is to use a continuous-flow mode with a sufficiently high solvent flow rate. The main drawbacks are that the extract may be too diluted for analytic measurements and higher costs are associated with the removal of high solvent volumes.

13.2.4 Other parameters (matrix, dispersants, solvent/sample ratio)

Other parameters, such as the physical state of the sample and the solvent-to-sample ratio, can also influence the extraction efficiency in PLE, and thus they need to be evaluated when aiming to optimize the process.

An important parameter to be considered in PLE is the solvent-to-sample ratio in the static extraction mode (see Section 13.3). The solvent-to-sample ratio should be as small as possible to avoid dilution of the extract but at the same time large enough to provide the highest possible extraction yield.

The matrix can be pretreated before extraction, for example, by drying and/or reducing particle size. Solid samples usually have to be ground, chopped, milled, and homogenized. The particle size affects mass transfer and should be optimized to maximize the contact surface; for instance, a larger surface area per unit mass results in better solvent accessibility to the analyte. Moreover, the migration rate of the analyte through the pores of the matrix increases by decreasing particle size. Additionally, the mechanical treatment employed to decrease particle size might break cell walls and cell structure, which enhances diffusion of the analyte. However, the particle size should be large enough to avoid channeling effects (i.e., agglomeration of particles).

Dispersants (i.e., sea sand and glass beads) and agitation are employed sometimes during the extraction to favor uniform distribution of the sample and extractant, which could increase the extraction yield.

Moisture content is another parameter that can influence the extraction yield. Drying the sample might induce shrinkage of the cells, which can be detrimental for extracting the target compounds from inside the cell. Some studies show that crude samples with high moisture content could improve the extraction yields compared with dried samples [19, 20]. On the contrary, considering certain solvents and

matrices, the presence of water may compete with the extraction solvent and decrease the extraction ratio.

Furthermore, mixing the solvent with some organic and inorganic solvents, surfactants, and additives may facilitate the solubility of the analytes in the extracting phase, as well as affect the physical properties of the matrix and the desorption of analytes. For instance, changing the pH of the medium can help break down noncovalent bonds between the analyte and the matrix, although conventional acid or base hydrolysis should be used carefully given the risk of breaking covalent bonds in the analytes of interest.

13.3 Instrumentation

The instrumental requirements needed to carry out a PLE process are relatively simple (Fig. 13.1). Therefore, although there are a number of commercially available instruments, it is simple to build a homemade extraction system, and many applications employ this approach. Anyhow, it must be taken into account that, given the high pressures (set to between 35 and 200 bar) and temperatures (from room temperature to 200°C) typically employed, corrosive-resistant materials have to be used. Basic instrumentation may differ depending on whether a static (Fig. 13.1A) or dynamic (Fig. 13.1B) process is implemented. Basically, the instrumentation consists of a solvent reservoir, a pump, an oven containing the extraction cell, different valves and restrictors, and a collecting vial.

Firstly, the solvent reservoir is coupled to the high-pressure pump. The pump introduces the solvent into the system and helps to push the extract out once the

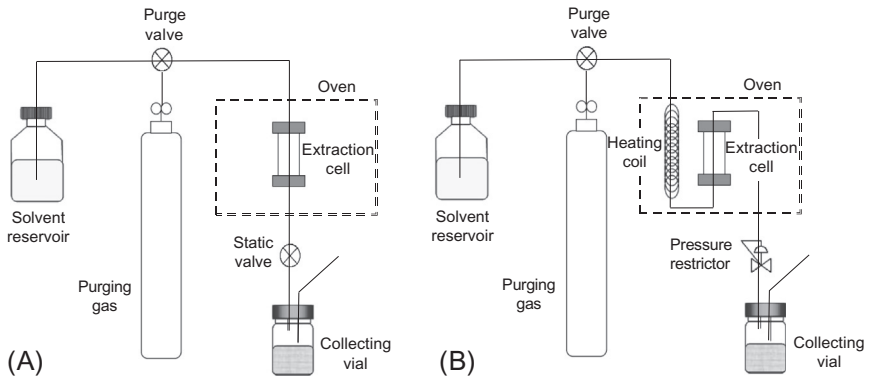

Fig. 13.1 Schematic PLE system. Configurations for development of (A) Static and (B) Dynamic PLE procedures.

process is finished. The extraction process takes place inside the extraction cell. A filter paper is inserted into the stainless steel extraction cell followed by the sample, sometimes mixed with a dispersing agent, if needed. Then the cell is automatically or manually placed into the oven. Different valves and restrictors are essential to control the extraction pressure. Finally, the collection vial is placed at the end of the extraction system.

The instrumentation can be more or less sophisticated, depending on the process requirements. For instance, a solvent controller is needed when several solvent reservoirs are available (for obtaining online solvent mixtures), or an inert gas (commonly nitrogen) circuit can help to flush out solvent from the lines after extraction. Moreover, a cooling bath can be utilized for the collection vial; this lowers the temperature of the extractant to minimize thermal degradation. Dynamic PLE also requires a slightly more sophisticated high-pressure pump to control the solvent flow rate, solvent preheating coils, and a pressure restrictor (back pressure regulator) or a micrometering valve rather than a static open/close valve used in static PLE.

13.4 Applications

13.4.1 Contaminants, toxins and residues

13.4.1.1 Organic Contaminants

Since the introduction of PLE as an official US Environmental Protection Agency (EPA) method for the determination of persistent organic pollutants (POPs) in environmental solid samples, PLE has become a popular green extraction technique for the analysis of a broad variety of organic contaminants such as personal care products and pharmaceuticals (PPCPs), flame retardants, endocrine-disrupting chemicals (EDCs), and nanoparticles (NPs), widely distributed in environmental samples [8]. Most PLE applications are focused on the extraction of organic contaminants present in sediments and sewage sludge [21–23]. Analytic methods based on PLE were developed for the extraction of persistent organic pollutants (POPs) from nonbiological samples (e.g., soil, sediment, sludge, and dust), including PCBs, PCDDs, PCDFs [24, 25], PBDEs [26], and PAHs [27]. In addition, a number of emerging contaminants such as nitrosamines [28], alkyl phenols, bisphenol A [29], and UV filters [30] have also been determined in the same type of samples [21]. Other contaminants, described as endocrine disruptors (EDs), were determined by PLE to form sludge samples, including BP congeners and BPA [31, 32], hormonal steroids [33], and flame retardants such as BFRs and chlorinated flame retardants [34, 35].

As for other extraction techniques, the coextraction of nontarget analytes from the sample matrix can interfere in the determination of the target contaminants. For this reason, a postextraction cleanup, using different types of SPE cartridges, gel permeation chromatography (GPC), or packed chromatographic columns, is typically required prior to the determination step [35]. In some applications, PLE procedures incorporate appropriate adsorbents in the extraction cell to retain interferences from the matrix, such as sulfur, a typical elemental interference in soil and sediment matrices [21]. The possibility of including in-cell cleanup significantly increases sample throughput. Thus, silica gel was shown to be an effective sorbent to obtain clean extracts in the analysis of nonpolar compound (e.g., PAHs and flame retardants PBDEs). Silica was also used to retain polar interferences in the detection of benzothiazoles from indoor particles. Activated carbon was satisfactory for the determination of UV filters by removing sulfur under reducing conditions [23].

Personal care products (PCPs), such as cosmetic preservatives, UV filters, or fragrance allergens, are emerging contaminants that have been analyzed not only in environmental samples but also in cosmetics and personal care products. In this case, PLE was demonstrated to be an efficient strategy to extract PCPs from complex cosmetic matrices [36, 37], since PLE-based methods allow simultaneous in-cell derivatization and extraction of multiclass cosmetic preservatives (e.g., parabens, triclosan, bronidox, bronopol, and IPBC) prior to GC-MS analysis [38]. Parabens and triclosan were also determined in dust, after first removing nonpolar interferences with hexane under low temperature and pressure and extracting the target analytes with polar solvents [39].

POPs and ECs were also determined in air samples by PLE. Here, air sampling is performed by filters that can be easily transferred to PLE cells, acting as an integrated preconcentration and cleanup step [8].

13.4.1.2 Pesticides

Although the applications of PLE were originally focused on the extraction of environmental contaminants, the advantages of this technique have also been explored for the analysis of biological and food samples. Several applications for the determination of pesticide residues have been reported, since PLE allows the simultaneous extraction of different types of residues with a wide range of polarities. Thus, PLE is used for the analysis of vegetable and animal tissue samples [40]. Pesticides are frequently present in nonfat foods such as fruits, vegetables, and cereal-based food, with medium or high water content. Therefore, the addition of a drying agent (e.g., sodium sulfate or diatomaceous earth) is frequently required [41, 42].

A wide variety of pesticide residues were determined in different agricultural and food matrices by PLE, including honey [43], organophosphorus pesticide residues in corn [44], pyrethroid residues in complex feed samples [40], multiclass pesticides in

food commodities and grain, or herbicides in soybeans [42]. Accurate determination of common organochlorine, organophosphorus, and pyrethroid pesticide residues in herbal samples like tea can be obtained by PLE coupled to gas chromatography/high-resolution isotope dilution mass spectrometry [45]. The use of PLE followed by gel permeation chromatography (GPC) and SPE (ENVI-Carb tubes coupled to LC-Alumina-N tubes) overcomes the analytic complexity of tea sample matrices due to the high amount of caffeine, pigments, polyphenols, etc. Several pesticides along with other lyophilic contaminants (PCBs and PBDEs) can be simultaneously extracted and determined from lipid-rich matrices applying a selective PLE approach. This involves placing the homogenized lipid sample in the extraction cell on top of basic alumina, silica gel, and Florisil and extracting the target compounds using 1:1 (v/v) dichloromethane/hexane in a single automated step [46].

Comparative assessment of PLE performance and other pressurized sample preparation procedures such as supercritical fluid extraction (SFE) or the traditional Soxhlet extraction revealed higher recoveries of pesticides by PLE. When compared with conventional analytic techniques, such as QuEChERS and buffered ethyl acetate extraction, PLE methods also provided superior performance for the extraction of pesticide residues [42]. In these cases the removal of lipids and other coextractable materials was achieved by adding fat-retaining sorbents to the PLE cell, such as Florisil, alumina, or sulfuric acid-impregnated silica gel [40]. Fig. 13.2 illustrates the capacity of PLE to remove matrix coextractives compared with conventional extraction techniques for yam samples.

13.4.1.3 Toxins

Mycotoxins are a type of toxins produced by various fungi of high toxicological importance. Mycotoxins such as aflatoxins, ochratoxin A, or zearalenone were determined in different food commodities by PLE [47, 48]. A cleanup step is frequently recommended before chromatographic analysis of mycotoxins. In this regard, a molecularly imprinted polymer solid-phase extraction (MISPE), based on tailor-made materials with specific binding sites complementary to the target compounds, was more selective than typical conventional SPE sorbents. Following this strategy, two *Alternaria mycotoxins* (i.e., alternariol and alternariol monomethyl ether) were successfully determined in tomato samples with low volumes of MeOH (8 mL per sample) and short extraction times (13 min per sample) [49].

13.4.1.4 Metals

Metal and organometallic species have been determined using PLE. Employing dispersing agents based on ENVI-Carb, speciation of polar arsenic species was carried out in seafood [50]. The recovery of four arsenic species was evaluated using silica, C-18, sea sand, diatomaceous earth, and alumina as cleanup adsorbent, being C-18

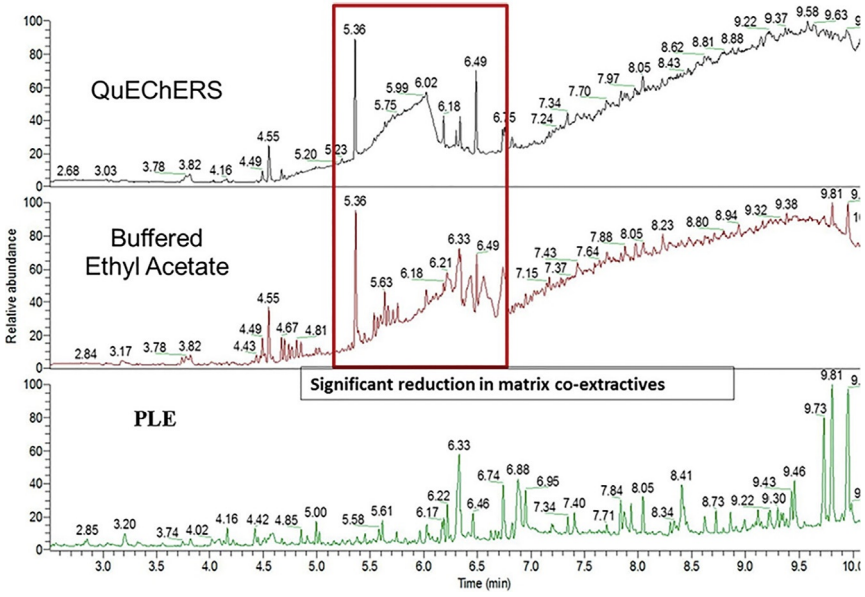

Fig. 13.2 A section of total ion chromatogram showing comparison of the amount of matrix coextractives across various extraction techniques in yam. *(Reprinted from Khan Z, Kamble N, Bhongale A, Girme M, Bahadur Chauhan V, Banerjee K. Analysis of pesticide residues in tuber crops using pressurised liquid extraction and gas chromatography-tandem mass spectrometry. Food Chem 2018;241:250–57. with permission of Elsevier.)*

the most appropriate for the target analytes. On the other hand, ENVI-Carb was used as a cleanup sorbent and dispersing agent for the extraction of Mg, Al, Ti, Cu, Ag, Sn, and Pb in lubricating oils without any additional cleanup step [21, 51].

13.4.1.5 Antibiotics and Other Pharmaceuticals

The release of pharmaceutical products into the environment has raised issues concerning their occurrence and fate and their effects on the biota. Antibiotics are an important group of pharmaceutical products widely used in human and animal health care, which are reported to be ubiquitous compounds in the aquatic environment. PLE is a reliable technique for the extraction of antibiotics and other drug residues associated with suspended solid matter [52]. Multiresidue analysis of sulfonamide antibiotics and their acetylated metabolites in soils and sewage sludge can be performed using fully automated PLE methods [53, 54]. In these methods, a subsequent SPE step for preconcentration and purification is required, considering the complexity of sludge samples, using, for instance, a hydrophilic-lipophilic balanced polymer. The extraction of quinolone and sulfonamide residues, such as lomefloxacin, enoxacin, sarafloxacin,

enrofloxacin, sulfadiazine, sulfamethoxydiazine, and sulfadimethoxypyrimidine, in fish (e.g., sardine samples) and shrimp was carried out by PLE using diatomaceous earth as a dispersing agent and acetonitrile as the extraction solvent [55].

13.4.2 Matrix components

The feasibility for the extraction of several families of compounds such as polyphenols, terpenoids, lipids, and essential oils from different matrices was studied by PLE. Different approaches were evaluated: as individual, sequential, or integrated processes according to biorefinery or in online extraction and separation systems, as shown in Fig. 13.3 by some illustrative examples. Some PLE applications of matrix components are described in the succeeding text.

13.4.2.1 Polyphenols

PLE is one of the most employed techniques for the extraction of polyphenolic compounds from different sources such as foods, vegetable products, marine products, and agroindustrial by-products. The hydroethanolic mixtures (EtOH > 50%), acidified or not, is the preferred solvent for the extraction of polyphenolics by PLE. Furthermore the temperature ranges are usually 40–60 and 75–220°C for thermolabile and thermostable phenolic compounds, respectively. For example, Bodoira et al. [56] optimized PLE extraction to obtain antioxidant phenolic compounds from defatted peanut skins. Optimal extraction was achieved using aqueous ethanol (60.5% v/v) at 220°C for 12.2 min. Under these conditions, extracts with high phenolic yield and chemical diversity (phenolic acids and glycosidic flavonoids) were obtained. In another study the extraction of polyphenolic compounds with antioxidant activity from *Rubus fruticosus* L. residues using PLE was carried out [57]. Anthocyanins were the major compounds recovered under the following PLE conditions: ethanol/water (50:50) as extraction solvent at 100°C for 30 min in the dynamic extraction mode.

PLE has been used in a sequential and integrated extraction processes for polyphenols. One example is the extraction of monomeric anthocyanins and other phenolic compounds from grape (*Vitis vinifera* L. cv. Syrah) marc by sequential PLE [58]. The extraction was separated into two sequential steps to recover different groups of compounds. The first step was performed at 40°C with water/ethanol (50% w/w) at pH 2.0 as solvent and the second step at 100°C with water/ethanol (50% w/w). This process provided two different extracts: one rich in anthocyanins (first step) and the second rich in other phenolic compounds (second step). The mild temperature of the first step prevented thermal degradation of anthocyanins before the second step, and the low pH helped increasing their extraction yield. In the second step an efficient extraction of phenolic compounds was observed, since high

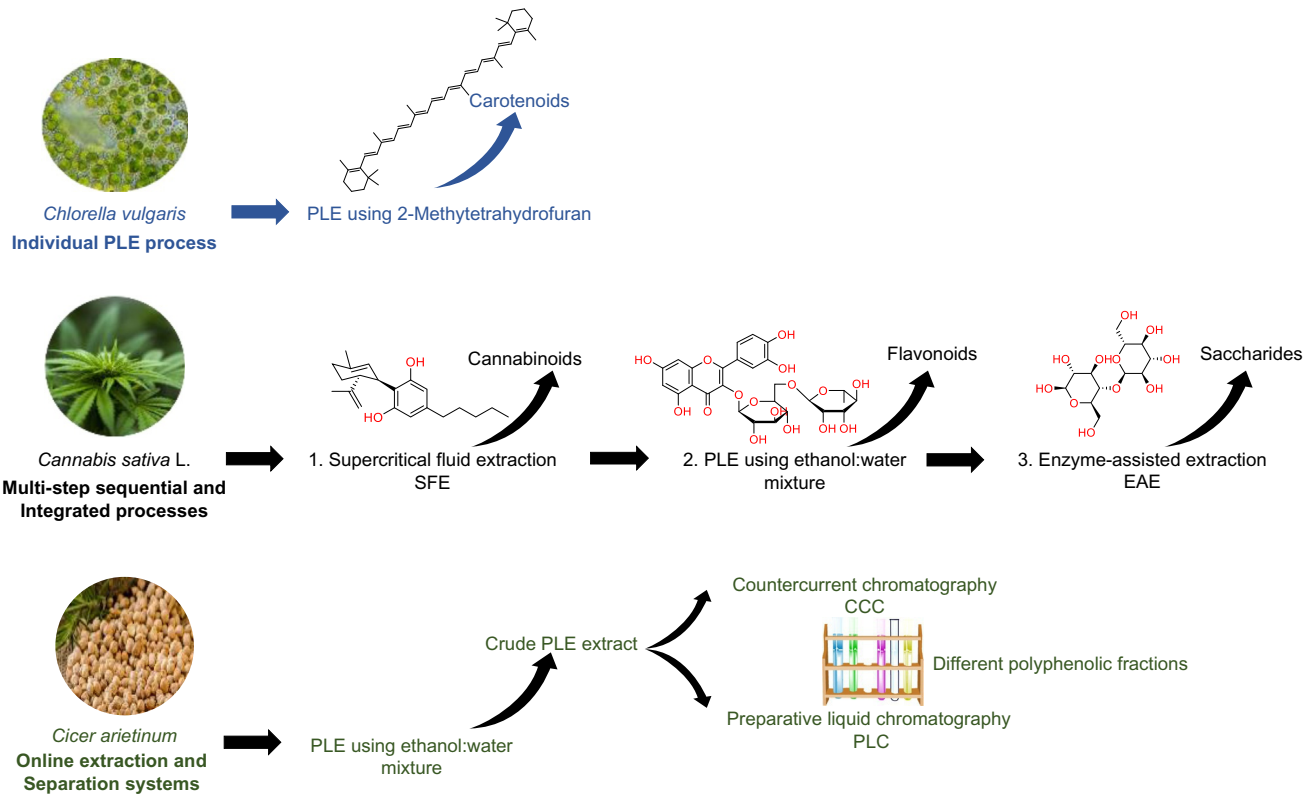

Fig. 13.3 Different approaches tested for matrix components extraction using PLE.

temperatures intensified the extraction of heat-resistant phenolics. Another interesting example is the recovery of biflavonoids and anthocyanins from Brazilian pepper (*Schinus terebinthifolius* Raddi) dried fruits after a defatting step, in a sequential PLE process [59]. The first step was performed using petroleum ether at 60°C for 6 min. In a second step the extraction of phenolic compounds was performed through three static extraction cycles (10 min per cycle) using acidified ethanol (5% v/v acetic acid) at 75 and 100°C for drupes and exocarps of dried fruits, respectively. This sequential PLE process contributed to the selective extraction of phenolic compounds such as binaringenin, biapigenin, and methylated anthocyanin glycosides (pelargonidin and cyanidin derivatives). An application of PLE as an integrated process is demonstrated by the extraction of anthocyanins from blackberry (*Rubus fruticosus*), blueberry (*Vaccinium myrtillus*), and grumichama (*Eugenia brasiliensis*) using combined ultrasonic-assisted extraction (UAE) and PLE [60]. In this process, samples were subjected to a preliminary ultrasound treatment before PLE extraction. The samples were mixed with hydroethanolic solutions (50% or 70% ethanol v/v) as extraction solvent and treated in an ultrasonic bath for 8 min at 80°C. The samples were then extracted by PLE at 80°C for 30 min using the same sample and solvent as for the ultrasound treatment. This integrated process provided extracts with high antioxidant activity and an important content of anthocyanin compounds compared with individual UAE and PLE processes.

PLE was also employed in biorefinery processes for extracting polyphenolic compounds. For instance, Kitrytė et al. [61] biorefined *Cannabis sativa* L. threshing residues by consecutive supercritical carbon dioxide (SFE-CO_2) and PLE and enzyme-assisted extractions (EAE). In the SFE-CO_2 process, lipophilic fraction rich in cannabidiol and cannabidiolic acid was obtained, while in PLE and EAE processes, flavonoid- and saccharide-enriched fractions were obtained, respectively. The PLE biorefining process was divided into two sequential steps. In the first step, acetone was used as a solvent, while in the second step a hydroethanolic solution (EtOH/H_2O 4:1 v/v) was employed. Each step was performed for 45 min (3 cycles × 15 min) at 100°C. The PLE process generated two fractions with different flavonoid content and antioxidant activity, contributing to the general efficiency of the biorefinery scheme.

PLE was also used in online extraction and separation systems for the extraction of polyphenolic compounds. In this sense, Zhang et al. [62] assembled a continuous online system comprising PLE coupled to countercurrent chromatography (CCC) and preparative liquid chromatography (PLC) for the extraction and isolation of bioactive compounds (human aromatase inhibitors) from *Cicer arietinum* seeds. The PLE extraction was performed using aqueous ethanol (60% v/v) at 80°C for 5 min. Subsequently, the PLE extract was loaded into the CCC and PLC sample loops. The CCC and PLC separations were optimized according to the polarity of the bioactive compounds previously characterized in the PLE extract.

The complementarity between CCC and PLC allowed the isolation of 11 bioactive flavonoid-type compounds. This novel continuous extraction and online isolation method is effective and can be applied to other bioactive compounds in various food or herbal plants.

13.4.2.2 Terpenoids

In recent years a wide variety of terpenoid-type compounds have been extracted from diverse sources, such as herbal plants and microorganisms, using PLE. However, due to its chemical diversity and polarity, different solvents and temperature ranges are required. Solvents frequently employed are water, ethanol, hydroethanolic mixtures, and ethyl acetate. Novel and renewable solvents, such as 2-methyltetrahydrofuran, have also been used. Temperatures ranged from 40°C to 160°C. Some related applications are described in the succeeding text.

Bursać-Kovačević et al. [63] optimized different PHWE parameters, such as time, temperature, and the number of cycles for the recovery of terpenoid compounds, like steviol glycosides and carotenoids, and other bioactive compounds from *Stevia rebaudiana* Bertoni leaves. Optimal conditions for the extraction of terpenoids were 160°C and 30 min (10 min × cycle), demonstrating that PHWE could be efficiently used for the recovery of thermally labile and nonpolar to polar components in stevia leaves.

Other terpenoids, such as carotenoids, have been recovered from microorganisms using PLE. One particular case is the extraction of the hydroxylated and nonhydroxylated salinixanthin forms and other carotenoids from *Rhodothermus marinus*, a marine bacterium, under PLE conditions using ethanol as solvent at 100°C for 6 min (3 cycles × 2 min). In a further study, PLE was used to extract carotenoids and chlorophylls from the microalgae *Chlamydomonas* sp. [64]. In this case the main carotenoid identified in the PLE extract using the most selective conditions (100% ethanol, 40°C for 20 min) was lutein, although this extract was also rich in the chlorophyll pheophytin.

PLE was used for chemical ecology studies related to the production of terpenoids. For example, Castro-Puyana et al. [65] analyzed different culture conditions (effect of nitrogen, light intensity, and carbon supply) on the total carotenoid and carotenoid composition of *Neochloris oleoabundans* microalgae using PLE as a reference extraction technique. In addition, the antiproliferative activity of PLE extracts against human cancer colon cells was evaluated. Extractions were carried out using ethanol at 100°C and 20-min static extraction time. PLE contributed to establish appropriate culture conditions to produce considerable amounts of carotenoids (i.e., lutein, carotenoids monoesters, and violaxanthin) with antiproliferative activity.

PLE is a versatile technique for the extraction of terpenoids due to the possibility of using alternative solvents according to their chemical features. In this sense,

2-methyltetrahydrofuran (MTHF) was evaluated for the first time for the extraction of selected carotenoids from *Chlorella vulgaris* under PLE conditions [66]. A mixture of MTFH/EtOH (50:50 v/v) at 110°C for 30 min was used for the extraction of carotenoids (β-carotene and lycopene) and xanthophylls (violaxanthin, astaxanthin, lutein, and canthaxanthin).

PLE was also integrated to a multianalytic platform to isolate and characterize high-added value compounds from natural sources. For instance a multianalytic platform based on the combination of PLE, liquid chromatography (LC), and gas chromatography quadrupole time-of-flight mass spectrometry GC-q-TOF-MS(/MS), in vitro antioxidant assays and Hansen solubility parameters (HSP), was proposed to obtain withanolide-rich extracts from *Physalis peruviana* L. calyces [67]. In this study, 4β-hydroxywithanolide E and withanolide E were selected as target compounds and PLE solvents selected based on the HSP approach. Ethanol, ethyl acetate, and their mixtures together with extraction temperature were evaluated with respect to withanolide content in the PLE extracts. An ethanol/ethyl acetate mixture (75:25 v/v) at 125°C was optimal to obtain extracts with the highest withanolide content.

The search for terpenoids with biological activity has boosted the development of integrated strategies to improve process selectivity toward the recovery of target compounds. Sánchez-Camargo et al. [68] developed an integrated PLE followed by supercritical antisolvent fractionation (SAF) at pilot plant scale and compared the process with other sub- and supercritical processes to obtain carnosic acid and carnosol-enriched rosemary (*Rosmarinus officinalis* L.) extracts with antiproliferative activity (on colon cancer cell lines). For the PLE + SAF process, a hydroethanolic extract was obtained under PLE conditions (EtOH/H_2O 80:20 v/v, 150°C, and 20 min); then the PLE extract was diluted with water and fractionated using supercritical carbon dioxide (40°C and 100 bar), based on the antisolvent properties of sc-CO_2 in aqueous systems. PLE + SAF fractions presented high content of phenolic terpenes and showed strong antiproliferative activity.

13.4.2.3 Lipids

One of the main applications of PLE is the extraction of lipids. Lipids of diverse chemical structure and sources have been extracted by PLE using low- or medium-polarity solvents, including hexane, (+)-limonene, ethyl acetate, methyl acetate, ethanol, and hydroethanolic mixtures. Generally the temperature employed for lipid extraction ranged from 90 to 220°C. Some applications of PLE for lipid extraction are illustrated in the succeeding text.

PLE was evaluated as a green process for isolating edible oils to overcome problems associated with conventional extraction processes using toxic organic solvents. Recently, PLE, microwave-assisted extraction (MAE), and ultrasound-assisted

extraction (UAE) were evaluated for the efficient extraction of ω3-rich oil from *Echium plantagineum* seeds using hexane-free processing methods [69]. In the PLE process, different solvents (ethyl acetate, hexane, ethanol, water, and mixtures of ethanol/water) and temperatures (60–200°C) were evaluated. Ethanol at 150°C provided the best ω-3 fatty acid recovery (31.2%) compared with MAE (21.2%) and UAE (29.1%); the results obtained were close to those achieved by Soxhlet extraction with hexane (31.3%).

PLE was used for nutritional quality studies of lipids. For this application, it is important to assure the chemical integrity and maximum extractability of the target compounds. As an example, PLE was applied to extract fatty acids from *Laminaria ochroleuca* using four solvents (hexane, ethyl acetate, ethanol, and ethanol/water mixture 1:1) [70]. The PLE performance was evaluated measuring the fatty acid extraction yield and the nutritional quality of extracts. The extract obtained under PLE conditions using ethanol/water mixture at 120°C showed high unsaturated (55%) and saturated fatty acid (45%) recoveries, while the PLE extract obtained using ethanol at 120°C had a major nutritional quality according to its ω-6/ω-3 fatty acid ratios: 0.75 and 1.05 atherogenic and thrombogenic indexes, respectively.

Alternative biobased solvents, such as (+)-limonene and α-pinene, were used for lipid extraction by PLE. (+)-Limonene represents a major by-product and is considered generally recognized as safe (GRAS) by the US Food and Drug Administration. In a recent application the (+)-limonene performance for lipid recovery from different microalgae (*Arthrospira platensis*, *Phormidium* sp., *Anabaena planctonica*, and *Stigeoclonium* sp.) was studied using PLE [71]. The limonene/ethanol (1:1 v/v) mixture under PLE conditions (200°C for 15 min) was a selective solvent to obtain lipid extracts enriched in valuable fatty acids from the evaluated sources.

The sequential PLE approach was also used for the fractionation of lipids. In a recent study, Castejón and Señoráns [72] developed a four-step sequential method using PLE for the extraction and fractionation of lipid compounds from *Nannochloropsis gaditana*. This method was based on increasing the temperature progressively and decreasing solvent polarity through the sequential steps. In the first and second steps, the polar compounds (i.e., carbohydrates and peptides) were eliminated using water and hydroethanolic mixture (EtOH 5% v/v) at 90°C. In the third and fourth steps, lipid compounds were fractionated using hexane/ethanol mixture (3:1 v/v) at 120 and 150°C, respectively. This method allowed fractions enriched in neutral and polar lipids like triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, and glycolipids to be obtained.

Fatty acids are interesting for the biodiesel industries; consequently in recent years, alternative sources and extraction processes have been studied. For example, PLE with methyl acetate was used for oil extraction from *Crambe abyssinica* H. seeds [73]. The

PLE at 140°C in a dynamic process (solvent flow 3.0 mL min^{-1} × 30 min) provided a high extraction efficiency with a fatty acid composition similar to commercial *C. abyssinica* oil, which is obtained by mechanical pressing. Thus, the oil obtained by PLE had good quality and can be considered suitable for biodiesel production.

13.4.2.4 Essential Oils

PLE has also been used to extract essential oils (EOs) from plants. PHWE has been the technique employed most frequently for this application, since it is highly efficient for EO recovery and its "green and clean" status [74, 75] . In PHWE the temperature for EO recovery usually ranged from 50 to 200°C owing to their great chemical heterogeneity (terpenoids, alcohols, ethers, oxides, aldehydes, ketones, esters, amines, phenols, heterocycles, among others).

The potential of PHWE for EO recovery compared with hydrodistillation and Soxhlet has been evaluated. Khajenoori et al. [76] optimized PHWE conditions (flow and temperature) for EO extraction from *Matricaria chamomilla* L. leaves. At optimal PHWE conditions (150°C and 4 min mL^{-1} for 120 min) the best quality EOs (β-trans-farnesene, α-bisabolone oxides A, and α-bisabolol oxides A–B content) and yield (14%) were obtained compared with hydrodistillation and Soxhlet. This approach was also applied to obtain EO from *Coriandrum sativum* L. seeds [77]; the best PHWE conditions were 125°C, 0.5-mm particle size, and 2.0 mL min^{-1} of water flow. PHWE process showed an important EO yield (14.1%); however, hydrodistillation (21.7%) and Soxhlet (19.4%, using hexane as solvent) methods presented the best performance. Nevertheless, it is worth mentioning that higher quality EOs are obtained by PHWE, since small amounts of hydrocarbons are extracted. In another study, different extraction techniques (hydrodistillation, Soxhlet, SFE, and PHWE) were used for extracting EOs from *C. sativum* seeds [78]. In this case, SFE (sc-CO_2 at 40°C and 300 bar for 4 h) presented the best quality and yield; however, under PHWE conditions (200°C for 20 min), it was possible to obtain an extract of EOs rich in polyphenolic compounds with a higher added value.

Solvents other than water have also been explored for EO extraction under PLE conditions. In a recent study [79], ethanol, ethyl acetate, and hexane were evaluated to efficiently extract α-bisabolol-rich oil from *Eremanthus erythropappus* wood, using PLE and UAE techniques. α-Bisabolol is an important component of EOs found in several plants and is used in dermatologic formulations, decorative cosmetics, fine fragrances, shampoos, among others. Under PLE conditions (55°C for 20-min time extraction), the highest purity in terms of α-bisabolol content was obtained using hexane (64.23%), while UAE using the same solvent provided the highest yield.

13.5 Conclusions

Throughout this chapter, a general overview about the principles, the main factors involved, and some of the most interesting and important applications of PLE are presented. A general description of the instrumentation is also provided from the perspective of encouraging the readers to build their own system and to evaluate the improved performance of pressurized systems in terms of extraction efficiency, speed, and low solvent consumption. Applications were selected covering a wide range of analytes of interest in food and environmental samples. By no means is the information provided exhaustive, but gives some clues about the most common parameters and conditions for selected applications. Undoubtedly, even if not every new/different matrix and target analyte are carefully studied and conditions optimized, readers can gather some useful ideas and approaches helpful in their own extraction optimization from the information provided. Thus, recent applications for the analysis of pesticides, metals, drug residues, and toxins are presented, together with some examples for the extraction of valuable natural compounds (polyphenols, terpenoids, essential oils, and lipids) from different natural matrices.

It is clear that from the first applications of PLE back in early 1990s, it has evolved considerably increasing not only the number and diversity of applications but also the possibilities of use. In this sense, interesting analytic applications including the online hyphenation of PLE with separation techniques are being developed to reduce sample preparation steps; this concept is expected to increase in the near future with more sophisticated systems able to meet the requirements of high throughput in control laboratories. On the other hand, PLE for the isolation of valuable compounds can benefit from the chemical engineering processes, developing more efficient and selective systems, with several steps integrated within the system (pretreatment, extraction, and reaction), while increasing efficiency through the use of, for instance, intensified processes based on PLE + US, PLE + EAE, etc. In this sense, it is worth mentioning that PLE has already been employed with success as one of the steps in biorefinery processes. Without any doubt, PLE applications and developments will be increasing in the near future, placing this extraction technique (or process, at large scale) as a sustainable alternative to fulfill the energy and environmental challenges that the world has in front of it.

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Chapter 14

Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction

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Chapter Outline

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14.1 Introduction

In 2003, Anastassiades and Lehotay, along with their coworkers, published a fast isolation method in which a good number of multiclass pesticides were extracted from fruits and vegetables employing acetonitrile (MeCN) extraction/partitioning and dispersive solid-phase extraction (dSPE) [1]. That method was named as QuEChERS, an acronym created from the words *quick, easy, cheap, effective, rugged, and safe*, inherent characteristics of the procedure and the basis of its outstanding success. Furthermore and of equal importance, this work also introduced the concept of "dSPE" for cleanup purposes.

After its publication, it immediately caught the attention of the scientific community, and a good number of applications in the pesticide analysis field quickly followed, especially for the analysis of other fruits and vegetables, since it demonstrated in a good number of occasions a higher recovery and reproducibility than existing methods. At the same time and as a result of the specificity of each analyte and sample matrix, some modifications appeared: the use of buffers to avoid pH degradation of certain pesticides [2, 3], the introduction of new dSPE sorbents and salts, or even its application to analytes other than pesticides and to other matrices, among others. The method acquired such a dimension that one of its versions was adopted as an AOAC official method [3] and another as a standard method of the European Committee for Standardization [2], both widely used nowadays especially in regulatory laboratories. However, there are more QuEChERS versions than desirable today, with many vendors offering different QuEChERS kits adapted to the user's needs and requests. Such kits have also greatly contributed to its expansion and wider application, since the salts and sorbents do not need to be weighed in the laboratory with the consequent effort and loss of time.

The QuEChERS method is frequently included as part of "green analytical chemistry" procedures [4, 5]. Indeed, it requires relatively small amounts of low-toxicity and nonhalogenated solvents and reagents as well as laboratory ware and no external energy supply (though in some cases the application of ultrasounds for a short period of time might be helpful). Besides low cost and a short time, a reasonable number of samples can be extracted simultaneously.

In recent years the QuEChERS method is being forced to face an important objective: its miniaturization and possible automation, which are not easy tasks, especially the latter. Several attempts have been made in both directions providing a new horizon for the method.

The aim of this chapter is to provide a general description of the origin and evolution of the QuEChERS method, the main modifications that currently exist, and its wide application range, which is no longer only focused on pesticide analysis. Since there are a large number of manuscripts that have used this method and many of them claim to apply it when only the partitioning step or the dSPE step alone are used, this chapter is focused on those applications that use the full method exclusively.

14.2 The Original Method

The original QuEChERS method was introduced in the contexts of evolving needs to develop more selective and sensitive methods for the determination of pesticide residues, based on the increasingly restrictive legislation related to environmental and agri-food safety. In particular, pesticides were the first to be highly regulated due to the widespread use of these compounds and to the hazardous effects of many of them.

Many methods were developed to extract pesticide residues from food commodities during recent decades. The traditional or classical methods typically consist of the mere use of a single organic solvent, solvent mixture, or an aqueous/organic solvent mixture with the addition of NaCl to induce phase separation. In this sense the first method to be widely applied since its introduction was the Mills method [6], which consists of an MeCN extraction followed by partitioning with nonpolar solvents such as hexane or petroleum ether. This method is especially effective for the extraction of nonpolar pesticides. In 1999, to facilitate the extraction of more the polar pesticides that were being introduced, Luke et al. [7] proposed the use of acetone rather than MeCN for the initial extraction step together with a partition step employing petroleum ether and dichloromethane to remove residual water from the organic phase and coextracted polar material. The addition of small amounts of anhydrous Na_2SO_4 also helped to remove the water from the organic phase. However, the use of chlorinated solvents quickly fell into disuse due to their adverse effects on the environment and human health. In contrast, other solvents such as ethyl acetate or cyclohexane in the partitioning step [8] or the addition of different salts, namely, NaCl or anhydrous $MgSO_4$, and fructose [9], won many adherents. Nevertheless the organic phase could still contain water residues, which are harmful for the subsequent chromatographic step (in particular, when gas chromatography (GC) was used) and even for possible specific cleanup steps based on conventional solid-phase extraction (SPE) procedures [10]. In 2002, only one year before the publication of the first QuEChERS article, Schenck and coworkers together with the father of the QuEChERS method, Dr. Steven J. Lehotay [11], demonstrated that anhydrous $MgSO_4$ exerts a stronger drying power compared with anhydrous Na_2SO_4 in organic extracts and that MeCN was more easily and effectively separated from water than acetone.

Based on these findings, Anastassiades, Lehotay, Štajnbaher, and Schenck [1] developed the QuEChERS method in 2003 for the multiresidue analysis of pesticides in fruits and vegetables as a user-friendly alternative to traditional methods characterized by multiple stages and large amounts of sample and solvents. This approach involves sample extraction with an organic solvent after suitable homogenization, the novel use of anhydrous $MgSO_4$ together with NaCl for salting-out induced partitioning of water and a minimal and very fast cleanup step called dSPE. Particularly a relatively small amount of MeCN (normally between 10 and 15 mL) is used to extract an aqueous-based sample in a ratio of 1 g/mL, so that the analytes of interest

are largely transferred into the organic phase, while the undesirable coextracted material remains in the aqueous phase that is discarded. MeCN was the selected organic solvent over acetone and ethyl acetate since it efficiently isolates a broad range of pesticides (nonpolar and relatively polar analytes) while minimizing the amount of lipophilic material. Besides, since MeCN is water-soluble, it allows a good penetration into the aqueous phase of the samples. This step also includes the use of 4 g of anhydrous $MgSO_4$ to induce phase separation by saturation and heat generation around 40°C (as a result of the exothermic hydration reaction) that aids in the extraction of nonpolar pesticides (avoiding the use of nonpolar solvents) and 1 g of NaCl to reduce the amount of polar interferences coextracted. The combined use of these salts showed better results in terms of recovery, phase separation, the amount of coextracted matrix, and peak shapes than their individual use or using other salts such as LiCl, $MgCl_2$, $NaNO_3$, and Na_2SO_4 or even fructose. The sample is stirred, centrifuged, and an aliquot of the organic extract is subjected to the dSPE procedure. The great virtue of the dSPE approach is that, in contrast to traditional SPE, the extraction of the analytes or of the matrix components (it depends on its use) is carried out in the bulk solution. Hence dSPE does not require the use of vacuum manifolds, columns, and preconditioning steps as well as the collection of solvent fractions, evaporation of the solvents, etc. In summary, it does not require a tedious and long procedure and the exhaustive attention of the analyst. As dSPE sorbents, only 25 mg of primary secondary amine (PSA) was needed and was more effective for sample cleanup than a methacrylate-divinylbenzene copolymer sorbent; graphitized carbon black (GCB); neutral alumina; a strong anion exchanger; and silica-based cyanopropyl, aminopropyl, and octadecylsilane (C_{18}) chemically bonded sorbents. The PSA was used together with 150 mg of anhydrous $MgSO_4$ to remove polar matrix interferences (including sugars, organic acids, and pigments) and traces of water from a 1-mL sample aliquot. Finally an aliquot of the resulting extract is transferred to a vial and injected into a gas or liquid chromatograph with preferably mass spectrometry (MS) detection as low-purity extracts are obtained. The original QuEChERS method allowed the analysis of a batch of 6–12 samples in 30–45 min at a cost of 1 USD per sample, providing typically high recovery values (85%–101% for 95% of the pesticides studied), good repeatability (RSDs $<5\%$), and high sample throughput and requiring less work than traditional methods, minimizing errors.

Concerning sample processing, multiple advances have been made with the fundamental objective of obtaining smaller subsamples to improve efficiency without detriment to representativeness or obtaining statistically reliable results when applying multiclass multiresidue methods. In fact, sample processing had been traditionally ignored compared with sample preparation and the instrument steps, but this is becoming increasingly crucial as automated high-throughput analysis is being implemented [12]. In the first article of the QuEChERS method, attention was paid to the

proper comminution of samples to achieve a suitable degree of homogeneity, but it was not new. de Kok et al. [13] were using 15-g subsamples in the mini-Luke method since the 1980s. Likewise the QuEChERS method uses smaller subsamples (10–15 g for wet samples and 2–5 g for dried samples) with the same quality compared with larger subsamples (50–100 g) typically used in more traditional methods [14, 15]. Particularly, representative subsamples of 2 g can be obtained through cryogenic processing using dry ice or liquid nitrogen, providing more reliable results and avoiding the loss of the most volatile analytes when treating samples at room temperature [16–18]. As a spectacular example, Fussell et al. [18] demonstrated that the degree of variability obtained for the extraction of chlorpyrifos in tomato for a subsample size of 5 g chopped with dry ice was similar to that of a sample of 110 g chopped at room temperature.

14.3 First Modifications

Since the first described the QuEChERS method has become the reference method for the analysis of pesticide residues worldwide in a large number of sample types with different characteristics [19], but with some exceptions, for example, certain highly polar pesticides, such as glyphosate, caused by a poor or no partition into the organic layer. Moreover the QuEChERS method has been expanded to other types of analytes demonstrating its potential greater utility. In fact, due to the possibility of its easy modification in terms of sample size, extraction solvent, type, and amount of salts and sorbents, the QuEChERS approach could be considered as a sample preparation concept rather than a particular method. Accordingly, it serves as a template for further modifications depending on the properties of the analytes, the matrix composition, and the equipment available. Hence an incessant number of enhancements/modifications have emerged as well as a wide spectrum of combinations, in many cases as a result of personal preferences, but without transcendence or continuity. In this way, there is a need to harmonize through collaborative studies the large number of suggested QuEChERS protocols. In any case individual efforts have focused on providing an effective initial extraction to maximize selectivity and recovery and subsequent cleanup of the extracts from matrices of different complexity to diminish the matrix effect.

The first and most significant modification to date was the introduction of buffers in the initial extraction to avoid the loss of certain pH-sensitive pesticides. In fact, in the original study, pH had already been considered as a crucial aspect that affects recovery. It was studied in the range 2.5–7.0 by using H_2SO_4 or K_2CO_3 solutions and adjusted to around 4, particularly for matrices with intrinsic high pH as a compromise between those pesticides that can be lost at low pH and those less stable at basic pH. Later, Lehotay et al. [20] validated the original QuEChERS method for the

determination of 229 pesticides in fruits and vegetables using GC-MS and LC-MS/MS and also demonstrated that the use of PSA in the dSPE step in nonacidic matrices increases the pH of the final extracts to basic values with the consequent hydrolysis of pesticides such as captan, folpet, chlorothalonil, and dichlofluanid. To solve the problem of pH-dependent degradation of pesticides and to expand the matrices covered, a strong acetate buffer (pH 4.8) and a weaker citrate buffer (pH 5–5.5) were incorporated in the general protocol, resulting in the AOAC Official 2007.1 Method (AOAC method) [3] and in the European Standard Method EN 15662 (EN method) [2], respectively. Fig. 14.1 illustrates the main steps of three primary QuEChERS methods. The three versions were compared later by Lehotay et al. [21] to analyze 32 representative pesticides by GC-MS and LC-MS/MS in apple-blueberry sauce, peas, and limes. The results demonstrated that the overall recovery was close to 100% with RSD <10% for the majority of pesticides for all methods, with only a few exceptions. The recovery of pymetrozine was higher and more consistent for the AOAC method (82%, 7% RSD) than the original (30%, 100% RSD) and CEN method (30%, 51% RSD) in all matrices and for thiabendazole in limes, but none of these methods provided acceptable results for chlorothalonil, folpet, and tolylfluanid in peas. Moreover, all methods gave equivalent amounts of matrix coextractives, matrix effects, and signal-to-noise (S/N) ratios. In short the AOAC method exhibited some advantages compared with the other two methods in terms of higher and more consistent recovery values for the pH-dependent pesticides in fruit and vegetable matrices. The more concentrated acetic acid/acetate buffer in the AOAC method could decrease the efficiency of PSA in the cleanup step [22]. However, this depends on the particular composition of each matrix. For example, Anastassiades et al. [23] found that the AOAC method gave 0.25% of coextractives for red currants, whereas the CEN method gave 0.10% of coextractives, contrary to what Lehotay et al. [21] found in apple-blueberry sauce (original method 0.23%, AOAC method 0.13%, and CEN method 0.17%) using the same combination of sorbents in the dSPE step (50-mg PSA, 50-mg C_{18}, and 150-mg anhydrous $MgSO_4$ per milliliter of extract).

The C_{18} and GCB sorbents were tested from the beginning in the dSPE step of the QuEChERS method but with different objectives and consequences. On the one hand, C_{18} removes nonpolar interferences (e.g., lipids) from extracts of fat content of 2%–20% (fish, oil, avocado, cereals, etc.) that modify the ionization efficiency using MS detection, but does not influence recovery values, so it can only help in the dSPE step [24, 25]. Because of this, C_{18} has replaced low-temperature precipitation (freezing out), which is the simplest method for fat removal from extracts but which is also time-consuming and requires additional cleanup steps to remove residual fat components [26, 27]. On the other hand, GCB is used to remove pigments (e.g., chlorophyll and carotenoids in green vegetables), but the recovery of certain planar analytes, such as hexachlorobenzene, and polycyclic aromatic hydrocarbons (PAHs) and coplanar polychlorinated biphenyls (PCBs) as target analytes is reduced

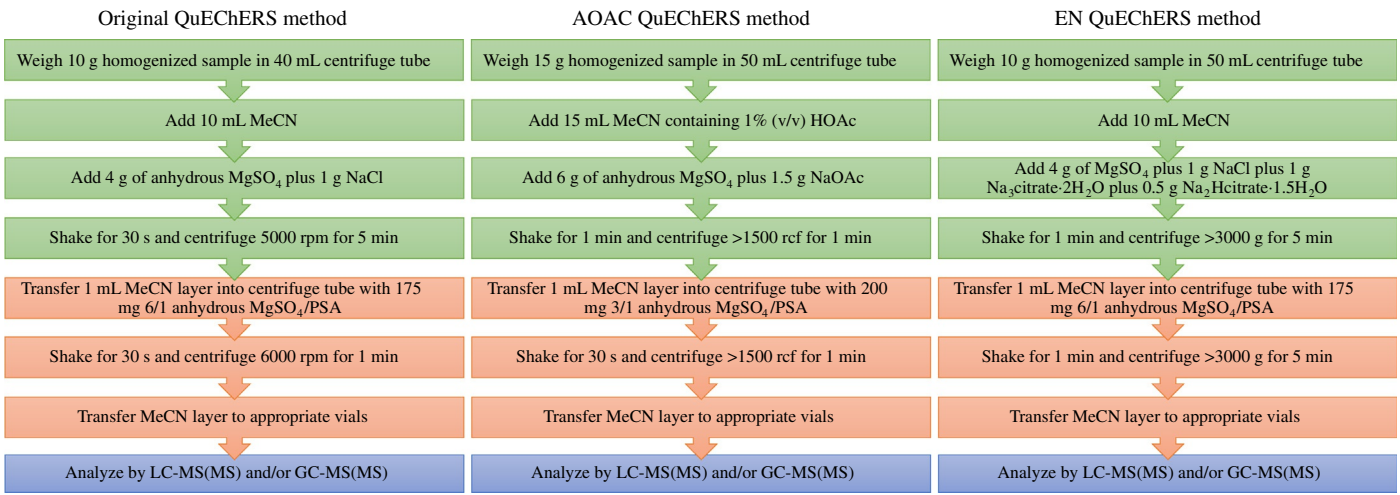

Fig. 14.1 Schematic flowchart for the main steps of three primary QuEChERS methods based on [1–3], respectively.

as more chlorophyll is removed [28]. More recently the so-called Z-Sep and Z-Sep+ sorbents were used as cleanup materials to enhance this step for the analysis of pesticide residues of commodities containing high amounts of fat [29, 30]. Both sorbents are based on zirconium dioxide and can replace the use of PSA and C_{18}, though Z-Sep is frequently used in combination with C_{18} for samples containing $<15\%$ of fat, while Z-Sep+ is a combination of Z-Sep and C_{18} dual bonded to silica and recommended for samples containing more than a 15% fat.

One of the main problems of the use of NaCl or $MgSO_4$ in QuEChERS is that trace amounts of such salts in the final extract make necessary the periodic maintenance of chromatographic instruments: that is, the replacement of liners in GC and the cleaning of the ion source in LC-MS. To solve this problem, an important modification was developed by González-Curbelo et al. [31] by using ammonium salts, since they are sufficiently volatile to avoid these problems. Also, ammonium can enhance the formation of analyte ions instead of undesirable sodium adducts for MS detection. Based on the previous work of Nanita and Padivitage [32] who compared different salts for pesticide extraction prior to flow injection MS/MS analysis (nine food and biological matrices were studied), González-Curbelo et al. demonstrated that ammonium formate is an excellent salt to induce phase separation between the MeCN extract and the water in the sample. Additionally, with the addition of formic acid, this provides suitable acidic buffering similar to the two official QuEChERS methods. Among its advantages compared with previous versions is that a single salt reagent is used for salting-out rather than a salt mixture. Moreover, compared with $MgSO_4$, ammonium formate has a lower vaporization temperature, which avoids the formation of undesirable solid deposit on the surfaces of the mass spectrometer [32, 33]. Specifically, 15 g of apple, lemon, or lettuce or 5 g of wheat grains was extracted with 15 mL of MeCN containing 5% (v/v) of formic acid and 7.5 g of ammonium formate with and without the dSPE procedure. For wheat grains, 15 mL of water was also added, and samples were shaken for 1 h to promote swelling of the sample to increase recovery, as recommended for low-water content matrices [34–36]. The method was fully validated and allowed adequate recovery of a wide range of GC- and LC-amenable pesticides. Table 14.1 summarizes the few applications known to date using this new version of the method. The majority of these have been developed by Lehotay and coworkers using only ammonium formate and MeCN (without buffer) mostly with the same ratio of sample/MeCN/ammonium formate. Regarding the coextractive material, Han et al. [39] showed that extractions using the ammonium formate version had a threefold less amount of coextractives than the original QuEChERS method (0.4% vs. 1.3%) in avocado but similar amounts for salmon and pork (0.6% and 0.1%, respectively). However, after the dSPE cleanup step, all final extracts were similar with 0.02%–0.04% coextractives.

TABLE 14.1 Some Examples of the Use of Ammonium Salts in the QuEChERS Method

		Extraction						
Analytes	Sample (Amount)	Solvents (Volume)	Salts (Amount)	Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
43 Pesticides	Apple, lemon, and lettuce (15 g) and wheat grains (5 g)	MeCN with 5% (v/v) formic acid (10 mL)	$HCOONH_4$, NH_4Cl or NH_4OAc (7.5 g)	$MgSO_4$ (150 mg), PSA (50 mg, 150 mg for wheat), C_{18} (50 mg) and GCB (7.5 mg) per milliliter of extract	(LP)GC-MS/MS and HPLC-MS/MS	70%–120% for almost all analytes	<5 μg/kg	[31]
42 Pesticides, 3 PAHs, 9 PCBs, and 5 FRs	Shrimps (10 g)	MeCN (10 mL)	$HCOONH_4$ (5 g)	(A) $MgSO_4$ (75 mg); (B) $MgSO_4$ (75 mg) and PSA, C_{18}, and Z-Sep (25 mg each); (C) $MgSO_4$ (75 mg) and PSA, C_{18}, Z-Sep, and CarbonX (25 mg each); (D) $MgSO_4$ (25 mg) and PSA (25 mg); (E) $MgSO_4$ (75 mg) and C_{18} (25 mg); (F) $MgSO_4$ (75 mg) and Z-Sep (25 mg); (G) $MgSO_4$ (75 mg) and CarbonX (25 mg). All cases also in mode filter-vial dSPE per 0.5-mL extract	(LP)GC-MS/MS and HPLC-MS/MS	70%–120% for 71% of the analytes	<5 μg/kg (<0.5 μg/kg for PCBs)	[37]

Continued

TABLE 14.1 Some Examples of the Use of Ammonium Salts in the QuEChERS Method—cont'd

Analytes	Sample (Amount)	Extraction		Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
		Solvents (Volume)	Salts (Amount)					
150 Pesticides, 15 PAHs, 14 PCBs, 6 PBDEs, and 22 FRs	Salmon and croaker (2 g) and NIST standard reference material 1947 (5 g)	MeCN (1 mL per gram of sample)	$HCOONH_4$ (5 g)	$MgSO_4$ (150 mg) and PSA, C_{18}, and Z-Sep (50 mg each) in mode filter-vial dSPE per 0.5-mL extract	(LP)GC-MS/MS	70%–120% for almost all analytes	<0.1 μg/kg (<0.01 μg/kg for PCBs)	[38]
65 Pesticides, 15 PAHs, 14 PCBs, 7 PBDE, and 16 FRs	Kale, pork, salmon, and avocado (1 g each per milliliter of MeCN)	MeCN (1 mL per gram of sample)	$HCOONH_4$ (0.5 g per gram of sample)	EMR-lipid (200 mg per milliliter of extract)	(LP)GC-MS/MS	70%–120% for 73% of the analytes in kale, 70% in pork, 65% in avocado, and 46% in salmon	<5 μg/kg	[39]
192 Pesticides, 14 PAHs, 7 PBDEs, 13 PCBs, and 17 FRs	Beef, chicken, and pork muscle (5 g)	MeCN (5 mL)	$HCOONH_4$ (2.5 g)	$MgSO_4$ (90 mg) and PSA, C_{18}, and Z-Sep (30 mg each) in mode filter-vial dSPE per 0.6-mL extract just for LPGC-MS/MS	(LP)GC-MS/MS and UHPLC-MS/MS	70%–120% for 82% of the analytes	<5 μg/kg (<0.5 μg/kg for PCBs)	[40]

19 Pharmaceuticals and PCPs	Surface and sewage waters (10 mL)	MeCN with 5% (v/v) HCOOH (10 mL)	$MgSO_4$ (4 g) and NH_4OAc (0.2 g)	NA	LC-MS/MS	73%–125%	0.002–0.25 μg/L	[41]
113 Pesticides	Green and ripe mangoes (10 g)	MeCN with 1% (v/v) HOAc (10 mL)	$MgSO_4$ (4 g) and NH_4OAc (1.7 g)	(A) PSA (400 mg), GCB (400 mg) and $MgSO_4$ (1200 mg); (B) PSA (150 mg), GCB (15 mg) and $MgSO_4$ (900 mg); (C) MWCNTs (60 mg); (D) Z-Sep+ (500 mg); (E) PSA (400 mg), GCB (80 mg), $MgSO_4$ (1200 mg) and Z-Sep+ (480 mg). All cases per 6-mL extract just for GC-MS/MS	GC-MS/MS and UHPLC-MS/MS	70%–120% for almost all analytes	<10 μg/kg	[42]

14.4 Recent Developments

The most important developments in recent years have focused on the introduction of other sorbents with a high matrix removal capacity. Several attempts have also been made to modify the extraction/partitioning step and the format in which the SPE procedure is carried out. In addition, progress has been made aimed at partial automation. Full automation would bring even further advantages.

14.4.1 New sorbents

The main modifications of the QuEChERS method is related to the use of new sorbents, many of which are also involved in conventional SPE methodologies. Among the different sorbents evaluated for the dSPE step (apart from the previously commented Z-Sep and Z-Sep+), the use of ChloroFiltr (UCT), CarbonX (United Science), Cleanert NANO (Agela), Oasis PRiME (water chromatography), Phree (Phenomenex), and EMR-Lipid (Agilent) should be highlighted.

CarbonX, ChloroFiltr, and Cleanert NANO were used to remove coextracted chlorophyll from plant matrices as for GCB [43–45] and to remove coextracted material from matrices of animal origin [37, 46]. CarbonX, which is a nonfriable form of GCB, is an efficient sorbent for the cleanup of QuEChERS extracts using filter-vial dSPE [37] or SPE minicartridges [45, 46]. In the first case a combination of 75 mg of anhydrous $MgSO_4$ plus 25 mg each of PSA, C_{18}, Z-Sep, and CarbonX and 75 mg of anhydrous $MgSO_4$ plus 25 mg of CarbonX are effective for cleanup of pesticides and environmental contaminants in shrimp [37]. However, similar to GCB, low recovery of planar compounds is obtained compared with other typical sorbent types. Even so, Carbon X adsorbs pesticides less strongly than GCB and is easier to work with. An automated mini-SPE method containing 20 mg of anhydrous $MgSO_4$, 12 mg of PSA and C_{18} each, and 1 mg of CarbonX (45 mg total) was more effective than 20.7 of mg C_{18}, 8.3 mg of Z-Sep, and 1 mg of CarbonX (30 mg total) for the cleanup of samples of kale, pork, and salmon after QuEChERS extraction [46]. CarbonX partially retained analytes with planar structures. As examples, hexachlorobenzene, polybromodiphenyl ether 183, and PCBs (126 and 169) yielded 80% relative recovery while still removing 95% of coextracted chlorophyll from QuEChERS extracts of kale. ChloroFiltr, a polymeric-based sorbent, was investigated for the selective removal of chlorophyll from green plant extracts using 150 mg of anhydrous $MgSO_4$, 50 mg of PSA, and 15 mg of ChloroFiltr per milliliter of extract in the dSPE mode without sacrificing the recovery of planar analytes [43, 44]. Larger amounts of ChloroFiltr were not recommended as it swells substantially in MeCN making it difficult to take aliquots of the supernatant [44]. Multiwalled carbon nanotubes (MWCNTs) have been widely used as sorbents in dSPE because of their extremely large

surface area and high porosity, which provide a high capacity for the removal of colorants and fatty acids from matrices as complex as garland [47], tea [48], or garlic [49]. A new commercial product based on MWCNTs, called Cleanert NANO, composed of functionalized MWCNTs with a deactivated surface to ensure the recovery of pesticide with a benzene ring, has been developed [50]. Only 10 mg of sorbent per milliliter of extract is required. Magnetic MWCNTs (Fe_3O_4-MWCNTs) were used as cleanup sorbents for the determination of veterinary drugs, pesticides, and mycotoxins in eggs [51]. An external magnet was utilized instead of the traditional centrifugation process, which simplifies the sample preparation process.

Oasis PRiME HLB [52] is a polymeric sorbent that can be used to remove phospholipids from fatty matrices, typically used packed in cartridges. After extraction an aliquot of the extract is loaded onto the cartridge for cleanup. However, in this case, the analytes and not the coextracted components are retained on the sorbent. The coextracted compounds pass through the cartridge and are discarded. The analytes are then eluted from the cartridge with a suitable solvent. The inclusion of a SPE step for extraction purposes clearly complicates the sample preparation procedure. This sorbent was used as earlier mentioned for pesticide analysis in spices [53] and fruit extracts [54].

Another sorbent used for this purpose is EMR-Lipid, introduced in 2015 by Agilent Technologies [55], suitable for the removal of phospholipids and proteins. However, it does not function as a solid sorbent in dSPE. After it dissolves to saturation in the extract solution, the extraction mechanism is based on size exclusion and hydrophobic interactions. Long-chain hydrocarbons associated with lipids fit within the EMR-Lipid structure, where they are trapped. The lipid/EMR-Lipid complex either is precipitated out of solution or remains in the aqueous phase during the final salting-out step. Han et al. [39] used this product in a multiclass multiresidue method for pesticides and environmental contaminants in kale, salmon, avocado, and pork. Sixty-five pesticides and 52 environmental contaminants were studied. When using this material, water should be added to the initial extract prior to EMR-Lipid cleanup, adding an additional step to the method complicating calculations, etc. Even though, it was found that EMR-Lipid efficiently removed 79%–98% of coextracted matrix components, providing clean extracts and low background in GC-MS/MS analysis. Fig. 14.2 illustrates the nonevaporated coextracts from kale, salmon, avocado, and pork before and after EMR-Lipid cleanup, where it can be seen that the amount of material present decrease considerably after EMR-Lipid application. It was also found that it removed up to 76% of coextracted chlorophyll without concomitant loss of planar analytes. Subsequently, EMR-Lipid was used for the cleanup QuEChERS extracts to determine pesticides in spices [53], edible vegetable oils [56], and virgin olive oil [57]; insecticides in honeybee [58]; and mycotoxins in edible nuts [59].

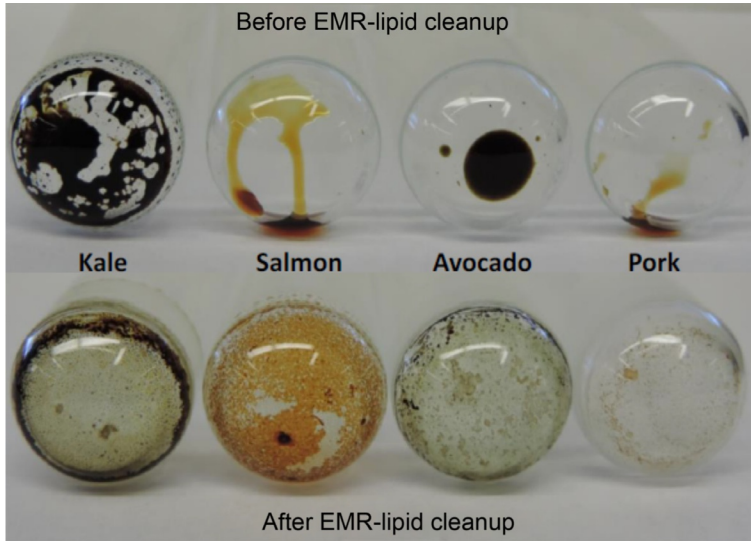

Fig. 14.2 Image of nonevaporated coextracts from different matrices before and after EMR-Lipid cleanup. *(From Han L, Matarrita J, Sapozhnikova Y, Lehotay SJ. Evaluation of a recent product to remove lipids and other matrix co-extractives in the analysis of pesticide residues and environmental contaminants in foods. J Chromatogr A. 2016;1449:17–29, with permission from Elsevier.)*

14.4.2 Extraction/partitioning modifications

The introduction of ammonium formate in 2014 [31] to promote extraction/partitioning in the first step of the QuEChERS method without the use of sodium or magnesium salts, whose presence in the final extract is disadvantageous for the subsequent chromatographic analysis, is an important modification that will likely continue to be used in the future. A further important development in the QuEChERS method is the so-called *quick polar pesticides* (QuPPe) method, which only involves a single extraction step (no dSPE procedure). This method evolved as a result of the poor capability of QuEChERS to extract polar pesticides with poor chromatographic behavior on reversed-phase liquid chromatography (RPLC) columns. Also, it mitigates losses in generic sample treatment procedures requiring specific sample preparation methods and analysis. The QuPPe method proposed by Anastassiades et al. [60] consists of the extraction of 10 g of each commodity (5 g if dried fruits, vegetables, etc. are selected to which water is added) with 10 mL of acidified methanol (MeOH) for 5–30 min, depending on the commodity (heating may also be necessary, i.e., for paraquat and diquat analysis). After centrifugation and filtering of the supernatant, it is injected into the chromatographic system. The QuPPe method is currently being updated to incorporate a larger number of pesticides. Such

modifications can be found in [60]. With the aim of extending the applicability of the QuPPe approach, it was later combined with the QuEChERS method by Robles-Molina et al. [61] for the extraction of 41 multiclass pesticides covering a wide range of physicochemical properties. After the extraction, parallel hydrophilic interaction liquid chromatography (HILIC)/RPLC and RPLC/aqueous normal phase LC were assessed using LC-MS/MS. In this case 10 g of sample (leek) was extracted/partitioned with 10 mL of MeOH containing 1% (v/v) formic acid, 10 mL of MeCN, 1.5 g of NaCl, and the corresponding amount of ultrapure water to achieve a final content of approximately 10 g depending on the vegetable. After agitation and centrifugation, two aliquots of the supernatant were diluted (1:7 dilution factor) by adding 1 mL of the supernatant, 6 mL of MeCN containing 0.1% (v/v) formic acid for HILIC analysis, or 6 mL of ultrapure water containing 0.1% (v/v) formic acid for the extract used for RPLC analysis, so that they matched the initial gradient composition of both methods. Recovery values were in the range 70%–120% for most pesticides. Only compounds with extreme log K_{ow} values (resmethrin and streptomycin) were not recovered at all.

Another way of avoiding the presence of sodium or magnesium salts that can lead to the maintenance problems previously discussed is to avoid using salts at all in the extraction but, instead, to freeze the extract to promote phase separation and to eliminate fats. For this purpose, Norli et al. [62] used disposable syringes and a freezing block consisting of a laboratory rack with 15-mL centrifuge tubes immersed in a polystyrene box filled with a freezing gel (see Fig. 14.3) to maintain the temperature of the MeCN extract at −24°C for 10 min. This approach was also used for the extraction of 22 organochlorine pesticides (OCPs) and 7 PCBs from fish (tilapia and salmon) and was based on earlier studies in which lipid removal from organic solvents was carried out in a similar way [63]. For that purpose, 6 mL of the MeCN extract was aspired into a disposable syringe with a polyethylene frit. The syringes were placed in the freezing device and kept for 2 h at −24°C. Afterward the syringe contents were poured into a tube containing 1.0 g of calcium chloride, shaken, and centrifuged. The supernatant was decanted into a new tube containing 900 mg of $MgSO_4$ and 150 mg of PSA, shaken, and centrifuged. After the freezing step, 69% of the lipids in tilapia and 61% in salmon were removed. Further reduction of coextractives up to 96% in tilapia and 87% in salmon were made with the dSPE step employing calcium chloride and PSA. Recovery values for tilapia ranged from 70% to 115% for all compounds, while for salmon they were in the range 43%–118% for the OCPs and 26%–65% for the PCBs.

Some years later, Shao et al. [64] developed a similar method in which phase separation of a water/MeCN mixture was induced by cooling to −16°C overnight (12 h). This procedure was referred to as "cold-induced aqueous MeCN phase separation (CIPS)." This group also evaluated the separation of aqueous solutions of MeOH, acetone, and 2-propanol, which failed to undergo a similar phase separation at −16°C.

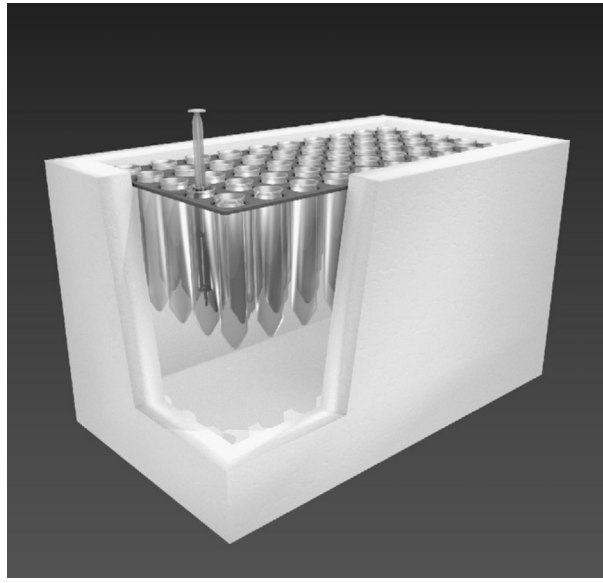

Fig. 14.3 Freezing block consisting of a laboratory rack with 15-mL centrifuge tubes immersed in a polystyrene box filled with freezing gel. *(From Norli HR, Christiansen A, Deribe E. Application of QuEChERS method for extraction of selectedpersistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry. J Chromatogr A 2011;1218:7234–41, with permission from Elsevier.)*

This was also true for the semimiscible solvents butanol and ethyl acetate, which failed to separate further beyond their initial partial phase separation at room temperature. The cooling phase separation proceeds as a descending cloud of microdroplets for MeCN/water mixtures. Shao et al. applied this method to the CIPS-QuEChERS procedure for the extraction of nine pesticides from salmon. Beef was also tested as a matrix but only to illustrate the separation of phases. After cooling the upper phase, which was rich in MeCN, 71.7% (v/v), was a clear liquid. The middle phase was ice and precipitated lipids, while the lower phase contained the residual matrix of undissolved salmon or meat. The salmon supernatant was treated with anhydrous $MgSO_4$ and C_{18} for the dSPE cleanup, with disparate recovery values, between 24% and 99%. These preliminary studies suggest that further study of CIPS-QuEChERS is worthwhile. However, the use of sorbents capable of efficiently extracting lipids (i.e., C_{18} and EMR-lipid) is recommended.

Freezing out or cryoprecipitation has also been used to eliminate fats prior to the dSPE step by freezing the extract at approximately −25°C for 1–2 h [65, 66]. However, this increases the sample pretreatment time. Moreover, it was demonstrated for pesticide analysis that the freezing-out step is unnecessary when carried out after the dSPE step using PSA and C_{18} since the amount of coextractives is equivalent [67].

14.4.3 Variations in the SPE format and automation

An interesting example of the path toward automation of part of the extraction process was developed by Kaewsuya et al. [68] using pipet tips fitted with filtration screens and containing PSA (25 mg), $MgSO_4$ (75 mg), and GCB (12.5 mg). In this case 250 multiclass pesticides were determined in carrots, tomatoes, green beans, broccoli, and celery. Thirty milliliters of MeCN was added to 30 g of each homogenized sample. The mixtures were shaken for 1 min followed by the addition of 3.0 g of NaCl and 12.0 g of anhydrous $MgSO_4$. The tubes were shaken again and centrifuged. Two hundred fifty microliters of the supernatant was slowly aspirated into the QuEChERS tips and then dispensed after a few seconds. This procedure was repeated three times into a clean vial. The QuEChERS tip was further eluted from the top with an additional volume of 250 μL of MeCN to ensure the pesticides were efficiently removed from the sorbent. The use of the tips avoided a second centrifugation step. High recovery (70%–117%) and good RSD values (<12%) were obtained for over 200 pesticides.

The use of magnetic materials, a current trend in analytic chemistry [69, 70], has also been used in the QuEChERS method as dSPE sorbent. The application of magnetic sorbents for dSPE was not developed until 1996, when Towler et al. [71] reported the recovery of different metals from seawater samples using manganese dioxide coated magnetite (Fe_3O_4) as the magnetic sorbent. However, the term magnetic SPE was only introduced 3 years later by Safariková et al. [72]. A clear example of the application of magnetic sorbents in QuEChERS occurs in the work of Li et al. [73] utilizing bare Fe_3O_4 nanoparticles. In this case 101 multiclass pesticides were analyzed in tomato, cucumber, orange, and apple. After optimizing the amount of magnetic nanoparticles (m-NPs) (40 mg were used for the cleanup of 1 mL of MeCN extract), recovery was evaluated. One hundred milligrams of anhydrous $MgSO_4$, 10 mg of GCB, and 50 mg of PSA were also added. Recoveries in the range 71.5%–111.7% (RSDs <10.5%) were obtained. The use of magnets to retain the sorbents simplifies the procedure by avoiding centrifugation and filtration of the extracts. A similar approach was developed by Zheng et al. [74] using Fe_3O_4 m-NPs combined with GCB and PSA as sorbents ("an aggregate wrap" of all of them) for the extraction of 10 target pesticides from cucumbers, gourds, cabbages, and tomatoes. Recovery values ranged from 69.9% to 125.0% with RSDs <9.8%. As described previously, magnetic MWCNTs have also been used for this purpose [51].

One of the problems of the QuEChERS method is that carryover of a portion of the dSPE sorbents during the removal of the supernatant. An approach to avoid this issue is the use of filter vials for the dSPE step [37] (see Fig. 14.4). In this case an aliquot is added to the receptacle half of the vial (outer/bottom part) that contains a mixture of the cleanup sorbents. After vortex the plunger half of the vial (inner/superior part), which contains a filter (i.e., 0.45 μm), is pressed down into the receptacle.

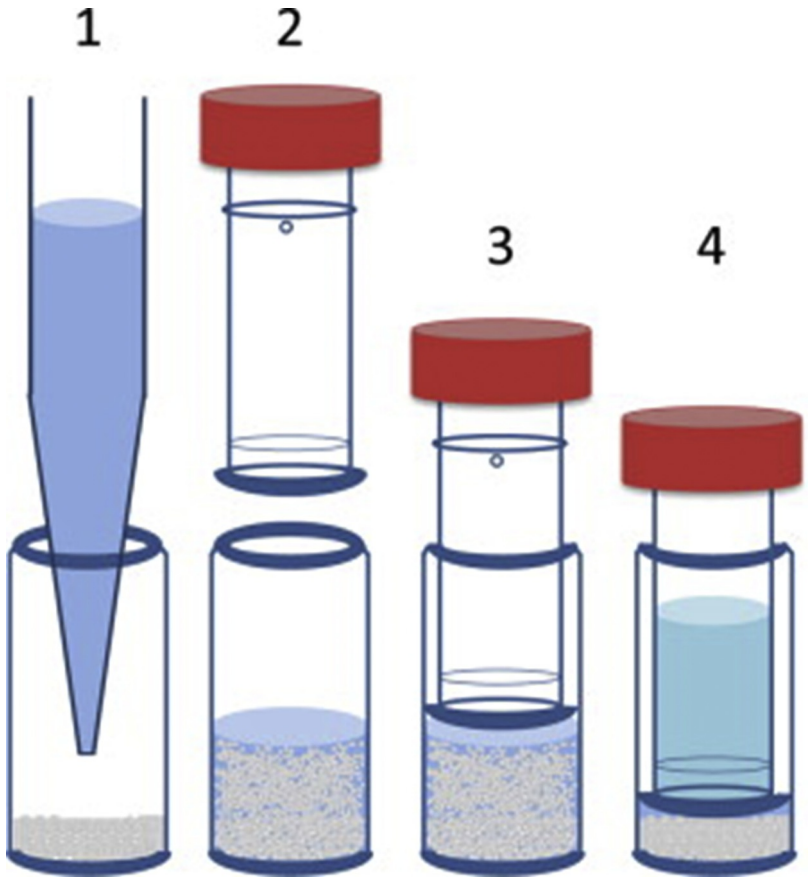

Fig. 14.4 Procedure for filter-vial dSPE: (1) pipette 0.5 mL of initial QuEChERS extract into bottom piece containing sorbents, (2) press filter plunger halfway into bottom piece, (3) shake for 30 s, and (4) depress filter plunger to filter extract for analysis. *(From Han L, Matarrita J, Sapozhnikova Y, Lehotay SJ. Streamlined sample cleanup using combined dispersive solid-phase extraction and in-vial filtration for analysis of pesticides and environmental pollutants in shrimp. Anal Chim Acta 2014;827:40–46, with permission from Elsevier.)*

In this way a clean extract ready for further analysis is obtained. It should be noted that the cleanup/filtration step can be carried out in parallel and that the amount of supernatant to be filtered and sorbents used is easily scaled. A similar approach can be developed with the commercially available SpinFiltr tubes from United Chemical Technologies [75]. However, in this case the sorbents are contained in the inner/upper tube that also has a 0.2-μm frit. Once the supernatant is introduced, vortex is applied followed by centrifugation. It is the centrifugation that promotes the final filtering.

The use of conventional SPE cartridges instead of the dSPE step, though more tedious and time-consuming, has also been proposed [76] since it provides a better

cleanup of the extracts while maintaining high recovery values. For this purpose, columns of aminopropyl (—NH_2) and PSA [77] or GCB and PSA [78], among others, have been used. In practice, dSPE is more convenient since it is easier, faster, and less expensive. However, if automation of the extraction process and the use of small cartridges to decrease the amounts of solvents and loaded extracts are considered, then SPE becomes more competitive. Such an approach was suggested by Morris and Schriner [45] who used an automated mini-SPE cartridge from ITSP Solutions for QuEChERS cleanup. This method was called "instrument top sample preparation" (ITSP) and utilizes a PAL HTS-xt robotic X-Y-Z autosampler from CTC Analytics (this autosampler is widely available nowadays in analytic laboratories). After testing different sorbent mixtures (30 mg of Z-Sep/C_{18}/CarbonX (2:5:0.24), 45 mg of PSA/C_{18}/CarbonX/$MgSO_4$ (3:3:0.25:5), 20 mg of HLB/Z-Sep/CarbonX (1:1:0.22), and 10 mg of Z-Sep or 30 mg of Z-Sep+ sorbents), the best results were obtained with Z-Sep/C_{18}/CarbonX sorbent eluted with a formate buffer in MeCN/MeOH (1:1). Such cartridges were applied for the extraction of 263 multiclass pesticides from avocado and citrus prior to (LC-MS/MS). In the case of avocado, the removal of 90% of the di- and triacylglycerols was obtained. Spiked recoveries were within the range 70%–120% with RSD of 20% for 243 of these pesticides in avocado and 254 in citrus. Lehotay et al. [46] further explored the application of automated mini-SPE cartridges during the extraction of pesticides and environmental contaminants from avocado, salmon, pork loin, and kale as representative matrices, obtaining good results.

14.5 Applications

As was suggested by Lehotay [79], QuEChERS can be considered as a "mega method" today, in the sense that the same fixed experimental conditions are appropriate for the simultaneous extraction of a wide variety of analytes (pesticides, pharmaceutical compounds, mycotoxins, PAHs, PCBs, etc.). Even though it remains uncommon to analyze an extremely large number of multiclass compounds, if needed, such an approach would be of high value.

Among the different target analytes to which the QuEChERS method has been applied, pesticides are, by far, the most studied, which is not unexpected, since the method was originally created for their analysis. This fact is clearly observed in several review articles devoted to the analysis of pesticide residues [80–83] or to the revision of the QuEChERS method [19, 84]. As an example, Table 14.2 compiles some works devoted to the analysis of a large number of pesticides from complex samples by a variety of analytic techniques. Nowadays, trends in this field (and also in the analysis of other compounds using QuEChERS) are focused on the extension of the application of the method to other analytes and to highly complex matrices, to the detailed study of matrix effects [94], and the long-term routine applications of the method.

TABLE 14.2 Some Examples of the Application of the QuEChERS Method in Pesticides Analysis

		Extraction						
Analytes	Sample (Amount)	Solvents (Volume)	Salts (Amount)	Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
205 Pesticides	Spice (2 g)	MeCN (10 mL)	$MgSO_4$ (4 g), NaCl (1 g), $Na_3Cit{\cdot}2H_2O$ (1 g), $Na_2HCit{\cdot}1.5H_2O$ (0.5 g)	EMR-lipid, EMR polish (0.2 g of NaCl and 0.8 g of $MgSO_4$)	GC-MS/MS	70–120 for 90% of the analytes	2 μg/kg	[53]
60 Pesticides	Crayfish and mantis shrimp (10 g)	MeCN (10 mL)	NaCl (3 g)	PSA (50 mg)	HPLC-MS/MS	70–120	0.4–10 μg/kg	[85]
40 Pesticides	Apple, banana, broccoli, celery, grape, green bean, peach, potato, orange, and squash (15, 10, 5, 2, and 1 g)	MeCN (1 mL per gram)	4/1 $MgSO_4$/NaCl (0.5 g per 1-g sample)	$MgSO_4$/PSA/C_{18}/CarbonX (20/12/12/1, w/w/w/w) (45 mg)	(LP)GC-MS/MS and UHPLC-MS/MS	70–120	–	[86]
69 Pesticides	Wheat and rice straws (2.0 g)	H_2O with 2% (v/v) formic acid (5 mL) MeCN (20 mL)	NaCl (3 g)	Wheat straw: $MgSO_4$ (30 mg) PSA (20 mg), Rice straw: $MgSO_4$ (30 mg) C_{18} (20 mg)	LC-MS/MS	70–120 for 90% of the analytes	40–200 μg/kg	[87]
60 Pesticides	Cinnamon bark (2 g)	MeCN (10 mL)	(A) $HCOONH_4$ (2.5 g), (B) NaCl (2.5 g)	$MgSO_4$ (150 mg) and C_{18} (50 mg)	LC-MS/MS	71–118 for 73% of the analytes	0.5 μg/kg	[88]

20 Pesticides	Apple, broccoli, shallot (5 g), and tea (1 g)	MeCN (20 mL)	$MgSO_4$ (2 g) and NaCl (2 g)	PVPP (150 mg), PSA (50 mg) and GCB (10 mg)	UPLC-MS/MS	73–106	1–2 μg/kg	[89]
35 Pesticides	Apple/pomarrosa (*Syzygium malaccense*), starfruit/carambola (*Averrhoa carambola*), yoyomo (*Spondias purpurea*), and papayuela (*Vasconcellea pubescens*) (15 g)	MeCN with 1% (v/v) HOAc (15 mL)	$MgSO_4$ (6 g) and NaOAc (1.5 g)	$MgSO_4$ (150 mg), PSA (50 mg), C_{18}(50 mg) and GCB (7.5 mg)	GC-MS/MS	70–120 for 95% of the analytes	5 μg/kg	[90]
170 Pesticides	Green pepper and cucumber samples (15 g)	MeOH with 1% (v/v) HOAc (15 mL)	$MgSO_4$ (6 g) and NaOAc (1.5 g)	$MgSO_4$ (112.5 mg) and PSA (18.75 mg)	LC-MS/MS	70–120 for 95% of the analytes	0.1 μg/kg	[91]
99 Pesticides	Groundnut, soybean, kidney bean, black bean, cowpea, chili pepper, Egusi seeds, coffee beans, cocoa beans, maize, white pepper, and Bambara nuts (5 g)	MeCN (15 mL)	$Na_2HCit{\cdot}1.5H_2O$ (0.75 g) $Na_3Cit{\cdot}2H_2O$ (1.5 g), NaCl (1.5 g), and $MgSO_4$ (6 g)	$MgSO_4$ (112.5 mg), PSA (37.5 mg) and C_{18} (18.75 mg)	LC-MS/MS and GC-ECD	70–120 for 60% of the analytes	0.0004 and 0.0537 mg/kg	[92]
43 Pesticides	Honey and honeybee (0.5 g)	(A) Honey: MeCN (5 mL), (B) honeybee MeCN (5 mL) and *n*-hexane (1.5 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (150 mg) and PSA (50 mg)	GC-MS/MS and LC-MS/MS	85–116	2.8 μg/kg	[93]

Table 14.3 summarizes some studies dealing with the extraction of pharmaceuticals, mycotoxins, and PAHs, the second group of most extracted compounds by QuEChERS after pesticides, and Table 14.4 miscellaneous compounds selected to illustrate the wide application of the method. In the first case (pharmaceutical analysis), this field is an important application area, since many pharmaceutical compounds are considered as emerging contaminants and their monitoring is especially relevant nowadays. Many drugs are also ionizable, and therefore suitable pH control may be required [19, 123]. Most of the applications of the QuEChERS method in this field frequently involve the simultaneously analysis of pharmaceuticals with other emerging contaminants.

For mycotoxins, all studies so far are focused on food samples, since food is the main route for human exposure. Most applications have been to cereals, since mycotoxin contamination is mainly associated with such matrices [19]. As previously indicated the addition of water is required in such cases to promote swelling to increase recovery.

Regarding PAHs, their extraction is mainly performed using the original method, without the addition of a buffer, since they are not ionizable [19]. In most cases the 16 PAHs included in the US EPA priority pollutants list have been determined, as well as the analysis of their metabolites on a few occasions [19].

For the majority of applications, MS/MS detection is routinely used, which is necessary since the method provides low selectivity (as previously indicated as a "mega method") and also to unequivocally confirm the presence of each compound, although "traditional" detectors have been used as well [124]. In most cases, either LC or GC is the analytic technique of choice for separation, although in a few applications, capillary electrophoresis (CE) has been used [125, 126]. The application of CE is probably hindered by the necessity of highly clean extracts with low conductivity, since high sample conductivity results in current breakdown. The fact that the QuEChERS method requires the use of salts and sorbents not readily compatible with CE limits current applications.

Finally, it should be highlighted that internal standards (ISs) are frequently used to correct for analyte losses during the extraction. Typically deuterated standards are used, although their high cost tends to limit the number employed. The presence of matrix components in the final extracts makes necessary the evaluation of the matrix effect [94] and the use of matrix-matched calibration to compensate for them. The use of analyte protectants [127, 128] also helps to reduce analyte tailing, and decomposition within the GC inlet by masking the active sites that generate nonvolatile compounds in the GC inlet is also recommended, especially for pesticide analysis.

TABLE 14.3 Some Examples of the Application of the QuEChERS Method in Pharmaceutical, Mycotoxin, and PAH Analysis

		Extraction						
Analytes	Sample (Amount)	Solvents (Volume)	Salts (Amount)	Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
Pharmaceuticals								
6 Pharmaceuticals	Soil (5 g)	(A) MeCN (10 mL), (B) MeCN/water 70:30 v/v with 5% (v/v) acetic acid (10 mL), (C) MeCN with 5% (v/v) water (10 mL), (D) MeCN/water 70:30 v/v with 5% (v/v) acetic acid (7,5 mL)	(A) $MgSO_4$ (4 g) and NaCl (1 g), (B) $MgSO_4$ (3 g) and NaCl (0.75 g) in version (D)	(A) PSA (25 mg), (B) C_{18} (25 mg)	HPLC-UV	83–113	3,5 µg/L	[95]
6 Pharmaceuticals	Lagoon cockle (*Cerastoderma glaucum*), coquina clam (*Donax trunculus*), manila clam (*Ruditapes philippinarum*), striped venus clam (*Chamelea gallina*), sword razor clam (*Ensis sp.*) and mussel (*Mytilus galloprovincialis*)	MeCN (10 mL)	(A) $MgSO_4$ (4 g), NaCl (1 g), $Na_3Cit{\cdot}2H_2O$ (1 g), $Na_2HCit{\cdot}1.5H_2O$ (0.5 g), (B) $MgSO_4$ (6 g) and NaOAc (1,5 g)	Silica gel (1 g) per milliliter of extract	LC-MS/MS	61–95	1 ng/g	[96]

Continued

TABLE 14.3 Some Examples of the Application of the QuEChERS Method in Pharmaceutical, Mycotoxin, and PAH Analysis—cont'd

		Extraction						
Analytes	Sample (Amount)	Solvents (Volume)	Salts (Amount)	Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
26 Pharmaceuticals	Human whole blood (1 mL)	EtOAc (3 mL)	Saturated carbonate buffer (45-g $NaHCO_3$, 30-g Na_2CO_3 in distilled water) 100 µL	$MgSO_4$ (150 mg) PSA (50 mg) per milliliter of extract	UHPLC-MS/MS	21–98	0.05 ng/mL	[97]
15 Emerging pollutants	Sediments (2 g)	MeCN with 1% (v/v) HOAc (10 mL)	$MgSO_4$ (6 g) and NaOAc (1.5 g)	$MgSO_4$ (900 mg) PSA (150 mg) and GCB (15 mg) per 6-mL extract	LC-MS/MS	40–98	0.5 ng/g	[98]
Mycotoxins								
Zearalenone	Maize and samples for animal feed (rabbit and hamster) (15 g)	MeCN with 1% (v/v) HOAc (15 mL)	$MgSO_4$ (6 g) and NaOAc (1.5 g)	$MgSO_4$ (150 mg) and PSA and C_{18} (50 mg each)	Automated fluorimetric sensor	93–107	15 µg/L	[99]
23 Mycotoxins	Wheat and maize (2 g)	MeCN with 5% (v/v) HCOOH (10 mL)	$MgSO_4$ (4 g) and NaOAc (1 g)	Ultrafree-MC	UPLC-MS/MS	60–98	0.13 µg/kg in wheat, 0.14 µg/kg in maize	[100]
23 Mycotoxins	Beer (15 mL)	MeCN (5 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (900 mg) and C_{18} (300 mg) per 6-mL extract	UPLC-MS/MS	70–110 for almost all analytes	0.002 µg/L	[101]

15 Mycotoxins	*Menthae haplocalycis* (1 g)	H_2O with 2% (v/v) HCOOH (5 mL), MeCN (5 mL)	$MgSO_4$ (2 g) and NaCl (1 g)	$MgSO_4$ (150 mg) and C_{18} (50 mg)	LC-MS/MS	67.1–103 for almost all analytes	0.007 μg/kg	[102]
11 Mycotoxins	Wheat, maize, and millet (2 g)	MeCN (10 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (600 mg) and PSA (200 mg)	DART-MS	84–118	50–150 μg/kg	[103]
11 Mycotoxins	Functional and medicinal herbs (2 g)	MeCN with 2% (v/v) HCOOH (20 mL)	$MgSO_4$ (4 g), $Na_3Cit{\cdot}2H_2O$ (1 g), NaCl (1 g) and $Na_2HCit{\cdot}1.5H_2O$ (0.5 g)	C_{18} (8.3 mg)	LC-MS/MS	52.4–91.2	0.25–2.5 μg/kg	[104]
15 Mycotoxins	Liquorice (2 g)	MeCN with 5% (v/v) HCOOH (15 mL)	$MgSO_4$ (4 g), $Na_3Cit{\cdot}2H_2O$ (1.5 g), NaCl (1 g) and $Na_2HCit{\cdot}1.5H_2O$ (1 g)	$MgSO_4$ (900 mg), PSA (150 mg), C_{18} (600 mg), and Si for the dSPE cleanup (150 mg) per 6-mL extract	UHPLC-MS/MS	81–103	0.05 μg/kg	[105]
Patulin	Strawberry (10 g)	MeCN with 1% (v/v) HOAc (10 mL)	$MgSO_4$ (4 g), $Na_3Cit{\cdot}2H_2O$ (1 g), NaCl (1 g) and $Na_2HCit{\cdot}1.5H_2O$ (0.5 g)	(A) $MgSO_4$ (150 mg), PSA (25 mg), GCB (7.5 g), (B) $MgSO_4$ (150 mg) and PSA (50 mg)	HPLC-DAD	96–103	1.5 and 5 μg/kg	[106]
PAHs								
12 PAHs	Baby foods (10 g)	MeCN (10 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (150 mg), PSA (25 mg) and C_{18} (25 mg)	GC-MS	72–112	1 μg/kg	[107]

Continued

TABLE 14.3 Some Examples of the Application of the QuEChERS Method in Pharmaceutical, Mycotoxin, and PAH Analysis—cont'd

		Extraction						
Analytes	Sample (Amount)	Solvents (Volume)	Salts (Amount)	Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
23 PAHs	Smoked meat (2 g)	Water (1,6 mL) and MeCN (2 mL)	$MgSO_4$ (3 g) and NaCl (0.5 g)	$MgSO_4$ (500 mg) and Z-Sep (100 mg)	GC-MS	74–117	0.3 and 0.9 μg/kg	[108]
5 PAHs	Strawberry, lemongrass, peppermint, and boldo (1 g)	Water (10 mL) and EtOAc (10 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (60 mg), PSA (20 mg) and silica gel (60 mg)	HPLC-FD	54–99	0.03–0.3 μg/kg	[109]
16 PAHs	Soil (5 g)	Hexane-acetone or EtOAc (2:1 v/v) (30 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (150 mg), PSA (50 mg), C_{18} (50 mg) clinoptilolite (50 mg), Florisil (50 mg) and diatomaceous earth (50 mg)	GC-MS	23–109	0.60–1.53 μg/kg	[110]
16 PAHs	Vegetables (10 g)	MeCN (30 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (150 mg) and PSA (50 mg)	GC-MS	71–108	0.012 μg/kg	[111]
24 PAHs	Fish tissues, feeds, and feed ingredients (1 g)	MeCN (2 mL)	$MgSO_4$ (0.8 g)	$MgSO_4$ (150 mg), PSA (50 mg) and C_{18} (50 mg)	GC-MS/MS	70–120	0.5–2 μg/kg	[112]

12 PAHs	Curry spice powder (2 g), salmon, mussels, shrimps, bacon, cutlets, wheat flour, infant formula, infant follow-up formula, and infant foods (10, 5, 2, and 1 g, respectively)	Water (5 mL) and MeCN (10 mL)	(A) MgSO4 (4 g), NaCl (1 g), $Na_2HCit{\cdot}1.5H_2O$ (0.5) and $Na_3Cit{\cdot}2H_2O$ (1 g), (B) EMR-lipid dSPE	(A) $MgSO_4$ (900 mg), PSA (150 mg) and C_{18} (150 mg) per 8-mL extract, (B) EMR polish containing (1:4, NaCl/$MgSO_4$) (2 g)	GC-MS/MS	50–120	$\leq$0.3 and $\leq$0.9 μg/kg	[113]

TABLE 14.4 Some Examples of the Application of the QuEChERS Method for Multiresidue Analysis

		Extraction						
Analytes	Sample (Amount)	Solvents (Volume)	Salts (amount)	Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
16 PAHs, 12 PCBs, and 7 OCPs	Spiked estuarine and marine sediments (10 g)	MeCN with 1% (v/v) formic acid (10 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	$MgSO_4$ (150 mg) and PSA (50 mg)	GC-MS	62–131	0.01 μg/kg	[114]
16 PAHs, 7 PBDEs, 12 PCBs, and 17 OCPs	Blood of subadult green turtles (*Chelonia mydas*) (1 g)	MeCN (3 mL) and water (1 mL)	$MgSO_4$ (1 g) and NaCl (0.2 g)	$MgSO_4$ (150 mg), PSA (50 mg), C_{18} (50 mg)	GC-MS/MS	60–107 for 51% of the analytes	0.1–2 μg/L	[115]
65 Pesticides and 200 environmental contaminants (PCBs, PAHs, PBDEs, and other flame retardants)	Cattle, swine, and poultry muscle tissues (2 g)	Water (1.6 mL) and MeCN (2 mL)	$MgSO_4$ (0.8 g) and NaCl (0.2 g)	0.2 μm PVD containing (45 mg) of 20/12/12/1 (w/w/w/w) $MgSO_4$ and PSA, C_{18}	(LP)GC-MS/MS and UHPLC-MS/MS	70–120 for 82% of the analytes	<5 μg/kg	[116]
16 PAHs, 12 PCBs, and 9 OCPs	Sediment (5 g)	Hexane/acetone, dichloromethane/acetone (20 mL)	$MgSO_4$ (4 g), NaCl (1 g), $Na_3Cit{\cdot}2H_2O$ (1 g) and $Na_2HCit{\cdot}1.5H_2O$ (0.5 g)	$MgSO_4$ (150 mg) and PSA (50 mg)	GC-MS	PAHs, 60–103; PCBs, 76–131; and OCPs, 81–137	0.02 μg/kg	[117]

90 Pesticides, 16 PAHs, and 22 PCBs	Honey (5 g)	MeCN (10 mL)	$MgSO_4$ (4 g), NaCl (1 g), $Na_3Cit{\cdot}2H_2O$ (1 g), $Na_2HCit{\cdot}1.5H_2O$ (0.5 g)	PSA (50 mg)	LC-MS/MS	60–103	3 μg/kg	[118]
41 PCBs, 24 PBDEs, and 17 PCDD/Fs	Blue mussels (*M. edulis*) and Atlantic salmon (*Salmo salar*) (6 g)	EtOAc (10 mL)	$MgSO_4$ (4 g) and NaCl (2 g)	SPE column: 2 g of silica (deactivated with 2% H_2O), 1 cm of Na_2SO_4	GC-MS	70–100	0.05 μg/kg for PCBs, 0.2 μg/kg for PAHs and PBDEs and 1 ng/kg for PCDD/Fs	[119]
6 PCBs, 15 OCPs, 7 PBDEs, 4 PAHs, and 17 PFASs	Mussels and clams (5 g)	HPLC-MS: Hexane/acetone 4/1 v/v (10 mL), GC-MS/MS: MeCN (10 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	Z-Sep (50 mg)	HPLC-MS and GC-MS/MS	70–120	0.005 μg/kg	[120]
7 PAHs, 7 PBDEs, 7 PCBs, 5 PBT chemicals, 7 ECCs	White sturgeon (*Acipenser transmontanus*) (5 g)	MeCN (5 mL)	$MgSO_4$ (2 g) and NaCl (0.5 g)	$MgSO_4$ (150 mg), PSA (50 mg) and C_{18} (50 mg)	GC-MS	71%–98% for PAHs, 60%–107% for PBDEs and PCBs, 86%–107% for PBT chemicals, and 88%–107% for ECCs	15 μg/kg	[121]

Continued

TABLE 14.4 Some Examples of the Application of the QuEChERS Method for Multiresidue Analysis—cont'd

		Extraction						
Analytes	Sample (Amount)	Solvents (Volume)	Salts (amount)	Sorbents in the dSPE Step	Analytic Technique	Recovery (%)	LOQs	References
4 Pharmaceuticals, 4 pesticides, and 4 PCPs	Sludge (10 g)	MeCN with 1% (v/v) HOAc (10 mL)	$MgSO_4$ (4 g) and NaCl (1 g)	(A) C_{18} (50 mg), (B) PSA (50 mg), (C) PSA (50 mg), C_{18} (50 mg), (D) PSA (50 mg), C_{18} (50 mg), chitin (50 mg), and GCB (7,5 mg) per 2-mL extract	LC-MS/MS	50–120	0.1 μg/kg	[122]

14.6 Conclusions and Trends

There is no universal sample pretreatment procedure, since it greatly depends on the analyte, matrix, and the selected determination step. However, the QuEChERS method, including its possible modifications, has demonstrated an extremely wide applicability range, and it has been applied for the extraction of a wide variety of analytes (pesticides, pharmaceutical compounds, PAHs, mycotoxins, etc.) and matrices. It can be considered as a "mega method." Its inherent advantages (rapidity, simplicity, security, low cost, effectiveness, ruggedness, and high throughput) are among the most desired characteristics of any analytic method.

QuEChERS is more than a simple extraction procedure. It is, by far, the routine method most frequently employed in regulatory laboratories around the globe for pesticide residue analysis in food. It can be combined with either GC or LC and in some cases CE (limited by the high conductivity of the final extract).

Depending on each specific class of analyte and matrix, the sample amount and the combination of salts and sorbents should be changed accordingly. The commercialization of kits adapted to the user's needs has greatly facilitated this issue and increased its applicability.

Despite the current maturity of the QuEChERS method, it is still under development and in the process of being adapted to different and more complex scenarios. In particular, it is presently fighting its possible automation, which is still a challenge. In the coming years the introduction of new dSPE sorbents is likely, and the analysis of multiple analytes in complex matrices is expected to expand, maintaining a high interest in the method.

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Chapter 15

Single-Drop Microextraction

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Chapter Outline

Liquid-Phase Extraction. https://doi.org/10.1016/B978-0-12-816911-7.00015-3

15.1 Introduction

A liquid drop as a potential interface for sampling was first demonstrated experimentally by flowing across a 5 μL droplet, supported by a silica capillary tube, a gas stream containing ammonia and sulfur dioxide from air. The soluble constituents in the gas were diffused into an aqueous drop, and were inline detected spectrophotometrically utilizing the indophenol blue formation by ammonia and conductometrically by oxidation of sulfur dioxide to sulfuric acid [1]. This drop-based gas collection was further explored as a drop-to-drop preconcentration and matrix isolation module in which the sample (sodium dodecyl sulfate) and the reagent (methylene blue) were continuously added to the aqueous drop surrounding the chloroform microdrop, about 1.3 μL, when the ion-pair was extracted while the aqueous phase was aspirated away. Then, the aqueous drop was made clear by a wash solution and the color in the organic drop was measured by an optical fiber-based absorbance detector [2]. Use of fresh single-drop solvent for each extraction was a major advancement in avoiding the stubborn problem of analyte memory effects as observed with membranes.

Drop-based extraction was later developed as a single-drop microextraction (SDME) technique for gas chromatography (GC) in which a Teflon rod with a spherical recess drilled at one end to hold 8 μL of water-immiscible organic solvent (*n*-octane), containing *n*-dodecane as the internal standard, was kept immersed in a stirred aqueous sample of 4-methylacetophenone for a specified time. Thereafter, the probe was withdrawn and a 1 μL portion of extract was sampled with a microsyringe and injected into the GC for analysis [3]. The method was further simplified [4] by employing a microsyringe, whereby its needle was penetrated through the septum of a mini-vial to protrude the tip holding the drop of extraction solvent well in the aqueous sample. After stirring for a specified time, the drop was retracted and the extract injected into the GC. Thus the same GC microsyringe was used both for extraction and injection. This work also investigated the theoretical model for mass transfer performance of a suspended solvent drop as a function of experimental variables.

SDME is rarely an exhaustive process, and typically not taken to equilibrium to allow a reasonable extraction time. Due to the highly reduced ratio of extraction drop-to-sample volume in SDME, high enrichment or preconcentration of analytes is achieved. By careful selection of the extraction solvent for particular analytes, a favorable distribution of target analytes between the donor aqueous phase and acceptor organic solvent drop occurs in a specified time. This kinetic exclusion of most matrix substances results in better sample cleanup in SDME than in classical liquid-liquid extraction. In this regard, SDME also contrasts with the rival method of dispersive liquid-liquid microextraction (DLLME) [5, 6], where the emphasis is on attaining a large enrichment factor in the shortest time by exploiting the higher

surface area of extraction solvent droplets. The sensitivity of DLLME is due to high enrichment factors, whereas in SDME it is due to low background noise. Regardless of the extent of extraction, but determined by its precision, calibration is based on the extraction of aqueous standards under conditions identical to those for the sample. SDME has gained popularity on account of its low cost, use of common laboratory equipment, and immense reduction in sample size and amount of extraction solvents. Additional features of merit include applicability to both polar and nonpolar analytes, derivatization of analytes under green analytical methodology, availability of diverse modes of extraction, utilization of the whole extraction drop in analysis to gain optimum sensitivity, and easy full automation of methods.

Because the solvent drop is kept hanging at the needle tip, SDME in its original format has shortcomings of drop dislodgement, solvent evaporation, and partial miscibility with water. These problems become severe when the final analysis is based on GC, which is compatible with low-boiling and low-viscosity SDME solvents. There is a relatively narrow range for variation in stirring rate and temperature.

SDME is an attractive alternative to classical liquid-liquid extraction. Its original format of a single drop suspended at the needle tip placed in the aqueous sample solution has been creatively used to develop a variety of modes of extraction and an array of liquid-phase microextraction (LPME) techniques working on different principles. The major early advances in SDME were reviewed in 2002 [7], 2007 [8], and 2010 [9] with emphasis on implicit theoretical aspects and experimental parameters that influence the drop extraction process, and in 2011 [10] covering the comprehensive literature on the analytical applications of different modes of SDME. Trends in green aspects of analysis by different modes of SDME were described in 2015 [11], and the developments made in 2017–18 are discussed in a recent review [12].

15.2 Modes of SDME

The modes of extraction (Fig. 15.1) constitute different experimental formats in which SDME can be performed, each having its own object and advantage, and designed to reduce the limitations of original formats. Based on the phases participating in the extraction, SDME can principally be a two-phase or a three-phase process.

Direct immersion SDME (DI-SDME) is a commonly applicable mode of SDME in which the needle of a GC syringe containing 1–3 μL of a water-immiscible organic solvent is pierced through the septum of the vial until the tip position is below the meniscus of the sample solution [4]. A solvent drop is formed at the needle tip by depressing the plunger, and maintained in the stirred sample solution for a predetermined time. Thereafter, the drop is drawn back into the syringe and immediately injected for analysis. SDME is performed with water-immiscible and low-vapor

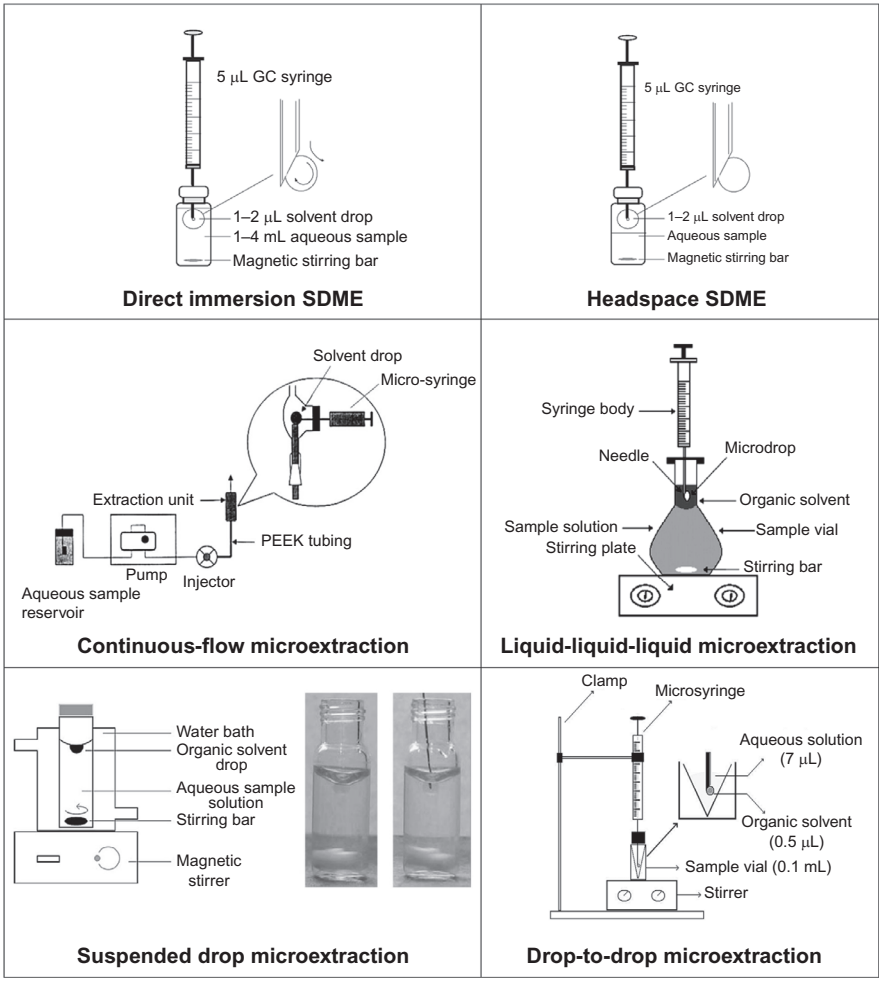

Fig. 15.1 Different modes of single-drop microextraction. *(Reproduced from Jain A, Verma KK. Recent advances in applications of single-drop microextraction: a review. Anal Chim Acta 2011;706:37–65; Yangcheng L, Quan L, Guangsheng L, Youyuan D. Directly suspended droplet microextraction. Anal Chim Acta 2006;566:259–64; Fan Z, Liu X. Determination of methylmercury and phenylmercury in water samples by liquid-liquid-liquid microextraction coupled with capillary electrophoresis. J Chromatogr A 2008;1180: 187–92 with permission from Elsevier; and from Wu H-F, Yen J-H, Chin C-C. Combining drop-to-drop solvent microextraction with gas chromatography/mass spectrometry using electronic ionization and self-ion/molecule reaction method to determine methoxyacetophenone isomers in one drop of water. Anal Chem 2006;78:1707–12; Liu W, Lee HK. Continuous-flow microextraction exceeding 1000-fold concentration of dilute analytes. Anal Chem 2000;72:4462–67 with permission from the American Chemical Society.)*

pressure organic solvents that have optimum extraction efficiency for the analytes, and at a stirring rate so that the sessile drop is not dislodged [8, 9]. Similarly, insoluble or particulate matter in the aqueous sample should also be avoided.

In *drop-to-drop microextraction (DDME)*, a microdrop of chloroform is placed inside a flowing aqueous sample drop containing analyte and reagent [2]. To reduce the consumption of analyte solution, an innovative system was proposed with the aqueous sample typically reduced to 7 μL and placed inside 0.5 μL of extraction solvent drop hanging at the needle tip of a microsyringe [13]. The equilibrium between the two phases is quickly established without stirring because of miniaturization and rapid mass transfer. Based on the small sample requirement, this method has the advantage of being able to work with small volumes of biological fluids with fast extraction and extreme simplicity of operation [14].

Another mode of small volume liquid-liquid extraction, which avoids the problem of accidentally dislodging the solvent drop in DI-SDME, is *dynamic LPME* [15]. This can be considered a simulated flow injection extraction and not strictly a drop-mode extraction. A plug of organic solvent followed by a plug of aqueous sample is drawn into the microsyringe. A renewable microfilm is formed along the inside walls of the microsyringe by pulling the plunger rapidly from the aqueous sample. Thus both the donor phase (sample) and the acceptor phase (extraction solvent) are in motion. The same process was repeated with fresh aliquots of aqueous sample, retaining the organic solvent in the syringe. Here, too, water immiscibility of the extraction solvent is necessary, but those that give unstable drops in DI-SDME, e.g., chloroform, can be employed [16].

A mechanism of diffusion and convection was used in *continuous flow microextraction* in which, instead of using portions of aqueous sample for extraction as in dynamic LPME, the microdrop organic solvent held at the tip of polyetheretherketone tubing is brought into contact with a continuously flowing aqueous sample solution propelled by a mechanical pump [17]. Enrichment factors of 260–1600 were reported for an aqueous sample volume of 3 mL circulated during a period of 10 min.

A technique that does not make use of a microsyringe to hold the extraction solvent is referred to as *directly suspended droplet microextraction (DSDME)* [18]. Stirring (typically 1000 rpm) the aqueous sample produces a vertical velocity gradient resulting in the formation of a vortex in the solution into which 5–100 μL of a water-immiscible organic solvent is added. The spinning solvent drop intensifies the mass transfer of analytes into the organic solvent. While the solution is still being stirred, a portion of the solvent drop is withdrawn for analysis. The drawback of drop dislodgement, the restriction to low stirring speeds, and small drop volumes, as in DI-SDME, are practically absent in DSDME. Nevertheless, there is an obvious problem in collecting extract from a small volume spinning organic drop without some aqueous sample entering the microsyringe. This inconvenience is amicably sorted out by using an organic solvent, such as 1-undecanol, which has a melting point close to

room temperature (13–15°C). After extraction, the sample vial is cooled in an ice bath to solidify the solvent drop and collected manually, a technique called *solidification of floating organic drop microextraction (SFODME)* [19, 20]. The solvent tablet is removed with a microspatula and thawed in a micro-vial before analysis.

The solvents used in SDME are water immiscible and as such not compatible with reversed-phase liquid chromatography or capillary electrophoresis. Analyte acid-base properties have been exploited for extraction and to facilitate the use of liquid chromatography. For basic analytes, the sample solution is adjusted to pH 13 and a polytetrafluoroethylene (PTFE) ring placed upon it. Then, a water-immiscible organic solvent lighter than water is delivered into the ring. An aqueous receiving phase of pH 2.1 is added carefully on to the organic membrane, and the sample solution is stirred. After a prescribed extraction time, a portion of the aqueous receiving phase is withdrawn for analysis by liquid chromatography [21, 22]. Alternatively, an aqueous drop, as a receiving phase, is formed in the organic solvent using a microsyringe [23]. This three-phase extraction system, aqueous-organic-aqueous, is called single-drop *liquid-liquid-liquid microextraction (LLLME)* or back-extraction. The donor sample solution is adjusted to a high pH to keep basic substances in their molecular form for their extraction into the organic solvent interface. The aqueous acceptor phase of low pH strips the basic analytes from the organic phase as protonated species. The back-extraction technique has a twofold advantage of additional sample cleanup due to the dual mass transfers and higher enrichment. In a still simpler format, which avoids a PTFE ring, a larger volume of low-density organic solvent is placed on top of an aqueous sample into which the aqueous receiving drop is formed using a microsyringe. Based on the acid-base reaction principle, the method was used for the extraction of antihistamines in human urine for analysis by micellar electrokinetic chromatography [24]. Extension to other principles was demonstrated by the extraction into toluene of organomercury compounds as their 1-(2-pyridylazo)-2-naphthol complexes, followed by back-extraction into aqueous cysteine. The acceptor drop was analyzed by capillary electrophoresis [25].

Another three-phase extraction technique is *headspace-SDME (HS-SDME)* applicable to volatile and semivolatile compounds, utilizing the same equipment as DI-SDME; the distinction being that the solvent drop is kept in the headspace above the aqueous sample meniscus where the chances of dislodging the drop are less frequent. This method can also be used for solid samples. In a modified version, *exposed dynamic HS-SDME*, the solvent drop is exposed in a repeated manner to the sample headspace, and after each exposure withdrawn back into the microsyringe barrel. Solvents used in HS-SDME need not be water immiscible as in DI-SDME; thus a wider choice of solvents of low volatility makes this mode of extraction more attractive. This feature prompted the development of HS-SDME for polar analytes, such as alcohols, by using ethylene glycol as the extraction solvent [26], and for nonpolar compounds—benzene, toluene, ethylbenzene, and xylenes—using 1-octanol

[27] or *n*-hexadecane [28]. Headspace extraction results in a high degree of cleanup for volatile analytes, and particulate matter and nonvolatile matrix substances in the aqueous sample are not a problem. Contrary to DI-SDME, headspace extractions can be performed at high stirring speeds and moderate temperatures, both of which promote higher mass transfer of volatile analytes to the headspace.

15.3 Attributes of Different Modes of Single-Drop Microextraction and New Strategies

15.3.1 Direct immersion single-drop microextraction

DI-SDME (two-phase mode) and HS-SDME (three-phase mode) are the most widely used extraction modes. The reason being the use of the same low-cost equipment and the two different approaches to locating the solvent drop with respect to the sample can amicably handle matrix interference and facilitates analyte preconcentration and chemical modifications of both polar and nonpolar analytes. However, another noteworthy difference is in the solvent used for extraction. Since the solvent drop is in direct contact with the aqueous sample in DI-SDME, the solvent used must be water immiscible and of low polarity similar to the analytes present in a relatively clean matrix. On the contrary, solvent for HS-SDME must be involatile and may be polar, e.g., water (with sodium hydroxide) for phenols [29], or semipolar to nonpolar [27, 28].

A new format of DI-SDME reverses the solvent polarity for the donor and acceptor phases for extraction of polar analytes from nonpolar matrices with a polar solvent drop, such as water [30]. Electroenhanced DI-SDME has been used to accelerate the mass transfer and in-drop derivatization of amphetamines (Fig. 15.2) [31]. A platinum wire and the syringe needle housing the extraction solvent and derivatization reagent for the solvent drop were dipped into the sample solution and used to complete the electrical circuit. The analytes as protonated amines migrated into the solvent drop and were simultaneously derivatized. The whole process was complete within 4 min. Many inconveniences associated with the syringe needle-supported microdrop were avoided by placing the microdrop of extraction solvent, denser than water, at the bottom of a centrifuge tube containing the aqueous sample. A magnetic stir bar was introduced above the tapered portion of the centrifuge tube with the help of a magnet, and the sample solution stirred at 1000 rpm. After extraction the stirring bar was removed using the magnet, and the extract collected [32]. This format avoided restrictions on drop size and the stirring rate. For 1 mL of aqueous sample and 3 μL of carbon tetrachloride as extraction solvent, better detection limits and enrichment factors were obtained than for conventional DI-SDME with a 1.5 μL toluene drop immersed in 3 mL of aqueous sample. Another system free

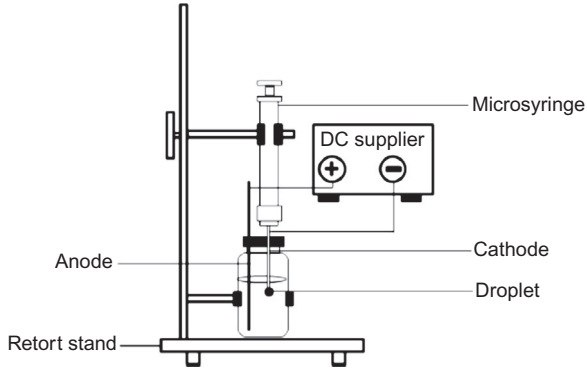

Fig. 15.2 Electroenhanced single-drop microextraction. *(Reproduced from Song A, Yang J. Efficient determination of amphetamine and methylamphetamine in human urine using electro-enhanced single-drop microextraction with in-drop derivatization and gas chromatography. Anal Chim Acta 2018; https://doi.org/10.1016/j.aca.2018.09.024 (web archive link) with permission from Elsevier.)*

from the drop dislodgement problem used an optical probe with an optical window to house the extraction solvent, and two optical fibers to connect to a radiation source and a detector. The probe tip was immersed in the aqueous sample and 40 µL of extraction solvent was delivered to the hole with the help of a microsyringe. Continuous absorbance measurements were used to follow the extraction of thiocyanate from the stirred solution [33].

Addition of salt (often sodium chloride) reduces the solubility of analytes in aqueous solution, and is a common practice in classical liquid-liquid extraction. Reduced recovery of analytes in DI-SDME has been widely reported. This effect is due to the reduced migration of analytes through the Nernst diffusion film built up between the donor aqueous phase and the acceptor organic phase [34]. In another example, this occurred due to a decrease in the drop volume of an ionic liquid extraction phase caused by salt formation and ion-exchange reactions increasing the water solubility of the extraction phase [35]. Salt addition also produced reduced extraction in dynamic liquid-phase microextraction [36]. In contrast, salt-saturated aqueous samples of polar analytes with addition of polar cosolvents, e.g., benzyl alcohol or a mixture of benzyl alcohol and 2-propanol, in DI-SDME enhanced the extraction of highly polar analytes [37, 38]. Reduction in the hydration sphere and formation of ion-pairs were believed to be responsible for increased analyte mass transfer. This method avoids the use of toxic organic solvents, and the extract can be analyzed by liquid chromatography.

Sample agitation has the effect of reducing the equilibrium extraction time and enhancing analyte recovery. Stirring rates of 200–800 rpm result in favorable mass

transfer due to decreased diffusion film thickness in stirred solutions [4]. Still faster stirring rates (1000 rpm) cause air bubble formation, partial dissolution of the solvent (toluene) drop, and dislodgement of the solvent drop in DI-SDME [39]. As expected, fast stirring of aqueous samples increased extraction rate in DSDME/SFODME [40, 41]. In place of mechanical stirring an efficient alternative approach was proposed to accelerate drop extraction. A plastic straw filled with a single drop of aqueous sample was dipped into a drop of ionic liquid acceptor phase, and the latter irradiated with surface acoustic waves. This caused the movement of ionic liquid around the sample drop and completed the extraction within 2 min [42]. Moreover, the proposed mechanism of donor-acceptor interaction thwarted the drop dislodgement inconvenience. The second arrangement used a conventional configuration for DI-SDME. Here, a drop of dilute nitric acid supported by a microsyringe was immersed into vegetable oil and placed in an ultrasonic bath at 46°C. The system was sonicated and the oxidative dissolution and extraction of cadmium was complete in 15 min [43]. The third option combined the advantages of continuous flow microwave extraction and SDME [44]. The powdered sample (organophosphorus pesticides in tea leaves) mixed with alumina was packed into an extraction vessel and connected to a flow line of 25% ethanol-water mixture. The effluent line was attached to a microchamber containing 5 μL of carbon tetrachloride housed in a microwave oven. The ethanol-water flow rate was set at 1 mL min^{-1} and the microwave oven was operated at 230 W. After 10 min the organic solvent drop in the microchamber was withdrawn for analysis (Fig. 15.3). Only a nonpolar solvent drop is stable in a continuous flow system, though dissolution of a small fraction is inevitable. For aqueous samples the

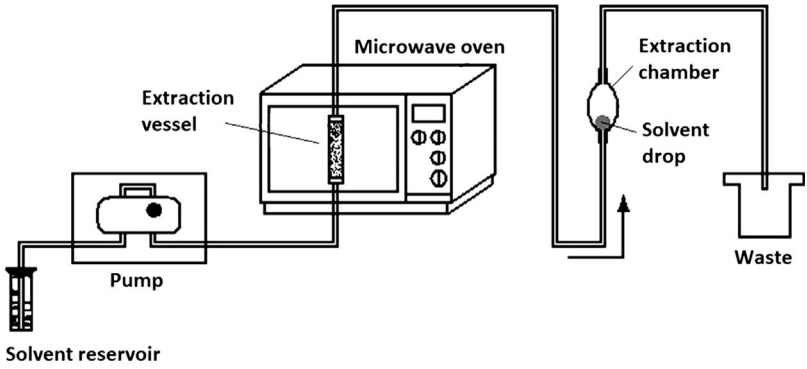

Fig. 15.3 Dynamic microwave-assisted extraction online coupled with single-drop microextraction. *(Reproduced from Wu L, Hu M, Li Z, Song Y, Zhang H, Yu A, et al. Dynamic microwave-assisted extraction online coupled with single drop microextraction of organophosphorus pesticides in tea samples. J Chromatogr A 2015;1407:42–51 with permission from Elsevier.)*

enrichment factors are significantly lower than for DI-SDME [45]. Continuous flow extraction has the advantage of being adaptable to microfluidic devices, and with microwave assistance, to solid samples without any preextraction of analytes by the classical liquid-based method.

Bubble formation is regarded as a nuisance in SDME procedures and efforts have been made to avoid the problem. Accidental bubble formation has been related to solvent evaporation and working at elevated temperatures leading to variable results [4]. Air bubbles larger than the drop size intentionally incorporated in the extraction produced high enrichment factors when compared with when either the bubble size was small or no bubbles were utilized [46]. The effect was related to the increased surface area of the drop and to the thin film phenomenon (Fig. 15.4). A 70- to 135-fold enrichment was reported for a chlorobenzene drop of 1 μL containing a 1 μL air bubble [47].

In another approach, column cleanup of an aqueous sample and continuous flow of effluent past a single solvent drop for extraction and enrichment was used. The

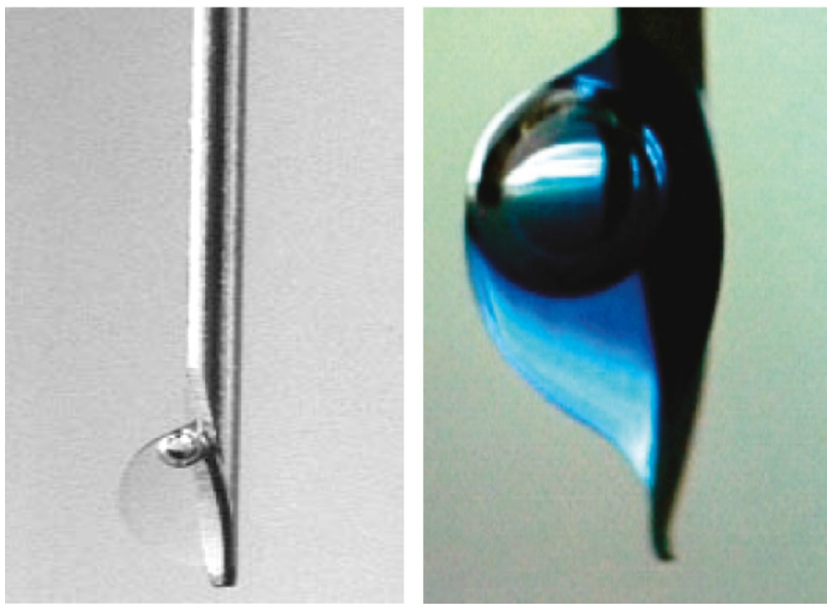

Fig. 15.4 Air bubble in single-drop microextraction. Accidently formed, *left panel*, and intentionally incorporated air bubble in extraction solvent drop, *right panel*. *(Reproduced from Jeannot MA, Cantwell FF. Mass transfer characteristics of solvent extraction into a single drop at the tip of syringe needle. Anal Chem 1997;69:235–39; Williams DBG, George MJ, Meyer R, Marjanovic L. Bubbles in solvent microextraction: the influence of intentionally introduced bubbles on extraction efficiency. Anal Chem 2011;83: 6713–16 with permission from the American Chemical Society.)*

barrel of a microsyringe was enclosed in a tubular liner, which in turn was placed inside a wide extraction vessel terminating into a narrow tube. With the needle of the microsyringe protruding into the narrow tube, the space between the liner and extraction vessel was packed with a macroporous resin. The aqueous sample was passed through the resin at 0.5 mL min^{-1} and then through the narrow tube of the extraction vessel where a solvent drop was formed at the needle tip [48].

Focused laser beam irradiation of certain aqueous solutions containing an organic solvent, e.g., 1-butanol, induced a local phase separation resulting in the formation of a single picoliter-dimension drop of organic solvent optically trapped at the focal point of the laser beam. Analytes present in the surrounding aqueous solution were promptly extracted in the drop of organic solvent [49]. This SDME technique was found useful for the detection of ultratrace amounts of analytes.

15.3.2 Drop-to-drop single-drop microextraction

The principal aim of drop-to-drop SDME is phase transfer because the volume of aqueous sample and extraction phase are mostly comparable. This allows sample cleanup without analyte enrichment. As the extraction is carried out without external aid, solvent selectivity is of utmost importance. To avoid drop dislodgement, the acceptor drop was protected in a hollow fiber supported by a microsyringe and placed in the donor aqueous sample. After extraction the acceptor phase was withdrawn into the syringe [50]. In a novel drop-to-drop LPME device based on a digital microfluidic chip, 2-nL-volume drops of immiscible liquids, one of which was an ionic liquid acceptor phase, were formed, merged, and mixed for extraction by driving along electrodes. Thereafter, the extracting ionic liquid drop was separated for real-time image-based concentration measurement [51].

15.3.3 Headspace single-drop microextraction

Temperature plays two important roles in HS-SDME. First, it accelerates mass transfer of analytes from the aqueous sample to the headspace, and then to the solvent extraction drop placed in the headspace. Thus it is necessary to increase the temperature for higher extraction recovery. Second, extraction solvent evaporation is higher at elevated temperatures. To maintain a balance between these two opposing effects of temperature, modified temperature gradient systems for HS-SDME have been proposed for volatile and semivolatile analytes in which an in-vial temperature gradient is generated between the donor aqueous sample and the acceptor microdrop at 50 and 4°C [52] and 80 and −20°C, respectively (Fig. 15.5) [53]. In the latter system, the enrichment factor was about four times higher than that attained in the classical HS-SDME. Temperature gradient HS-SDME was utilized in a simple sensor for ammonia where the donor

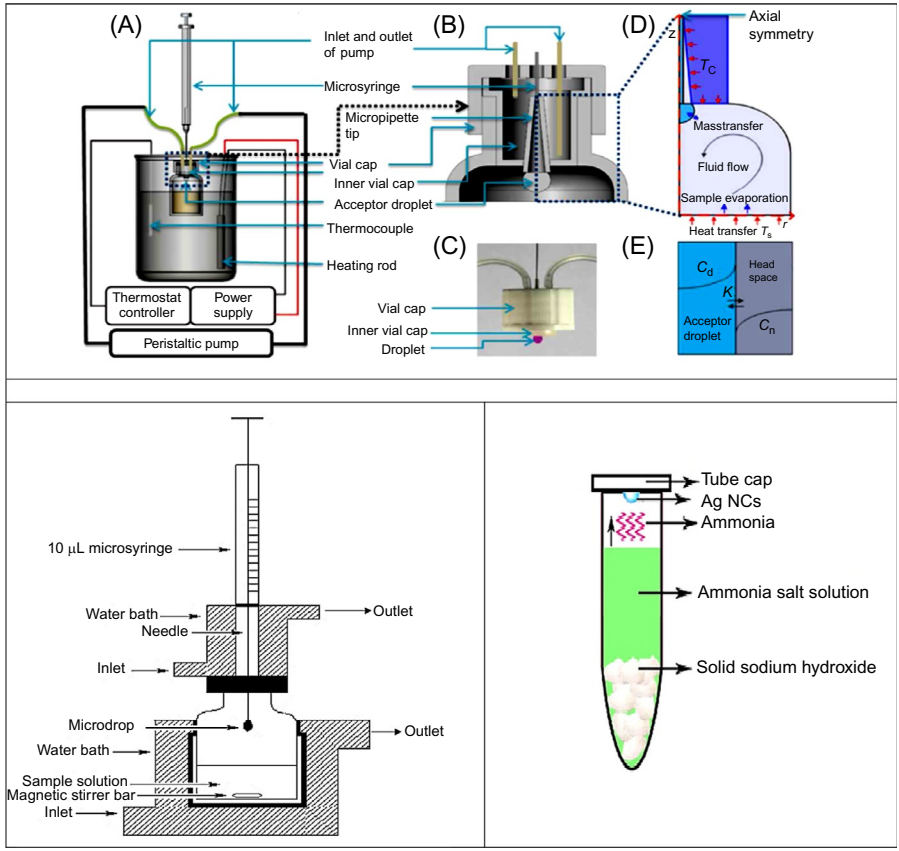

Fig. 15.5 In-vial temperature gradient headspace single-drop microextraction. *Top panel*, (A) In-vial temperature gradient unit, (B) Inner vial cap, and (C) Image of in-vial headspace acceptor extraction drop (with phenolphthalein in alkaline solution), (D) Two-dimensional geometry with the boundary conditions in the model, T_S and T_C, temperature of sample matrix and cooling liquid, respectively, (E) Mass transfer at the interface. *Bottom left panel*, temperature gradient produced by placement of extraction vial in a thermostatic bath. *Bottom right panel*, by addition of aqueous sample solution to solid sodium hydroxide in extraction tube. (Top panel: *Reproduced from Jahan S, Zhang Q, Pratush A, Xie H, Xiao H, Fan L, Cao C. In-vial temperature gradient headspace single drop microextraction designed by multi-physics simulation. Anal Chem 2016;88:10490–98 with permission from the American Chemical Society.* Bottom panels: *Reproduced from Yamini Y, Hojjati M, Haji-Hosseini M, Shamsipur M. Headspace solvent microextraction: a new method applied to the preconcentration of 2-butoxyethanol from aqueous solutions into a single microdrop. Talanta 2004;62:265–70; Dong JX, Gao ZF, Zhang Y, Li BL, Li NB, Luo HQ. A selective and sensitive optical sensor for dissolved ammonia detected via agglomeration of fluorescent Ag nanocluster and temperature gradient headspace single drop microextraction. Biosens Bioelectron 2017;91:155–61 with permission from Elsevier.)*

ammonium salt solution was added over solid sodium hydroxide. During dissolution of the solid, the temperature rose to about 50°C, assisting the transfer of ammonia to the headspace, where it was extracted by a silver nanocluster droplet (Fig. 15.5) [54].

In temperature-controlled HS-SDME, a flask containing the aqueous sample is heated in a domestic microwave oven while the solvent drop is located in a sleeve of polypropylene hollow fiber in the inner jacket of a water condenser. The latter was connected to a flask through a hole in the oven [55]. A different setup used 25 μL of extraction solvent in a knot-shaped hollow fiber with a 13 μL portion held at the center of the knot. The device supported by two microsyringes was kept in the headspace of the sample solution. After extraction for 20 min at 95°C, sufficient solvent was left to provide a preconcentrated extract for injection into the GC [56]. Headspace extractions are rapid due to larger diffusion coefficients in the gas phase when compared to liquids, and the larger drop interface increases the rate of mass transfer into the solvent drop. In comparison studies, HS-SDME was found more sensitive than DI-SDME [57].

A technique based on lab-in-syringe utilized a piston shaft modified with an auxiliary drilled channel for the formation of an extraction solvent drop in the syringe void above the aqueous sample [58]. This allowed direct automation by variation of the void pressure for generation of analyte vapor, precisely controlled formation of extraction solvent drops, on-drop optical measurement, and waste disposal from the syringe. In a capillary electrophoresis method, the acceptor phase was placed inside the tip of a fused-silica capillary prefilled with a basic run buffer. The capillary tip was positioned in the headspace of the acidic donor solution to extract volatile acidic analytes, subsequently analyzed by electrophoresis [59]. Since no acceptor drop was formed, the acceptor phase had a robust nature against the extraction temperature and extended extraction time in comparison to conventional HS-SDME. Although the analyte extracted mass was small due to the narrow capillary tip, enrichment factors of 450–1100 were still attained. A physically stable extraction drop system applied a magnetic ionic liquid as an extracting solvent on one end of a small neodymium (Nd) magnet that was fixed to the sample vial cap using another Nd magnet [60]. The sample vial was capped to expose the extraction drop in the headspace of the aqueous sample, which was stirred at 1500 rpm. After extraction for 10 min, the lower magnet with the extract was placed in a thermal desorption tube for gas chromatography-mass spectrometry (GC-MS). The optical probe described for DI-SDME [33] has also been used for HS-SDME [61] by placing iron(III)-1,10-phenanthroline in the solvent hole of the probe being kept above an acidified solution of sulfite (Fig. 15.6). During extraction, iron(III) was reduced to iron(II) producing a colored complex. The online monitoring of absorbance avoided the cotransfer of the sample solution for subsequent analysis.

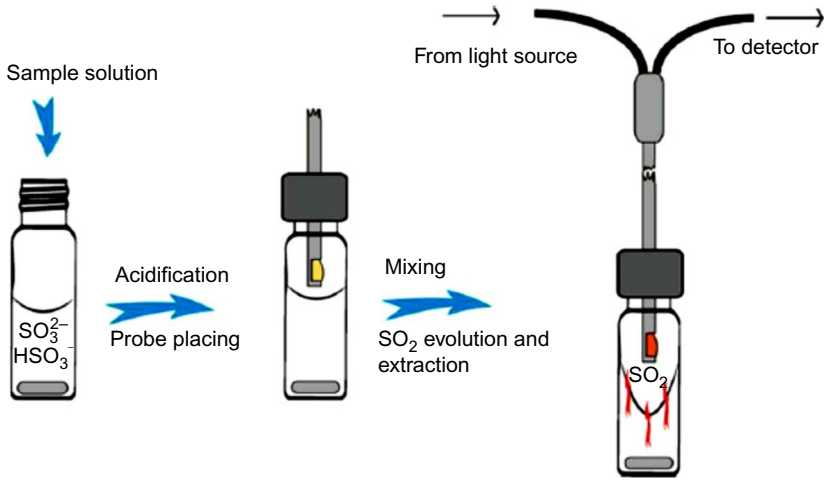

Fig. 15.6 Optical probe as the microdrop holder in headspace single-drop microextraction. *(Reproduced from Zaruba S, Vishnikin AB, Skrlikova J, Andruch V. Using an optical probe as the microdrop holder in headspace single drop microextraction: determination of sulfite in food samples. Anal Chem 2016;88:10296–300 with permission from the American Chemical Society.)*

A combination of a pneumatic nebulizer and HS-SDME effectively transferred the volatile/semivolatile analytes from an aqueous solution to the gas phase. For extraction the vapors were carried to the solvent drop placed in the water condenser [62].

15.3.4 Liquid-liquid-liquid microextraction

LLLME is applicable to ionizable organic compounds, e.g., phenol, carboxylic acids, and amines, and is compatible with reversed-phase liquid chromatography and capillary electrophoresis for analysis because the extracts are in aqueous solution. Adjusting the pH of the donor phase for acids or bases allows mass transfer to the water-immiscible organic solvent interface with organic solvents of lower density than water. Subsequently, the analytes are transformed to their ionizable species in the acceptor phase placed as a microdrop at the organic solvent interface and maintained basic for acids and acidic for bases.

Besides acids and bases, LLLME has utilized other principles for extraction of analytes from an aqueous phase into an organic solvent and their back-extraction [10]. New configurations have been suggested to perform three-phase SDME in elegant ways. A single drop of aqueous acceptor phase enclosed in a thin layer of octanol was constructed at a capillary tip by providing forward and reverse pressure in the capillary electrophoresis (CE) instrument. LLLME of acidic analytes was carried out from an acidic donor sample to the basic acceptor phase through an octanol membrane [63]. In Fig. 15.7

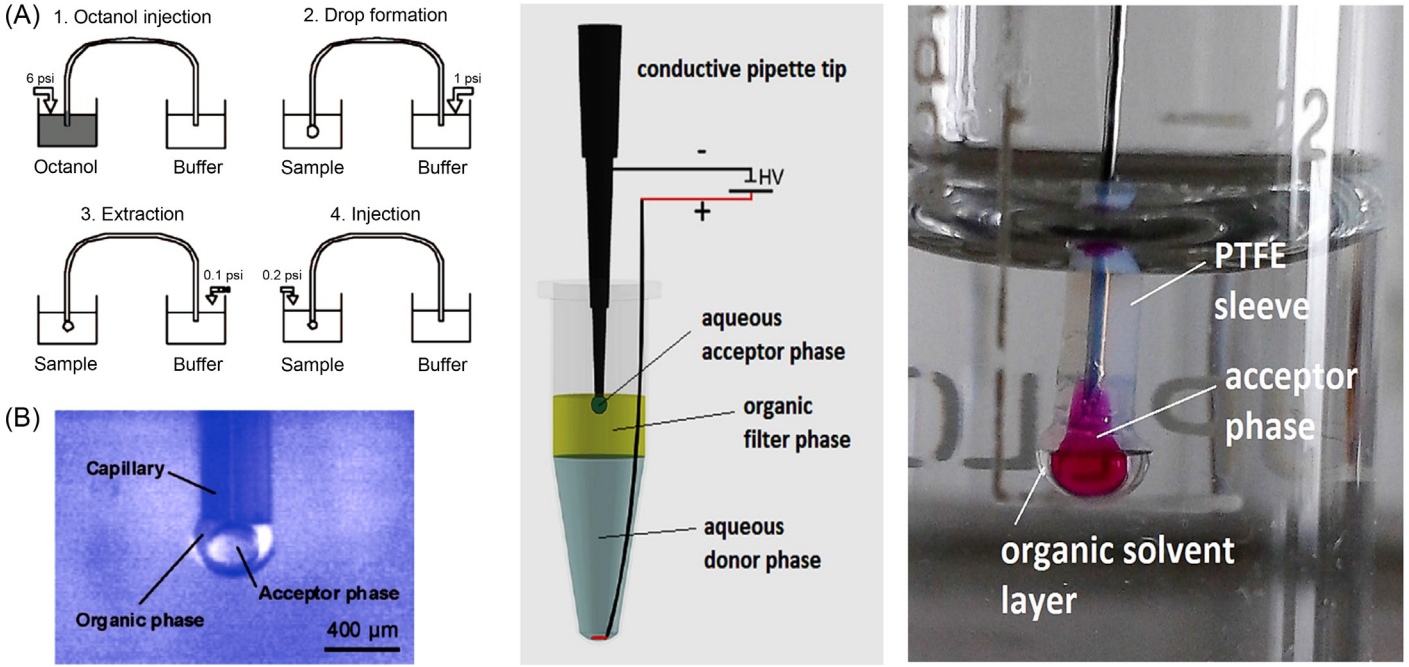

Fig. 15.7 Three-phase single-drop microextraction technique for sample preconcentration. *Left panel*, using a commercial capillary electrophoresis instrument. *Middle panel*, by electroextraction. *Right panel*, three-phase extraction by using a compound drop in which the aqueous acceptor phase was covered with the organic solvent layer. To visualize shapes of compound drops, extraction of phenolphthalein was carried out using dilute sodium hydroxide as the acceptor phase. *(*Left and Middle panels: *Reproduced from Choi K, Kim SJ, Jin YG, Jang YO, Kim J-S, Chung DS. Single drop microextraction using commercial capillary electrophoresis instruments. Anal Chem 2009;81:225–30; Raterink R-J, Lindenburg PW, Vreeken RJ, Hankemeier T. Three-phase electroextraction: a new (online) sample purification and enrichment method for bioanalysis. Anal Chem 2013;85:7762–68 with permission from the American Chemical Society*. Right panel: *Authors' unpublished studies.)*

the image of an approximately 30 nL drop used in CE is shown. After extraction, the organic phase was detached when the capillary was transferred from the donor vial to the run buffer vial. In a modified system, application of an electric field between the donor and acceptor phase accelerated the migration and enrichment of analytes from the donor aqueous phase through the immiscible solvent layer into an aqueous acceptor microdrop (Fig. 15.7) [64]. The organic solvent layer acted as a cleanup filter that did not allow proteins to migrate into the acceptor phase. Another simplified LLLME system utilized a compound droplet developed using a conventional microsyringe [65]. For extraction, microliter volumes of acceptor and organic phases were withdrawn in sequence into the syringe and the needle slid into a short poly(methyl methacrylate) tube connected to a 5 mm long flat tip fused-silica capillary. This needle modification was necessary due to the poor adhesion between stainless steel and the organic solvent film. The modified needle was immersed into the aqueous sample and the syringe plunger slowly depressed to form a compound drop in which the aqueous acceptor phase was covered with a film of organic phase. A funnel-shaped PTFE sleeve on the microsyringe needle tip was also found to work well in the authors' laboratory (Fig. 15.7). After extraction, the acceptor phase was retracted into the syringe while leaving the organic phase outside the capillary as waste.

15.4 Solvent Drop Protection

SDME is a convenient method for accomplishing analyte extraction, cleanup, and preconcentration using conventional laboratory equipment. Because of simple methods of operation it has an edge over other miniaturized extraction methods. The main critical point, however, is instability of the extraction drop, which limits drop size, stirring speed, and extraction time. Capillary electrophoresis in-tube microextraction located the extraction phase inside the tip of the capillary and utilized it without forming a drop [59]. A stir membrane extraction method used an independent device to hold a short PTFE membrane filled with 50 µL of ethyl acetate-toluene, 1:1 (v/v), as extraction solvent in the stirred aqueous sample. After extraction, 2 µL of extract was analyzed by GC-MS [66]. Another method used a magnetic stirrer with a groove on its PTFE coating to hold 9 µL of extraction solvent. The extraction was performed by stirring the paddle at 350 rpm for 45 min, and a portion of the extract was then analyzed by GC-MS [67]. A stainless-steel ballpoint tip is a convenient tool to hold 12 µL of carbon tetrachloride for extraction. It was placed into an aqueous sample solution stirred at 1000 rpm for 30 min. A 5 µL aliquot of the extraction solvent was removed for analysis [68]. Hydrophobic melamine foam was used as an extraction solvent holder, which was prepared by soaking pieces of foam in an *n*-hexane solution of octadecyltrichlorosilane and the surplus silylating reagent

removed by washing with methanol. The dried foam was impregnated with 100 μL of *n*-hexane and placed in the aqueous sample for 10 min with stirring at 1500 rpm [69]. The extract was collected for analysis by compressing the foam with a pair of tweezers.

A host of methods utilized the principle of modifying the microsyringe needle to assist in stabilizing the solvent microdrop. Microsyringes with a larger cross-section at the needle tip served to increase the adhesion force between the flat needle tip and the organic solvent drop, and allowed DI-SDME for at least 80 min with magnetic stirring at 1700 rpm without any drop (0.9 μL) dislodgement [70]. A plastic membrane on a wire holder [71], copper foam with porous nanostructured walls fabricated on a copper wire by an electrochemical process [72], and a stainless-steel net on a PTFE holder [73] have been employed as protective devices for the solvent drop (1.1–15 μL) in HS-SDME for 5–10 min at a stirring rate of 1000 or 1300 rpm.

When a PTFE sleeve or a microdevice is used at the needle tip, it is first necessary to withdraw the extraction solvent in the syringe and pierce the septum vial cap with the needle. The device was then attached to the needle of a microsyringe, the vial capped, and the solvent microdrop formed. In DI-SDME the needle tip was fixed with a silicone ring to provide increased adhesion for a 5 μL *n*-hexane drop for 45 min extraction at a stirring rate of 200 rpm [74]. Two reports used PTFE sleeves fitted to a blunt needle tip for HS-SDME. In the GC method, extraction was carried out with an ionic liquid (5 μL) drop with stirring at 1580 rpm for 37 min [75]; in the liquid chromatography method, a 1-butanol (7 μL) drop with stirring at 1000 rpm for 15 min was used [76]. In the latter case it was necessary to angle cut the PTFE sleeve tip to give a stable drop. In another method, a PTFE sleeve was used to hold 20 μL of ionic liquid with a lower stirring rate of 240 rpm for 10 min [77].

The attached sleeve could terminate in a small funnel to hold a larger volume of extraction solvent (drop volume, μL/extraction time, min/stirring rate, rpm, given in that order in parentheses), toluene (3.5/30/1000) [78], 2:1 (v/v) *n*-hexane-acetic acid (4/15/900) [79], 1-butanol (10/20/1000) [80], chlorobenzene (15/40/1250) [81], and 1-octanol (20/40/600) [82]. The significant experimental variables for a bell-shaped extraction device were optimized using a central composite design [83]. An extraction solvent volume up to 400 μL was used in a microfunnel by stirring at 240 rpm for 90 min [84]. Another method used 30 μL of coacervative solvent (decanoic acid and tetrabutylammonium hydroxide 2:1 mol basis) in DI-SDME at a stirring rate of 300 rpm for 60 min [85]. Use of an ionic liquid (12 μL) in HS-SDME allowed extraction at an elevated temperature of 80°C for 25 min with stirring at 1000 rpm [86]. However, another report placed a 1.2 μL drop of ionic liquid in a water condenser above a heated sample for 30 min, ostensibly to avoid the loss of extracted volatile analytes [87].

15.5 Solvents for Single-Drop Microextraction

First, as a general requirement, the solvent should have sufficient viscosity to avoid drop dislodgement and should not interfere with the analytes' peaks in the determination step. Second, the solvent should have low toxicity. Miscibility with the mobile phase and transparency in the ultraviolet (UV) region are desired when the final analysis is done by liquid chromatography with UV detection. For GC it is preferable for the solvent peak to elute either before or after the analytes. It is important to adjust the injection volume of the extraction solvent because large solvent peaks are a disadvantage. Solvent purity is always a vexing problem because not all common solvents for SDME are of high purity. Partial solvent evaporation or water miscibility leads to poor precision, and as such hygroscopic solvents should not be used in SDME. Care is required in using mixtures of organic solvents because the drop composition could change during extraction due to differences in boiling points in HS-SDME or water miscibility in DI-SDME. Many methods use a derivatizing reagent in the extraction solvent to avoid the loss of volatile analytes. A list of solvents that are commonly used in SDME is given in Table 15.1. A recent trend is the replacement of toxic

TABLE 15.1 Extraction Solvents for SDME

SDME Mode	Solvent[a]	Boiling Point (°C)	Technique	Target Analytes
DI-SDME	Toluene	110	GC	Carbamates, OPPs, nitroaromatics, PAHs, polychlorobenzenes, organochlorines, pesticides, chloroacetanilide herbicides, pyrethroid, nicotine, nicotinic acid, aromatic amines, metal complexes
	n-Hexane/isooctane	69/116	GC	Organochlorine pesticides, phoxim, polychlorobenzenes, anesthetics
	1-Octanol	195	GC/HPLC	Triazine herbicides, dopamines, surfactants, haloacetic acids, BTEX
	Chloroform (carbon tetrachloride/ dichloromethane)	61 (77/40)	GC	Tobacco alkaloids, OPPs, caffeine, BTEX, phenols, metal complexes, PAHs, fatty acid esters
	Anisole	154	GC	PAHs
	Chlorobenzene	132	GC	PAHs

TABLE 15.1 Extraction Solvents for SDME—cont'd

SDME Mode	Solvent	Boiling Point (°C)	Technique	Target Analytes
	Magnetic ionic liquids		GC	BTEX, phenols, UV filters, halomethanes, aromatics
	Ionic liquids, $[C_8mim][PF_6]$		HPLC	Sulfonamides
HS-SDME	Water	100	HPLC/CE	Phenols, amphetamines
	Water (with a reagent)		Colorimetry	Sulfur dioxide, chlorine, hydrogen cyanide, bromine, arsine, organomercury, hydrogen sulfide, nitrogen oxides
	Water (β-cyclodextrin)		HPLC	PAHs
	Water (H_3PO_4)		CE/ colorimetry	Ammonia
	Dimethylformamide	153	Colorimetry	Iodine, formaldehyde
	1-Butanol	117	HPLC	Carbonyl compounds, halophenols
	Benzyl alcohol	206	GC	Fire accelerants, methyl-*tert*-butylether, aliphatic amines
	n-Hexadecane	287	GC	BTEX
	1-Octanol	195	GC	Trihalomethanes, pesticides, trihaloanisoles
	n-Octane	125	GC	2-Phenoxyethanol
	n-Butyl acetate	126	GC	Short chain fatty acids
	Ethylene glycol	197	GC	Alcohols
	Ionic liquids, $[C_6mim][BF_4]$, $[C_8mim][PF_6]$		HPLC/GC	PAHs, phenols, chlorobenzenes, trihalomethanes, organochlorine pesticides, chloroanilines, aromatic amines, synthetic musk
	Magnetic ionic liquids, $[C_2mim]_2[Co(NCS)_4]$		GC	Chlorobenzenes
	Deep eutectic solvent (magnetic bucky gel)		GC	BTEX

Continued

TABLE 15.1 Extraction Solvents for SDME—cont'd

SDME Mode	Solvent	Boiling Point (°C)	Technique	Target Analytes
LLLME	Ethyl acetate	77	HPLC	Aromatic amines, patulin
	n-Hexane/1-octanol	68/195	HPLC	Aromatic amines, phenols, parabens
			Colorimetry	Iodine
	Toluene	110	HPLC	Phenols, fluoroquinolones
	Benzene	80	HPLC	Local anesthetics
	Cyclohexane	81	GC	Chloroquine
	1-Nonanol/[C_4mim][PF6]	213/—	GC	Phthalate esters

BTEX, benzene, toluene, ethylbenzene, xylenes; *DI-SDME*, direct immersion single-drop microextraction; GC, gas chromatography; *HPLC*, high-performance liquid chromatography; *HS-SDME*, headspace single-drop microextraction; *LLLME*, liquid-liquid-liquid microextraction; *PAHs*, polycyclic aromatic hydrocarbons; *OPPs*, organophosphate pesticides; *SDME*, single-drop microextraction.
[a] [C_8mim][PF_6], 1-octyl-methylimidazolium hexafluorophosphate; [C_6mim][BF_4], 1-hexyl-3-methylimidazolium tetrafluoroborate; and [C_2mim]$_2$[Co(NCS)$_4$], 1-ethyl-3-methylimidazolium tetraisothiocyanatocobaltate(II).

solvents by selective, environmentally friendly solvents [88] and additives [89]. Ionic liquids and their derivatives [90] and deep eutectic solvents fall into these categories [90, 91].

Ionic liquids are ionic compounds with melting points below 100°C and have found acceptance as designer solvents in extraction technology. Typically, they consist of a large asymmetric organic cation (e.g., 1,3-diakylimidazolium, pyrrolidinium, or phosphonium) and a small organic or inorganic anion (e.g., bis(trifluoromethylsulfonyl)imide or hexafluorophosphate). Among their favorable properties for SDME are low or negligible vapor pressure, high viscosity, and high thermal stability. They possess good solubilizing properties for organic and inorganic substances and are miscible with organic solvents. These properties make ionic liquids good alternatives to conventional organic solvents used in SDME. They allow formation of larger and more stable solvent drops for better extraction and improved precision, and facilitate the use of higher temperatures and longer extraction times for increased analyte recovery. In addition, magnetic ionic liquids with paramagnetic anions, e.g., tetrachloroferrate(III) or tetrachloromanganate(II), allow the manipulation of the extraction solvent by a magnet [92]. Deep eutectic solvents are analogs of ionic liquids containing two or more compounds consisting of, typically, a quaternary ammonium or phosphonium halide salt as a hydrogen bond acceptor and an amine, carbohydrate, alcohol, or carboxylic acid as a hydrogen bond donor. The deep eutectic solvents are generally less expensive than ionic liquids and are considered greener solvents [91].

Ionic liquids have been used in DI-SDME to determine cadmium(II) as its dithiocarbamate complex [77] and sulfonamides [93], both from aqueous samples; in HS-SDME for haloaromatic compounds [75, 94], volatile organic compounds from seeds [87] and fruit juices [95], and camphor from drugs [86]; and in LLLME as an interface solvent for the determination of phenols [96]. Ionic liquids can be injected directly for reversed-phase liquid chromatography [86, 93] but additional steps are required for GC to avoid column damage or residue buildup in the injection port liner [77, 95]. Alternatively, solvent extracts can be injected by the thermal desorption technique [75, 87, 97] or through an interface where ionic liquids are retained and the analytes effectively transferred to the separation column (Fig. 15.8) [98]. Direct injection of ionic liquid extracts using a programmable temperature vaporization (PTV) injector is a suitable approach without the need for back-extraction of the analytes into an organic solvent. In GC-ion trap-MS/MS methods a PTV injector operating at a constant

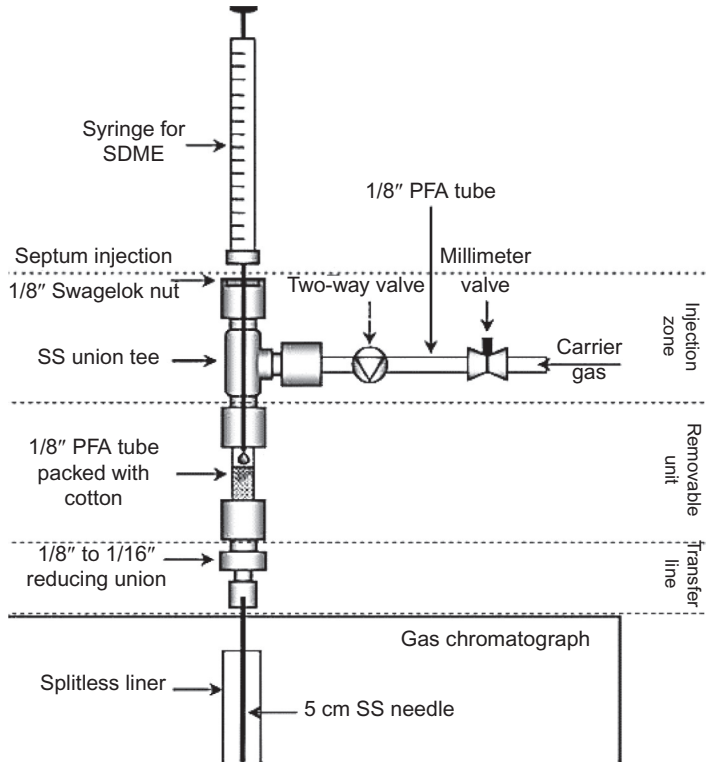

Fig. 15.8 Direct coupling of ionic liquid based single-drop microextraction and GC-MS. *(Reproduced from Aguilera-Herrador E, Lucena R, Cardenas S, Valcarcel M. Direct coupling of ionic liquid based single-drop microextraction and GC/MS. Anal Chem 2008; 80:793–800 with permission from the American Chemical Society.)*

temperature of 280°C with a large diameter liner (3.4 mm id), a glass wool plug, and a guard column were used to mitigate transfer of ionic liquids to the separation column [99]. Another approach using temperature-programmed heating for vaporization of the analytes and their transfer to the separation column was claimed to provide better chromatographic performance [100].

Salts and hydrochloric acid have a strong effect on the solubility of ionic liquids and could affect the performance of DI-SDME, where extraction of analytes is optimized by adjusting the salt concentration and pH of aqueous samples. For 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, the salting-out of the ionic liquid in aqueous medium occurred for salt concentrations higher than 0.2 mol kg^{-1}, and the effect was stronger for salts with higher valence cations [101]. The salting-out effect was further corroborated by varying the nature of salt and its concentration, and by the presence of hydrochloric acid in the aqueous medium on 1,3-dialkylimidazolium and *N*,*N*-dialkylpyrrolidinium bis(trifluoromethylsulfonyl)imide [102]. Hydrochloric acid caused a considerable increase in the aqueous solubility of the ionic liquids.

15.6 Automation

SDME methods are simple and advantageous on account of their high extraction efficiency, wide variety of available configurations and modes, and applicability to various analytes. However, a number of sequential operations are required in manual methods, which could significantly affect the quality of the final results. Moreover, events such as extraction drop dislodgement coerce repetitive experiments and delay the results. Drop instability and integration of constituent segments into a unified procedure are areas of major concern in realizing convenient SDME automation [103].

The factors affecting the precision and extraction efficiency of several fully automated LPME procedures, including SDME, have been investigated by autoperforming the events with a CTC CombiPal autosampler using Cycle Composer software [104]. The study showed that fully automated SDME was more accurate. Of the other modes of SDME the dynamic HS-LPME technique had the best performance, and the precision achieved eliminated the need for an internal standard. The autosampler and software also permitted fully automated extraction and derivatization by in-syringe dynamic LPME mode with GC-MS analysis [105]. Considerable saving of time and the potential to identify key variables/interactions for extraction parameters were added advantages. However, the extraction and sample agitation steps were conducted separately. The autosampler was equipped with a vortex agitator, which could homogenize the sample before or after the extraction. Later, online agitation was proposed for in-syringe dynamic LPME by attaching a magnetic mixer beneath the sample tray [106].

A GC-ion trap-MS/MS system with a PTV injector and a large internal diameter liner to deal with viscous liquids was developed for use with an ionic liquid as an extraction solvent for the automated HS-SDME of musk fragrances from environmental water samples [99] or for the initial extract obtained by pressurized liquid extraction (water and methanol, 1:1) of sewage sludge [107]. Ionic liquid extraction was necessary to remove fatty materials coextracted by pressurized liquid extraction.

The problem of the influence of complicated matrices, such as concrete, in the determination of ammonia was avoided by automatic stepwise injection using an HS-SDME system in which all additions were made by a peristaltic pump and solenoid valves [108]. The liberated ammonia was absorbed in a drop of dilute phosphoric acid by HS-SDME and delivered to the mixing chamber containing reagents for indophenol blue formation. The final step was spectrophotometric measurement in a flow cell. Another approach to automation utilized a syringe as a size-adaptable reaction chamber with a channel drilled in the piston for headspace reagent drop formation. A drop of bromothymol blue indicator was formed at the piston channel orifice in the syringe headspace, and on-drop color measurements were made by fiber optics [58].

Using two different commercial capillary electrophoresis instruments, a single drop of aqueous phase covered with a thin layer of organic phase was formed at the tip of the capillary by controlled bidirectional pressure changes to perform automatic LLLME [63]. Analytes from an acidic donor phase were concentrated into a basic acceptor phase. The thin film of organic solvent acted as an intermediate extraction phase. The acidic analytes were preconcentrated by 2000-fold within 10 min.

A fully automatic headspace bubble-in-drop microextraction system performed all extraction steps, namely formation of the solvent drop at the tip of the microsyringe needle, introduction of an air bubble, retrieval of the drop by the syringe, and injection into a GC-MS [109]. A 1 μL drop was found to hold up to 0.5 μL of air, but with larger air volumes the drop was unstable. Two automated lab-in-syringe techniques were based on an air bubble-stabilized hanging solvent drop containing either dithizone immersed into the sample solution for the extraction of lead(II) [110], or acidified dichromate exposed in the headspace of wine samples for determination of ethanol [111]. All operations were carried out in the barrel of an automated syringe pump, working as a chamber of adaptable size. Negative pressure in the syringe was used to create a vacuum in the headspace favoring vaporization of ethanol. Finally, the colored lead-dithizone complex or reduction in color intensity of dichromate was measured spectrophotometrically by pushing the drops into a flow-through cell [111]. A fully automated HS-SDME was based on a programmable lab-in-syringe platform for the extraction of mercury(II) and its mixing with stannous chloride to generate Hg^0 vapor inside the syringe under reduced pressure eliminating

analyte loss. An aqueous microdrop of dispersed Pd^0 exposed in the headspace was used to amalgamate Hg^0 for determination by electrothermal atomic absorption spectrometry [112].

15.7 Recent Applications

SDME has made remarkable progress in its range of applications, demonstrating incorporation of modified devices and materials for custom and immerging situations. Several representative examples are discussed next.

The LLLME was originally proposed for analytes acting as acids or bases, but subsequently other chemical principals were utilized. Formation of hydrophobic complexes of methyl- and phenylmercury with 1-(2-pyridylazo)-2-naphthol enabled phase transfer into toluene, from which a back-extraction into an aqueous drop by forming more stable complexes with cysteine was carried out [25]. Iodide was oxidized by 2-iodosobenzoate to iodine, which was extracted into hexane after adding sodium sulfate to the sample to decrease the solubility of iodine in water. An aqueous drop of potassium iodide and starch was made in the hexane layer to back-extract iodine as a blue starch-triiodide complex, which was determined by spectrophotometry [113]. Patulin is a toxic metabolite of fungi. Its contamination of food, especially apple juice, leads to many problems. Patulin is a polar compound that is fairly soluble in water as well as in ethyl acetate. Thus its transfer to an ethyl acetate phase layered over the aqueous sample was effected by adding sodium chloride to the donor phase to decrease the solubility of patulin in water. Finally, a drop of deionized water was placed in the organic phase to extract the patulin [114]. The method was also able to work efficiently with high sugar content in the matrix.

DI-SDME was used for analyzing multiclass pesticides in mango pulp. In the optimized procedure, the pulp was homogenized with 10% acetonitrile and centrifuged. The presence of acetonitrile was essential, ostensibly to diffuse the matrix-adsorbed pesticides to the bulk liquid phase for subsequent partition into the extraction solvent. A 2 μL drop of toluene in the supernatant solution was used for extraction. The GC-MS run of 27 pesticides was complete within 18 min [115].

Perchlorate is a competitive inhibitor of iodine uptake in the thyroid gland, and is a toxic contaminant of human milk. Milk samples were centrifuged at 16,000 rpm for 20 min to remove lipids. The clear and acidified solution was treated with cetyltrimethylammonium bromide, and the ion-pair formed was extracted into a 5 μL drop of methyl isobutyl ketone with stirring at 400 rpm for 10 min. The extract was analyzed for perchlorate by its absorption at 1076 cm^{-1} in attenuated total reflectance Fourier transform infrared spectroscopy [116]. The method is cost effective, provides high throughput, and was also applied to soil, water, and urine samples. Large amounts of anions, including iodate and bromate, did not affect the results.

Nanostructured materials have been employed in SDME for trapping target analytes with high extraction efficiency, and as luminescent probes for selective detection. A colorimetric HS-SDME method utilized the metallophilic interaction of Au nanoparticles with Hg^0 resulting in a color change from red to blue. The sample solution of Hg(II) was treated with tin(II) chloride to produce Hg^0 vapor in the headspace, which was extracted into a drop of an aqueous suspension of thioglycolic acid-functionalized Au nanoparticles for absorption with a change in color [117]. Quantum dots are colloidal semiconductor nanoparticles that have unique electronic and optical properties. Cadmium/selenide quantum dots stabilized by hexadecylamine in an octane-decane solution were used as luminescent probes for the determination of Se(IV) by HS-SDME. On treatment with sodium borohydride, the Se(IV) was converted to its volatile hydride and absorbed in the extraction phase. The decrease in fluorescence intensity, measured against a blank, was related to the Se(IV) concentration [118]. The inhibition of chemiluminescence due to the reaction of cadmium/selenide quantum dots with hydrogen peroxide was used for the determination of Sb, Se, and Cu. A preseparation of Sb as its dithiocarbamate complex by LLLME and its utilization in a cadmium/selenide quantum dot-hydrogen peroxide system afforded an ultrasensitive and selective method for Sb(III) [119].

Analyte derivatization is an important tool for the SDME of polar organic compounds and ionic substances. Processing of carbohydrate-rich food at elevated temperatures leads to the formation of acrylamide, which is a known human carcinogen. Acrylamide is highly water soluble and has a weak response to many detectors. Bromination with hydrobromic acid and ammonium persulfate produced 2,3-dibromopropanamide readily extracted into 1 μL of 1-octanol in DI-SDME. The brominated derivative was determined by a gas chromatography-electron capture detector (GC-ECD) [120]. Fluoride, which is otherwise reluctant to undergo a derivatization reaction, reacts with trimethylchlorosilane in acidic medium to form trimethylfluorosilane, which is volatile at ambient temperature. After HS-SDME in 0.8 μL of mesitylene, the derivative was analyzed by a gas chromatography-flame ionization detector [121]. HS-SDME was also utilized in the determination of iodide by its oxidation to iodine with hydrogen peroxide in acidic medium, and conversion to 2-iodo-3-pentanone by exposing iodine vapors to a 3 μL drop of 3-pentanone in 1-octanol (1:4, v/v). This method is suitable for the determination of total iodine in infant formula by GC-ECD [122].

A number of techniques are used for the extraction of essential oils from plant samples, including steam distillation or hydrodistillation, which are laborious and time consuming. Microwave distillation is a convenient and rapid method. Its efficiency was further enhanced by magnetite (Fe_3O_4) nanoparticles added to the plant material. The magnetite acts as a microwave absorption material to assist in the dry distillation of essential oils, which are simultaneously absorbed into 2 μL of ionic liquid extraction drop in HS-SDME for analysis by GC-MS [123]. Using 30 mg

of dry lavender together with 5 mg of magnetite in each run, the method was able to detect 36 essential oil components with 5 min of microwave irradiation and 20 min extraction time. For the extraction of compounds of a wide range of polarities in the same sample, DI-SDME with a 1.5 μL drop of 1-octanol was initially carried out on the sample solution to extract fewer volatile analytes; thereafter, the drop was retracted into the microsyringe and reexposed, but now in the void volume of the sample vial, for HS-SDME to extract more volatile compounds [124]. For the extraction of sulfonamides from water a mixture of 1-octanol in methanol was used for DLLME followed by addition of acetonitrile to deemulsify the solution with separation of a 1-octanol layer on top of the aqueous solution. A drop of sodium hydroxide was formed in the 1-octanol layer to back-extract sulfonamides by DI-SDME for analysis by high-performance liquid chromatography-ultraviolet detection [125]. A combination of HS-SDME and HS-solid-phase microextraction (SPME) provided an elegant solution for injecting large sample volumes into a GC as an alternative to PTV-GC for high sensitivity. A mixture of 13 phenols was methylated to increase their volatility and extracted by HS-SDME with a 7 μL drop of 1-butanol. The extract was then placed in a tapered sample vial and extracted by HS-SPME with a divinylbenzene/carboxen/polydimethylsiloxane (30/50 μm)-coated fiber. Thus in a two-step process the analytes were transferred from a large volume extract to an SPME fiber and analyzed by GC-MS [126]. Compared to separate individual extractions, coupled HS-SDME-SPME provided higher sensitivity.

15.8 Conclusions

SDME is an established technique of microextraction applicable to a wide variety of compounds in complex matrices. A number of extraction modes are available to handle different sample types characterized by a high sample throughput and minimal cross-contamination. Since only common laboratory equipment and small quantities of organic solvents are typically used for SDME methods, rapid acceptance was facilitated. Modification of the microsyringe needle with PTFE sleeves, magnetic fields to immobilize magnetic ionic liquids, or an optical probe with a hole for extraction solvent have been suggested to overcome the limitation of drop dislodgement. Recent advances in the capability of SDME have been made by the exploration of newer solvents, such as ionic liquids and deep eutectic solvents, to conduct extraction at higher temperatures and for longer extraction times. These solvents also allow the use of larger solvent drops without the problem of instability. A major reason for the strong interest in SDME is the ease of automation and application to microfluidic devices for online analyses with extremely small sample volumes, low reagent and solvent consumption, and fast analyses. Lab-in-syringe automated headspace extraction systems coupled to GC have immense potential for further development.

In particular, combined microwave-assisted distillation and HS-SDME-GC-MS has simplified studies of the chemistry of plant materials.

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Chapter 16

Dispersive Liquid-Liquid Microextraction

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Chapter Outline

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16.1 Introduction

At its inception in the mid-1990s, liquid-phase microextraction (LPME) may have been considered one more interesting academic research topic [1]; however, it rapidly gained popularity with analytic chemists. More than 20 years on, LPME has become a powerful tool for environmental, food, clinical, pharmaceutical, and industrial research and development analysis. LPME, also referred to as solvent microextraction (SME), basically consists of two major modes: exposed solvent and protected solvent microextraction of liquid, solid, and gaseous samples with extraction solvent volumes generally ranging from 0.5 to 100 μLs [2]. The protected extraction solvent mode include includes two major mode variants: hollow fiber liquid-phase microextraction (HF-LPME) [2–4] and electromembrane microextraction (EME) [5, 6]. Both modes contain the extraction solvent within a porous membrane, typically a polypropylene hollow fiber. The advantages of these modes are the protection of the solvent from sample solid and protein contaminants. The disadvantage for HF-LPME is the relatively long extraction time (30–90 min). EME contains an electrode within the fiber and in the sample, and application of a DC current allows greatly reduced extraction times for charged analytes, and EME has become a preferred technique for biological samples [7]. The exposed extraction solvent mode consists of two main variants, as well: single-drop microextraction (SDME) and dispersive liquid-liquid microextraction (DLLME), the focus of this discussion. SDME has the advantages of simplicity and versatility, requiring only a standard analytic syringe and application in direct immersion and headspace modes [8]. The major drawbacks to this technique are relatively long extraction times (30 s–30 min) and evaporation, dissolution, and instability of the drop. HF-LPME and SDME are also typically equilibrium techniques, and, while these techniques

are reproducible and quite sensitive, not all of the analyte may be extracted from the sample [2]. DLLME, however, overcomes these problems and has resulted in an explosion of research and applications (citations easily exceeding 1000) since its introduction in 2006 [9, 10]. DLLME involves the dispersal of an extraction solvent in a liquid sample, resulting in a large extraction solvent surface area and almost instantaneous extraction with nearly 100% analyte recovery. Initial DLLME experiments involved dissolving a water-insoluble solvent (CCL_4, C_2Cl_4, $CHCl_3$, or other high-density chlorinated solvents) in a water-soluble dispersal solvent, such as methanol, and rapidly injecting the mixture into the aqueous sample, producing the dispersion. This was followed by centrifugation to break the emulsion, collection of the extraction solvent, and analysis. This methodology involves several difficulties, one of which includes the use of non-environmentally friendly solvents. To overcome these difficulties a myriad of DLLME variations have been developed, as outlined later. Unfortunately, these many DLLME variations have led to much confusion in choosing the best method for a particular sample. In addition, research groups have often given their DLLME variation a unique name, resulting in a plethora of impossible to remember acronyms for similar techniques [11]. It should also be remembered that, despite the popularity of DLLME, it may not be the most appropriate microextraction technique for a particular sample and analyte. As an example, the technique may not be appropriate for samples containing silt or protein, which may be more amenable to HF-LPME or EME. The technique is also not easily automated, unlike SDME. In some cases, DLLME has been combined with additional extraction and purification techniques to provide successful analytic methods. These points must be kept in mind when developing a method incorporating DLLME [11]. The following discussion is meant to resolve some of these confusing points and to give the reader a starting point roadmap for choosing a DLLME technique appropriate for particular samples. Given that there are a vast number of DLLME reference sources, a selected few are tabulated here to simplify the search for additional information, definitions of important acronyms are provided, and a description of the most popular DLLME variants and experimental conditions is provided. These sources contain a near-complete compendium of all DLLME research and applications, including some important methodologies not covered here. This is followed by a discussion of the important principles involved in choosing, developing, and implementing an analytic method utilizing DLLME consistent with green analytical chemistry (GAC) principles.

Table 16.1 lists useful general references for DLLME and LPME reviews [10–26] and articles concerning safety and environmental impacts of ionic liquids, deep eutectic solvents, and traditional laboratory solvents [27–33], as well as a review on the role of green analytical chemistry in extraction [34]. These publications are recommended as good starting points for a better understanding of the scope

TABLE 16.1 Recommended General DLLME References and Publications

Reference Categories	References
Comprehensive LPME (SME) theory and application: text	[2]
Applications of DLLME 2006–16: review	[10]
Choosing an appropriate LPME method: review	[11]
Environmental analysis DLLME applications: reviews	[12–14]
Pharmaceutical and biomedical DLLME applications: review	[15]
Food safety applications: reviews	[16–19]
Forensics and toxicology DLLME applications: reviews	[20, 21]
DLLME modes: review	[22]
Solidification of floating organic drop: review	[23]
DLLME with ionic liquids and deep eutectic solvents: reviews	[24–26]
Ionic liquids and deep eutectic safety and environmental impacts: review, articles	[27–29]
Risk assessments of laboratory solvents: articles	[30–33]
Green analytical chemistry: review	[34]

of LPME and DLLME and a source for more than 1000 pertinent references for DLLME that have been published since 2006.

16.2 Nomenclature

DLLME, to many, refers to using a cosolvent to disperse an extraction solvent in an aqueous sample, and the term will be used here in that manner, since a generally accepted term for solvent dispersion DLLME is not available. However, all dispersion extractions, including those that do not use a dispersion solvent, are variations of DLLME. Thus the terminology used here, as presented in Table 16.2, will be based on the term DLLME, with additional preceding or following acronyms to indicate the exact modification of DLLME being used. This naming procedure is based in large part on the 2016 IUPAC glossary for extraction terms [35] and suggested terminology for DLLME in a 2016 paper by Sandrejova et al. [36] and a 2017 paper by Shishov et al. [37]. This approach will hopefully reduce the number of acronyms required for a literature searching process, when trying to find a suitable DLLME method. When appropriate, however, additional acronyms frequently used are defined, as an aid for literature searching, but will not be used otherwise. In addition,

TABLE 16.2 DLLME Terminology and Acronyms

Terminology	Acronym
Dispersive liquid-liquid microextraction	DLLME
Solvent-assisted liquid-liquid microextraction	DLLME
Air-assisted-	AA-
Effervescence-assisted-	EA-
Gas-assisted-	GA-
Ultrasound-assisted-	UA-
Vortex-assisted-	VA-
-Solidification of floating organic drop	-SFO
Ionic liquid-	IL-
Deep eutectic solvent-	DES-
Magnetic ionic liquid -	MIL-
Magnetic deep eutectic solvent-	MDES-

in each section describing a DLLME method, the full name (other than DLLME) may be spelled out again before using any acronyms, to avoid having the reader having to constantly refer back to this section.

DLLME is always used here as the name base, with acronyms appended to indicate the specific methodology used, and, in some cases, the type of extraction solvent (e.g., IL for ionic liquid or DES for deep eutectic solvent). Hyphened acronyms are placed before DLLME, with the exception of solidified floating drop (-SFO), which follows DLLME. The dispersion technique acronyms are placed directly before DLLME. Thus an appropriate DLLME method using vortex-assisted dispersion, along with ultrasonic-assisted dispersion, and a DES extraction solvent that is solidified upon cooling would be designated as DES-UA-VA-DLLME-SFO method. Whenever possible, abbreviations will be limited to the use of two or three acronyms, to avoid confusion.

Table 16.3 is a listing of additional acronyms used in this chapter to designate other analytic and instrumental analysis techniques.

16.3 Dispersion Methods

Table 16.4 lists the more commonly used DLLME dispersion generation modes. The advantages, disadvantages, and some recommendations for developing DLLME methods are covered in the following sections.

TABLE 16.3 Additional Acronyms Used in This Chapter

Terminology	Acronym
Liquid-phase microextraction	LPME
Solvent microextraction	SME
Single-drop microextraction	SDME
Hollow fiber liquid-phase microextraction	HF-LPME
Electromembrane extraction	EME
Liquid-liquid extraction	LLE
Quick, easy, cheap, effective, rugged, and safe	QuEChERS
Solid-phase extraction	SPE
Solid-phase microextraction	SPME
Gas chromatography	GC
Gas chromatography–mass spectroscopy	GC–MS
High-performance liquid chromatography	HPLC
Ultrahigh-performance liquid chromatography	UHPLC
Ultrahigh-performance liquid chromatography–mass spectroscopy	UHPLC–MS
Atomic absorption spectroscopy	AAS
Electrothermal atomic absorption spectroscopy	ETAAS

16.3.1 Solvent assisted dispersive liquid-liquid microextraction (DLLME)

DLLME, as first developed by Razee et al. in 2006 [9], involves dissolving a water-insoluble high-density halogenated solvent, such as CCl_4, $CHCl_3$, or C_2Cl_4 (10–100 μL), in a water-soluble disperser solvent (50–500 μL), such as ethanol, followed by rapid injection with a syringe into a water sample (typically 5–10 mL). The resulting dispersion is then broken by centrifugation, addition of salt or solvent. The extraction solvent is recovered from the bottom of the centrifuge tube with a syringe or pipette and analyzed, often by gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS). This methodology is simple and straightforward and remains popular [38–43], despite several problems [11, 23, 34], including the toxicity of the halogenated solvents; the fact that the extraction solvent is the bottom layer in the centrifuge tube; the need to evaporate and reconstitute the halogenated solvent

TABLE 16.4 Common Modes for DLLME Dispersion Generation

Dispersion Generation Method	Description
Solvent dispersion DLLME (DLLME)	A water-insoluble solvent is dissolved in a water-soluble dispersion agent and the solution injected rapidly into the sample
Vortex-assisted DLLME (VA-DLLME)	A water-insoluble solvent is added to the water sample and the mixture vortexed to produce a dispersion
Ultrasound-assisted DLLME (UA-DLLME)	A water-insoluble solvent is added to the water sample, and a dispersion is generated with ultrasound energy
Gas-assisted DLLME (GA-DLLME)	A water-insoluble solvent is added to the water sample, and a dispersion is generated by bubbling a gas into the mixture to generate the dispersion
Effervescence-assisted DLLME (EA-DLLME)	An acid and a carbonate salt are added to the water sample, and the resulting CO_2 bubbles generate the dispersion. The acid and base can be added in solid form as a tablet
Air-assisted DLLME (AA_DLLME)	A mixture of water-insoluble solvent and water sample are rapidly drawn into and expelled from a syringe with an attached needle, generating a dispersion
In situ DLLME	Soluble components of an IL or DES are added to the water sample to generate a dispersion of an insoluble IL or DES

in a solvent, such as acetonitrile (ACN) for analysis by high-performance liquid chromatography (HPLC), ultrahigh-performance liquid chromatography-mass spectrometry (UHPLC-MS), or atomic absorption spectroscopy (AAS); and increased solubility of the extraction solvent and analytes by the cosolvent effect. This last problem may sometimes be partially overcome by the addition of salt. These disadvantages led to the development of DLLME variations using low-density extraction solvents, such as cyclohexane and p-xylene, in which the solvent ends up as the top layer after centrifugation. However, this typically requires the use of a specially designed centrifuge tube with a restricted neck to concentrate the thin layer of extraction solvent into a vertical layer deep enough for recovery by syringe [44]. This problem was in turn overcome by using a liquid with a freezing point just below room temperature, such as 1-undecanol. Following centrifugation the solidified extraction liquid is retrieved with tweezers, transported to a sample vial, and analyzed [45]. This last technique is usually designated as dispersive liquid-liquid microextraction solidification of floating organic drop (DLLME-SFO). The disadvantage of this technique is that there are only a few solvents with compatible freezing point temperatures.

In the last decade the move toward more green solvents has led to the development of DLLME procedures using ionic liquids (ILs) and deep eutectic solvents (DESs), discussed in more detail later. When these solvents are used and utilized, the method name is often designated IL-DLLME or DES-DLLME [24–26]. These solvents hold great promise but have drawbacks as well, since many are relatively soluble in water. In addition, ILs are expensive, and some are potentially toxic and persistent in the environment [27]. Due to their low vapor pressures, ILs are largely incompatible with GC. Recently, however, less costly and more environmentally friendly hydrophobic DESs were developed, which are more compatible with DLLME and GC analysis [26], though even DESs pose some environmental concerns [28]. For small-scale or infrequent sample analysis, DLLME, which uses only microliters of solvent, can be considered environmentally friendly compared with traditional extraction techniques.

When following a published procedure as a starting point for developing a method, keep in mind that most procedures were carefully developed for specific analytes and samples, requiring a great deal of time and effort to perfect. Also, not every nuance of the procedure may have been published, as opposed to the complete experimental conditions found in standardized procedures. Thus it is rare that a published procedure can be duplicated or modified without some effort.

16.3.2 Vortex assisted-DLLME (VA-DLLME)

VA-DLLME involves the mechanical disruption of the extraction solvent with a vortex device (at 700–900 rpm) to produce dispersion, without the need of a dispersion solvent. This technique is also referred to as vortex-assisted liquid-liquid microextraction (VALLME) [46]. Since there is no need for a dispersion solvent, the problem of increased extraction solvent and analyte solubility is eliminated, resulting in the need for less extraction solvent and often elimination or reduction of salt addition. This also eliminates contamination of the extraction solvent with the dispersion solvent and simplifies instrumental analysis. The technique has been successfully used with high- and low-density solvents, as well as with ILs and DESs and with the SFO technique [46–48]. Interestingly the technique is also compatible with the so-called reversed DLLME in which a hydrophilic analyte or metal is extracted from mineral- or plant-based oils [49, 50]. Care is needed with high-viscosity solvents, especially ILs and DESs, in producing a true dispersion, and at times, it may be necessary to use a dispersive solvent or even ultrasound in addition to vortex. Vortex time and speed are important factors in these cases to achieve maximum extraction. In some cases, formation of an incomplete emulsion can be an advantage, since the semidispersion separates with gravity, on standing. A true dispersion must be broken with centrifugation or possibly salt addition and the solvent recovered from either the bottom or top of the centrifuge tube or with tweezers when using VA-DLLME with the SFO technique (VA-DLLME-SFO). These issues are covered in a recent review by Psillakis [46].

16.3.3 Ultrasound assisted-DLLME (UA-DLLME)

UA-DLLME is a clear example that DLLME isn't as simple and straightforward as it first appears. As the name implies, this technique is a means by which dispersions can be formed without the use of a dispersion solvent, with the same advantages as all nonsolvent dispersion techniques [51–53]. Some UA-DLLME procedures, however, use a dispersion solvent as a dispersion aid along with ultrasound, to enhance the dispersion process [51]. One interesting UA method involves the use of a reverse SOF method for extraction from water with toluene. Following centrifugation the water was frozen, leaving the toluene as liquid, which was removed for analysis [54]. The major disadvantages of using ultrasonic energy result from the heat generated and also potential analyte degradation, the fact that emulsions can be difficult to break in prolonged UA extraction, and requiring higher-speed centrifugation for longer times. These problems are generally controlled by cooling and appropriate application of ultrasonic power and time [2, 22]. Unfortunately, research publications commonly tend to omit this and other pertinent details, so care needs to be taken when adopting a published method, since extraction efficiencies are dependent on the type of ultrasound instrumentation (bath versus probe), power levels, and power duration. Do not confuse UA with ultrasonic-assisted extraction (USAE), which is a procedure for extracting analytes from solids, usually plant or animal tissue, and often used for this purpose before a DLLME procedure. A similar acronym, USAEME (ultrasonic-assisted emulsive microextraction), is sometimes used for UA-DLLME and another example of why acronyms should be used with care.

16.3.4 Gas assisted-DLLME (GA-DLLME) and effervescence assisted DLLLME (EA-DLLME)

In these techniques the sheering forces and mixing created by vigorous bubbling of air through the aqueous sample and extraction solvent mixture result in an emulsion or pseudoemulsion. GA-DLLME involves inserting a fine stream of gas bubbles into the sample [55]. The EA-DLLME procedure involves mixing the extraction solvent and sample with an acid (such as citric acid) and base, such as sodium carbonate, usually in the form of a tablet, to produce carbon dioxide bubbles [56]. Both techniques form emulsions without the aid of a dispersion solvent, although again the result may only be a pseudoemulsion. These procedures are fast and inexpensive, require only a centrifuge to break the emulsion, and were successfully applied with high- and low-density extraction solvents, including ILs and with SFO conditions.

16.3.5 Air-assisted-DLLME (AA-DLLME)

This relatively recent technique was developed by Farajzadeh and Moghaddam in 2012, who termed it air-assisted liquid-liquid microextraction (AALLME) [57]. The name given to the AA-DLLME procedure, which does not require dispersion

solvent, is likely a misnomer, since the addition of air is actually not involved in the dispersion process, as with GA-DLLME, although the vacuum created by rapidly pulling the sample and solvent into the syringe does produce bubbles of dissolved air or solvent and does increase mixing. The mixture of aqueous sample and extractant is rapidly pulled into and forced out of a syringe (usually 10 mL) through the needle 6–12 times. The sheering forces and turbulence encountered within the needle emulsify the mixture, which is then centrifuged to break up the emulsion. The procedure has been successfully used with all the common extraction solvents, including ILs, DESs, and SFO solvents [58–60].

16.3.6 In situ-DLLME

There are two commonly used methods for generating an in situ dispersion in an aqueous sample: in situ IL formation and in situ DES formation. A third technique, involving the use of acid-base chemistry to generate an insoluble acid or base dispersion, will not be discussed further [61].

16.3.6.1 In Situ-Ionic Liquid-DLLME (In-Situ-IL-DLLME)

An ionic liquid (IL) can be formed within the sample solution by a metathesis reaction, by the addition of water-soluble IL followed by a hydrophobic anion exchange reagent, forming a hydrophobic IL dispersion [62, 63]. Temperature control is sometimes necessary in this reaction, with the water-soluble IL forming at a high temperature and gradual cooling resulting in dispersion formation [24, 25]. ILs are quite viscous at low temperatures, and the rate of extraction is slow, but not enough to be significant. Too high a temperature results in IL dissolution. Typical temperatures range from 30°C to 80°C. The ILs employed are generally more dense than water.

16.3.6.2 In Situ-Deep Eutectic Solvent-DLLME (In Situ-DES-DLLME)

Analogous to the in situ generation of an IL, a hydrophobic DES can be formed by adding a hydrophobic hydrogen-bond donor and a hydrogen-bond acceptor to the sample solution, which results in the formation of a dispersion [64].

16.4 Extraction and Dispersion Solvents

While a wide variety of extraction and dispersion solvents are available, only a few are used in practice, due to toxicity, environmental, volatility, and solubility constraints. Some of the more commonly used extraction solvents are CH_2Cl_2, $CHCl_3$, C_2C_{l2}, cyclohexane, isooctane, 1-octanol, 1-undecanol, and 1-dodecanol, as well as a variety of hydrophobic ILs and DESs. The properties to consider when choosing an

extraction solvent include the Log K_{ow}, K_{ow}, boiling point, density and water solubility, and also viscosity for ILs and DESs. Also consider the safety information for these chemicals, to see if their use is allowed or advisable [30–33]. Log K_{ow} is the log of the partition constant between 1-octanol and water [2, 65]. It can be used as a surrogate measure of solvent polarity.

Extraction solvents are usually classified as either halogenated high-density or low-density solvents. This classification is further subclassified as SOF, IL, or DES solvents. Properties of many ILs and DESs, including K_{ow} and water solubility, may not be readily available. However, those used in DLLME can be assumed to have similar properties to $CHCl_3$ or 1-octanol.

16.4.1 Traditional extraction solvents

The requirements for DLLME solvents are essentially the same as for those of traditional macro liquid-liquid extraction (LLE) solvents: they must be water-insoluble, have complementary intermolecular interactions and polarity to the analytes, and be compatible with the method for analyte determination. The caveat to these requirements for DLLME is that solubility at the microliter level is more important and restrictive for solvent selection. Ethyl acetate (EtOAc), ethyl ether (Et_2O), chloroform ($CHCl_3$), 1-octanol, carbon tetrachloride (CCl_4), and cyclohexane (C_6H_{12}) were used in traditional LLE and some LPME methods. 1-Octanol, $CHCL_3$, CCl_4, and C_6H_{12} have traditionally been used to extract nonpolar analytes (such as polycyclic aromatic hydrocarbons, PAHs) from water, with LPME methods. On the other hand, diethyl ether and ethyl acetate, with smaller K_{ow} values and the ability to hydrogen bond, are popular for the extraction of polar analytes in LLE. These solvents are never used in DLLME methods, due to their water solubility. As an example, if a 10-mL sample were extracted with 100 μL of EtOAc, only ~10 μL of extraction solvent would be available for retrieval and analysis [2]. Despite the large K_{ow} value for 1-octanol, this solvent is also used mainly for the extraction of analytes of intermediate polarity. $CHCl_3$ remains popular as a DLLME extraction solvent, despite its volatility, water solubility, and toxicity, due to its intermediate polarity suitable for extracting intermediate and nonpolar analytes. A word of caution, however, under the same extraction conditions used for EtOAc extraction, nearly half the $CHCl_3$ remained dissolved in the sample, along with analyte. This is often partially corrected by using larger amounts of $CHCl_3$ or the addition of salt, which decreases the solubility of the $CHCl_3$. This may actually decrease the extraction efficiency, however, since large amounts of salt decrease mobility of analytes through the water [2] and increased solvent dilutes the extract.

CCl_4 is rarely used in DLLME methods anymore, and $CHCl_3$ should be limited to methods that are intended for low numbers of extractions. Recently a series of papers attempted to categorize more than 100 common solvents according to their

toxicological and environmental impacts, using data taken from the pharmaceutical industry and other sources [30–33]. The reader is advised to examine these lists before deciding on a solvent for a DLLME method.

16.4.2 Ionic liquid extraction solvents

Two additional classes of solvents have gained increasing importance in DLLME methods: ionic liquids (ILs) and deep eutectic solvents (DESs). ILs are ionic compounds typically composed of bulky cations and/or anions with a number of useful properties that have made them of interest to chemists. With a large number and variety of ions available, ILs with properties suitable for DLLME are easily synthesized. These include low volatility; solubility of organic and inorganic compounds; thermal stability; and compatibility with analytic instruments, including HPLC and atomic absorption spectroscopy (AAS). ILs have the potential to replace traditional organic solvents, especially the less environmentally friendly halogenated solvents, and have been applied to nearly all DLLME modes with good results [24, 25]. However, the low volatility of ILs means that they are incompatible with gas chromatography [2]. In addition, the high viscosity of ILs can lead to the necessity of using a dispersion solvent, even in modes such as UA-DLLME [51]. Also the toxicity of ILs containing imidazolium cations or fluorine-containing anions, their environmental persistence, requires that these chemicals are used according to good laboratory practice for collection and proper disposal of IL solutions, including aqueous solutions [27].

16.4.3 Deep eutectic extraction solvents

More recently, deep eutectic solvents (DESs) have come to the forefront in DLLME procedures. DESs have many of the same properties as ILs, and in fact, some are usually considered a subclass of ILs. The difference is that DESs consist of components that are held together by hydrogen bonds and van der Waals forces, rather than ionic bonds [26]. Thus DESs can be designed to be compatible with HPLC and GC analysis: decomposing or dissociating within the GC inlet to components compatible with GC columns and detectors [66–68].These DESs fall into two main categories: DESs formed from choline chloride (a hydrogen bond acceptor) and a relatively hydrophobic hydrogen bond donor, such as 4-chlorophenol [66] and DESs formed by combining two hydrophobic naturally occurring chemicals, such as thymol and camphor [68]. In the first case the choline chloride decomposes in the GC inlet. In the second case the DES dissociates into the individual components. DESs, like ILs, can be designed to be compatible with organic and inorganic compounds in DLLME methods [26, 69]. As with ILs, DESs are viscous solvents and pose similar restrictions to ILs in developing methods. DESs, on the other hand, are believed to be more environmentally friendly than ILs and can be easily and inexpensively synthesized from sustainable.

16.4.4 Magnetic extraction solvents

Magnetic ILs (MILs) and DESs (MDESs) are recent approaches to DLLME, either with or without the need for dispersion solvents and without the need for centrifugation for breaking the dispersion. Incorporation of nickel, iron, or manganese salts into the IL or DES solvents allows separation of the DLLME dispersion and isolation of extraction solvent from the sample solution with a strong magnet. Both dispersion solvent and nondispersion solvent DLLME modes have been used. After separation with a magnet and decanting of the aqueous sample, the extracts are either extracted from the MIL or MDES with a solvent for HPLC [70, 71] or GC [72, 73] analysis.

16.4.5 Dispersion solvents

Since DLLME is normally used to extract analytes from aqueous samples, the requirements for a dispersion solvent include solubility of the analyte in the extraction solvent and in turn solubility of the dispersion solvent in water. While a number of solvents meet these requirements and many have been used, the most commonly used dispersion solvents are ethanol, methanol, acetone, and acetonitrile (ACN). Whenever possible, ethanol, methanol, or acetone is the best choice, since these are more acceptable green solvents [30–33]. DLLME can also be used in so-called reversed modes, in which the analytes are present in a plant, animal, or mineral oil sample. In these cases the dispersion solvent is usually a hydrocarbon, and the extraction solvent is water-based (usually acidic or basic). The use of a dispersion solvent in DLLME can result in the increased solubility of the extraction solvent and analyte in the aqueous phase. For this reason the required volumes of sample, extraction solvent, and dispersion solvent are usually determined by preliminary experiments for developing a successful solvent dispersion DLLME method.

16.5 Techniques for Breaking the Dispersion

16.5.1 Centrifugation

Centrifugation is by far the most common method for breaking the emulsion in DLLME procedures [2]. It is also often the most practical method despite making full automation of a method more difficult without the use of specialized integrated robotic instrumentation. Typical methods use centrifugation speeds of 3000–5000 rpm for 2–5 min, though some procedures call for higher speeds and longer times, especially those using UA-DLLME. When using an extraction solvent more dense than water, the solvent is displaced to the bottom of the centrifuge tube. When using larger centrifuge tubes (10–25 mL), this can make use of an autosampler more difficult to remove the solvent. Low-density extraction solvents, such as

hydrocarbons, float to the top of the tube but form a very thin layer of solvent on top of the sample, resulting in the need of a specially designed centrifuge tube to collect the solvent. This requirement is eliminated when using the solidified organic drop (SFO) procedure with solvents such as 1-undecanol, 1-dodecanol, and cyclohexane. It should be mentioned that mechanical emulsification techniques, including VA-DLLME and AA-DLLME, may not produce a true, stable emulsion, with the result that the layers may separate upon standing, though this has not posed significant difficulties in achieving acceptable extractions. Much effort has gone into the development of other means of breaking the emulsion, with the most common being solvent-terminated deemulsification, and salting out, though the use of magnetic ILs and DESs is also implemented.

16.5.2 Addition of solvent (solvent demulsification)

This procedure involves the addition of a solvent, usually the dispersion solvent, to the emulsion to separate the layers [74]. Unfortunately, this often leads to additional solubility of analyte and extraction solvent in the aqueous sample, requiring larger amounts of extraction solvent. However, when successful, the technique does lend itself to full automation using a standard autosampler [44, 75].

16.5.3 Salting out

The addition of a salt (typically NaCl) to the aqueous sample lowers the water solubility of chemicals with K_{ow} values less than ~1000 and can also cause disruption of stable emulsions. DLLME procedures to break the emulsion have been developed by the addition of salt to the emulsion as a concentrated solution or solid [76] or by passing the emulsion through a column containing salt [77].

16.6 Derivatives and Complexes

DLLME is a means for separation, purification, and concentration of hydrophobic compounds from water. Analytes that are not hydrophobic must be converted to a hydrophobic form, by changes in pH, or by modifying their structure by derivatization [78–81], or by complexing with hydrophobic ligands [82–84]. Modification of pH is used for extraction of acids and bases. Inorganic materials must be complexed, and hydrophilic organic compounds derivatized before extraction. In situ or in-injector derivatization is preferred where possible [78]. One interesting in-injector derivatization for carboxylic acids involves extraction with a choline chloride, 4-methylphenol DES, with the decomposition of the choline chloride in the injector resulting in the formation of the methyl ester of the carboxylic acid, which is compatible with GC analysis [79].

16.7 DLLME Combined With Other Extraction Techniques

DLLME has been used to further concentrate and purify samples subjected to other extraction techniques initially, such as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) technique; solid-phase extraction (SPE); and solid-phase microextraction (SPME). These techniques and others are described in two recent reviews by Sajid et al. [85, 86].

16.8 DLLME Automation

Various attempts have been made to partially or fully automate DLLME methods. These fall roughly into two classes: those involving syringe pump or valve systems and those involving the use of commercial robotic autosampler systems. Automation involving syringe pumps or valve systems is described by Alexovic et al. [87]. While these techniques have yielded completely automated procedures, the instrumentation required is often complicated and laboratory-assembled. To date, these approaches have not seeded commercial interest.

The approach using a two-syringe robotic autosampler was successfully applied to the full automation of DLLME by Guo and Lee using solvent-terminated DLLME [44, 75] and a low-density solvent dispersion system in which the dispersion (or pseudodispersion) was broken by agitation of the sample [88]. More recently, automation of VA-DLLME for performing EPA method 8270 for water contaminants was achieved by integrating a vortex mixer, a centrifuge, a two-head autosampler, and a GC-MS into a single system [89].

16.9 Green Analytical Chemistry (GAC)

When choosing to adopt, modify, or build a new DLLME method, green analytical chemistry principles should be an important consideration, especially if it is to be widely used [34]. Recently, Galuszka et al. [90] published their version of the 12 principles of green analytical chemistry (GAC) as follows:

1. Direct analytic techniques should be applied to avoid sample treatment.
2. Minimal sample size and minimal number of samples are goals.
3. In situ measurements should be performed.
4. Integration of analytic processes and operations should be tailored to save energy and reduce the use of reagents.
5. Automated and miniaturized methods should be selected.

6. Derivatization should be avoided.
7. Generation of a large volume of analytic waste should be avoided, and proper management of analytic waste should be provided.
8. Multianalyte or multiparameter methods are preferred versus methods using one analyte at a time.
9. The use of energy should be minimized.
10. Reagents obtained from renewable sources should be preferred.
11. Toxic reagents should be eliminated or replaced.
12. The safety of the operator should be increased.

The authors concluded that the key issues that need to be addressed by a green analytical chemistry method fall into four categories:

1. Elimination or reduction of the use of chemical substances
2. Minimization of energy consumption
3. Proper management of analytic waste
4. Increased safety for the operator

It is, of course, difficult or nearly impossible to fulfill all 12 principles in a successful LPME method, although DLLME methods greatly reduce the volumes of sample, reagents, and waste, especially of hazardous chemicals. Sample preparation time, energy requirements, manpower requirements, and analytic expense are significantly reduced. Finally, some DLLME procedures are potentially compatible with automation, thus lending themselves to high-throughput methods.

16.10 Employing an Appropriate DLLME Mode

Given the number of DLLME modes available, with numerous permutations and parameters available for each, the task of choosing an appropriate method for a specific type of sample is daunting, to say the least. One definite necessity in this choice is to understand the chemical factors involved in the DLLME procedure, and this is best understood by examining the equilibrium equations associated with microextractions. There are five important equations involved, covering the effects of solubility, concentration, volumes, and mass transfer [2]. The rate constant (k) for the extraction at equilibrium is given by the following equations:

$$\frac{1}{\beta_{oo}} = \frac{1}{\beta_o} + \frac{K_{ow}}{\beta_w} \tag{16.1}$$

$$k = \frac{A_i \beta_{oo} [K_{ow}(V_o/V_w) + 1]}{V_o} \tag{16.2}$$

where

A_i = the interfacial area between the organic and aqueous layers
β_{oo} = the overall mass transfer coefficient for the organic phase in cm/s
β_o = the mass transfer coefficient for the organic phase in cm/s
β_w = the mass transfer coefficient for the aqueous phase in cm/s
V_o = volume of the organic extractant
V_w = volume of the aqueous phase
K_{ow} = distribution ratio between the organic and aqueous phases.

At equilibrium or near equilibrium, the following concentration equation applies:

$$C_o = \frac{K_{ow} C_w^0}{1 + K_{ow}(V_o / V_w)} \tag{16.3}$$

where

C_o = concentration of the analyte at equilibrium in the extraction solvent
C_w^0 = initial concentration of the analyte in the extracted (aqueous) phase

This equation can be rearranged to represent the amount of analyte (n) extracted into the extraction solvent at equilibrium as follows:

$$n = \frac{K_{ow} V_o C_w^0 V_w}{K_{ow} V_o + V_w} \tag{16.4}$$

Eqs. (16.1) and (16.2) clearly show that the rate of extraction depends on the surface area of the extraction solvent. Thus the extraction efficiency of DLLME is very high, close to 100% in an appropriately designed experiment, since the extraction solvent surface area for an emulsion is immense.

These equations also illustrate that the equilibrium rate is directly related to the mass transfer coefficient of analyte through the sample (water) and into the extraction solvent. This is one reason why addition of salt to the sample to improve extraction yields, sometimes has the opposite effect. It is also the reason why reaching equilibrium in LPME extractions requires more time for viscous liquids like ILs and DESs than for traditional organic solvents such as p-xylene or $CHCl_3$. In the case of DLLME, however, because the surface area of the solvent is immense at dispersion, the difference in extraction time is negligible.

Also, note that the equilibrium time is minimized and k maximized, by increasing the volume of the extraction solvent and decreasing the volume of the sample. Again, for DLLME, these parameters can be ignored, due to the immense extraction solvent surface area.

To understand just how the remaining parameters will affect the extraction yield of specific analytes in DLLME, one needs to place real numbers into Eq. (16.4) to calculate extraction yields. The effect of the partition constant (K_{ow}) and volume of

the extraction solvent (V_o, in μL), concentration of the analyte in the sample (C_w^0, in ng/mL), and volume of the sample (V_w, in mL) for three representative analytes are shown later. In these calculations the octanol/water partition constant will be used, since it is readily available in the literature and close enough to the partition constant for other common solvents, for the calculations presented here [2, 65].

Analyte	K_{ow}	V_o	C_w^0	V_w	Yield, ng	Extracted %
Benzene	148	10.0	10.0	5.00	49.8	99.6
	148	100.	10.0	10.0	99.7	99.7
Phenol	28.8	10.0	10.0	5.00	49.1	98.3
	28.8	100.	10.0	10.0	99.7	99.7
D-Glucose	0.000575	10.0	10.0	5.0	0.0574	0.115
	0.000575	100.	10.0	10.0	0.572	0.572

A close examination clearly shows that hydrophobic analytes are nearly completely extracted with DLLME. In this case it is assumed that the phenol solution was acidified. The corollary, of course, is that hydrophilic analytes are poorly extracted. Thus hydrophilic analytes must be derivatized to make them more hydrophobic before DLLME. It is also seen that doubling the sample volume also doubles the amount of analyte available for analysis. However, this also involves increasing the volume of the extraction solvent and, in effect, resulting in a dilution of the extracted analytes and a lower detector response. Many reports juggle these numbers to achieve an absolute maximum limit of quantification (LOQ) and limit of detection (LOD), rather than working toward a method that takes into account the sensitivity of the available laboratory instrumentation, the concentration of analyte present in the sample, and therefore the amount of sample and extraction solvent needed.

These data are further skewed by one more important equation, which must be taken into account: extraction solvent solubility. Therefore it is useful to look at the effect of extraction solvent solubility in water on DLLME, with and without the addition of salt, when developing a method. The water solubility of three common DLLME solvents is given later [65] and upon addition of 10% (w/w) NaCl. Solubilities upon the addition of salt are calculated with Eq. (16.5) [2].

$$K_{ow(\text{salt})} = K_{ow} \times 10^{+S[\text{salt}]} \tag{16.5}$$

where

$K_{ow\ (\text{salt})} = K_{ow}$ value corrected for the addition of salt
S = the Setschenow constant for NaCl and a specific organic analyte [2], ranging from 0.15 for phenols to 0.4 for PCBs
[salt] = molar concentration of the added NaCl

Solvent	Solubility	Solubility	Solubility
	mg/mL water	μL/mL water	μL/mL water
	0% NaCl	0% NaCl	10% NaCl
$CHCl_3$	8.40	5.60	2.70
1-Octanol	0.54	0.65	0.26
Cyclohexane	0.055	0.071	0.027

From these data, it can be seen that when a sample is extracted with $CHCl_3$, less than half of the $CHCl_3$ can be recovered. Typically, actual recovered amounts available for analyses are around 20–30 μL. Where is the rest? Some has evaporated during the workup perhaps, but the majority is dissolved in the water, and it should also be understood that there is also water dissolved in the recovered $CHCl_3$. Is all of the extracted analyte in the recovered 20–30 μL of $CHCl_3$? Again, probably not—it is still in the water. Thus, while 100% of the analyte may have been extracted, less than 100% is actually available for analysis, and for GC, typically only 1–2 μL is actually analyzed. This is seldom made clear in published procedures.

It is possible to significantly reduce the solubility of the extracting solvent or analyte in water by the addition of salt. As can be seen, addition of 10% (w/w) salt significantly decreases the solvent solubility. In many publications, however, as little as 2% (w/w) salt is added, before finding a negative yield enhancement. This is probably due to the effect on the water mass transfer coefficient. The 2% (w/w) of salt has so little effect on the yield that it should be avoided. In fact, calculations show that salt addition is not appropriate for decreasing the solubility of analytes such as polycyclic aromatic hydrocarbons (PAHs) or chlorinated pesticides with K_{ow} values greater than 1000 when using DLLME and is perhaps best used for helping to break emulsions.

One last point worth repeating involves the use of a dispersion solvent in solvent-assisted DLLME or other procedures requiring a dispersion aid. The dispersion solvent increases the solubility of both the analyte and the extraction solvent in water, decreasing the volume of recovered extraction solvent and analyte. This is a major reason why so much effort has been placed on developing methods that do not use dispersion solvents.

16.11 Conclusions

DLLME is a method for the extraction, concentration, and purification of hydrophobic compounds from water or hydrophilic compounds from oils. When developing a

new DLLME method, either by modifying a published method or developing an original procedure, green analytical chemistry (GAC) principles should be adhered to when possible. In doing so a number of important steps should be followed:

1. Determine the amounts of extracted analytes needed for the determination step before adopting a literature method.
2. Sample, dispersion solvent, and extraction solvent volumes should be kept as low as possible, according to the requirements for the determination step.
3. A nondispersion solvent method is often a better choice, but the ultimate choice must be determined by first examining all methods for the analytes concerned, to see if any meet the manpower, cost, instrumentation, and green analytic requirements of the laboratory.
4. Examine the requirements for solvent use in the laboratory. Some laboratories ban the use of toxic, volatile, and chlorinated solvents.
5. Choose a nonpolar extraction solvent compatible with the instrumental analysis to be used.
6. The recently developed nonpolar ILs and DESs are useful alternatives to halogenated and volatile solvents and can be used with or without a dispersion solvent in a DLLME procedure, but also, be aware that they are 10–30 times more viscous than water.
7. ILs can be used directly with HPLC and AAS, but not with GC. Newly developed DESs, however, are compatible with GC analysis.
8. Keep in mind that good laboratory practice must be used for all chemicals, even for ILs and DESs, especially if a method is to be used on a large scale.
9. The addition of salt is not necessary, even counterproductive, for the extraction of analytes with K_{ow} values larger than 1000. Salt addition may be useful when using a solvent-assisted DLLME method. In any case, check the calculations before experimental confirmation.
10. Choose in situ derivatization and complexation procedures when possible.
11. While manual DLLME methods can be highly reproducible, the method should maximize automation where possible, to eliminate variability.

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Chapter 17

Extraction With Ionic Liquids-Organic Compounds

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Chapter Outline

17.1 Introduction

Ionic liquids (ILs) are a class of molten salts with melting points below 100°C, formed by the combination of organic cations and organic or inorganic anions

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[1]. Besides the characteristic negligible vapor pressure at room temperature, high conductivity, and thermal and electrochemical stability, the synthetic versatility is the most attractive feature of ILs. Thus the adequate design of these solvents by the selection of different ion moieties and the incorporation of functional groups in their structures leads to the preparation of ILs with specific and targeted physicochemical properties, such as solubility, viscosity, or interactions with other chemical species [2]. Due to this impressive tunability, several types of ILs have been described, including room-temperature ILs (RTILs), IL-based surfactants, polymeric ILs (PILs), magnetic ILs (MILs), and task-specific ILs (TSILs) [3]. Fig. 17.1 shows the most common IL cations and anions and representative examples of subclass of ILs depending on their composition.

All these subclasses of ILs exhibit the inherent properties of ILs, together with the main features of each group of derivatives: RTILs have melting points below room temperature; IL-based surfactants can be prepared by the incorporation of long alkyl chains in their structures, thus ensuring micellar properties when dissolved in water; PILs are a class of polyelectrolytes obtained by the self-assembly of IL monomers; MILs contain a paramagnetic component either in the cation or in the anion moiety; and TSILs are those ILs tailored for a particular application different from their use as solvents [2, 3].

ILs and their derivatives have been explored in numerous applications in different scientific fields [4], including synthesis and catalysis [5], energy storage and electrochemical applications [6], processing of biomass [7], gas separation and absorption [8], and extraction and separation techniques [9]. Among all these applications the use of ILs as extraction solvents for the separation and determination of organic, inorganic, and bioactive compounds merits citation, because the applications involving ILs have shown outstanding analytic performance while ensuring a decrease in terms of toxicity (particularly when compared with the conventional halogenated organic solvents, widely used in analytic sample preparation) [10–12].

The development of more sustainable extraction methods by reducing the amounts of organic solvents and wastes, by the incorporation of more environmentally friendly solvents, while minimizing the energy consumption and miniaturizing and automating the analytic techniques, is one of the trendiest research lines within analytical chemistry [13]. The incorporation of ILs in the extraction procedure allows meeting most of these requirements. In this sense, ILs have been widely exploited as extraction solvents in microwave-assisted extraction (MWAE), ultrasound-assisted extraction (USAE), and aqueous biphasic systems (ABS) [14]. In general, these methods are devoted to the isolation of chemical compounds from both solid and liquid samples and normally require volumes of ILs between 0.15 and 5 mL. However, most recent applications of ILs in sample preparation are devoted to their use in microextraction methods in which amounts of ILs ranging from 1 to 200 μL are used. Within microextraction approaches, ILs and their liquid derivatives

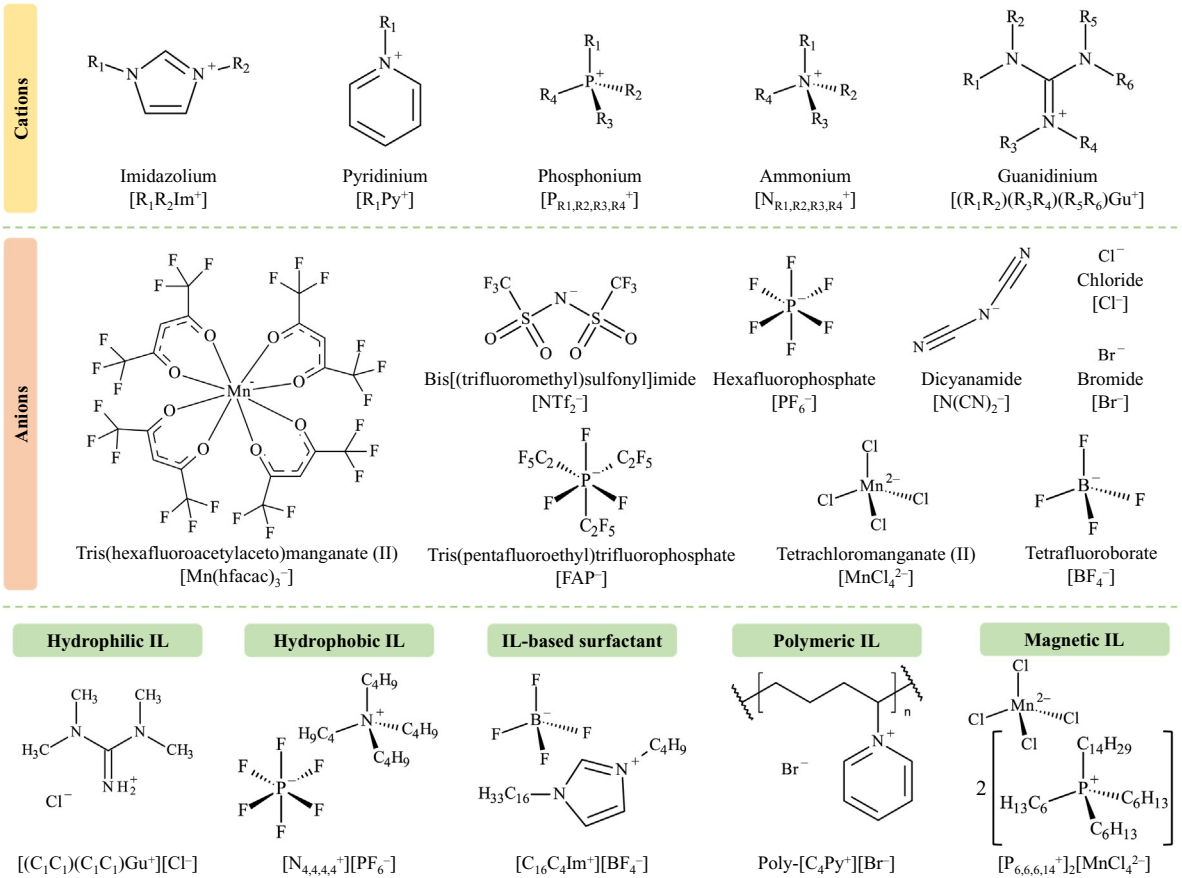

Fig. 17.1 Structures of the main cations and anions used to prepare ILs and representative examples of the chemical composition of main IL derivatives.

have been of particular interest in dispersive liquid-liquid microextraction (DLLME), single-drop microextraction (SDME), and hollow-fiber liquid-phase microextraction (HF-LPME), for the determination of a variety of compounds [14].

The aim of this chapter is to provide an overview of liquid-phase extraction methods (miniaturized or not) that have been developed using ILs (and derivatives) as extraction solvents, focusing on the extraction of organic compounds from environmental, food, and biological samples. Fig. 17.2 includes a summary of the studies reported up to date on this topic while specifying the percentages according to the nature of the organic compound targeted and highlighting the particular importance of these designer solvent in the different liquid-phase (micro or not) extraction methods.

17.2 Ionic Liquids in Liquid-Phase Extraction Methods

Main strategies for the determination of organic compounds that utilize ILs in non-miniaturized liquid-based extraction methods include MWAE, USAE, and ABS and are described in this section.

17.2.1 Microwave-assisted extraction

MWAE basically involves the utilization of microwave radiation to heat the extraction solvent, thus improving the dispersion of the solvent into the sample while accelerating the partitioning of the compounds from the liquid or solid sample to the solvent [15]. The extraction solvent in MWAE applications must be polar to absorb microwave energy, with methanol and ethyl acetate the solvents most commonly used. Aqueous solutions of hydrophilic ILs (rather than neat ILs) have been used as extraction solvents in MWAE with the aim of replacing organic solvents [16].

Fig. 17.3A includes an operational scheme of the MWAE procedure using ILs. In the studies reported, volumes of aqueous solution of the IL (clearly hydrophilic) between 4 and 100 mL are added to the vessel that contains the solid sample. Then the mixture is placed in the microwave oven for a certain time (quite short if compared with conventional liquid-phase extraction methods) at a specific power. Finally the solid is discarded, and the IL aqueous solution containing the extracted compounds is subjected to analysis. When the MWAE extraction method is not coupled to any further extraction/cleanup step, the resulting IL solution is diluted (usually 10 mL are diluted up to 50 mL) and/or directly injected into a liquid chromatography (LC) system.

All ILs utilized in MWAE are composed of dialkylimidazolium cation ($[R_1R_2Im^+]$), with 1-butyl-3-methylimidazolium ($[C_4C_1Im^+]$) the most commonly used [17–24], together with 1-hexyl-3-methylimidazolium ($[C_6C_1Im^+]$)

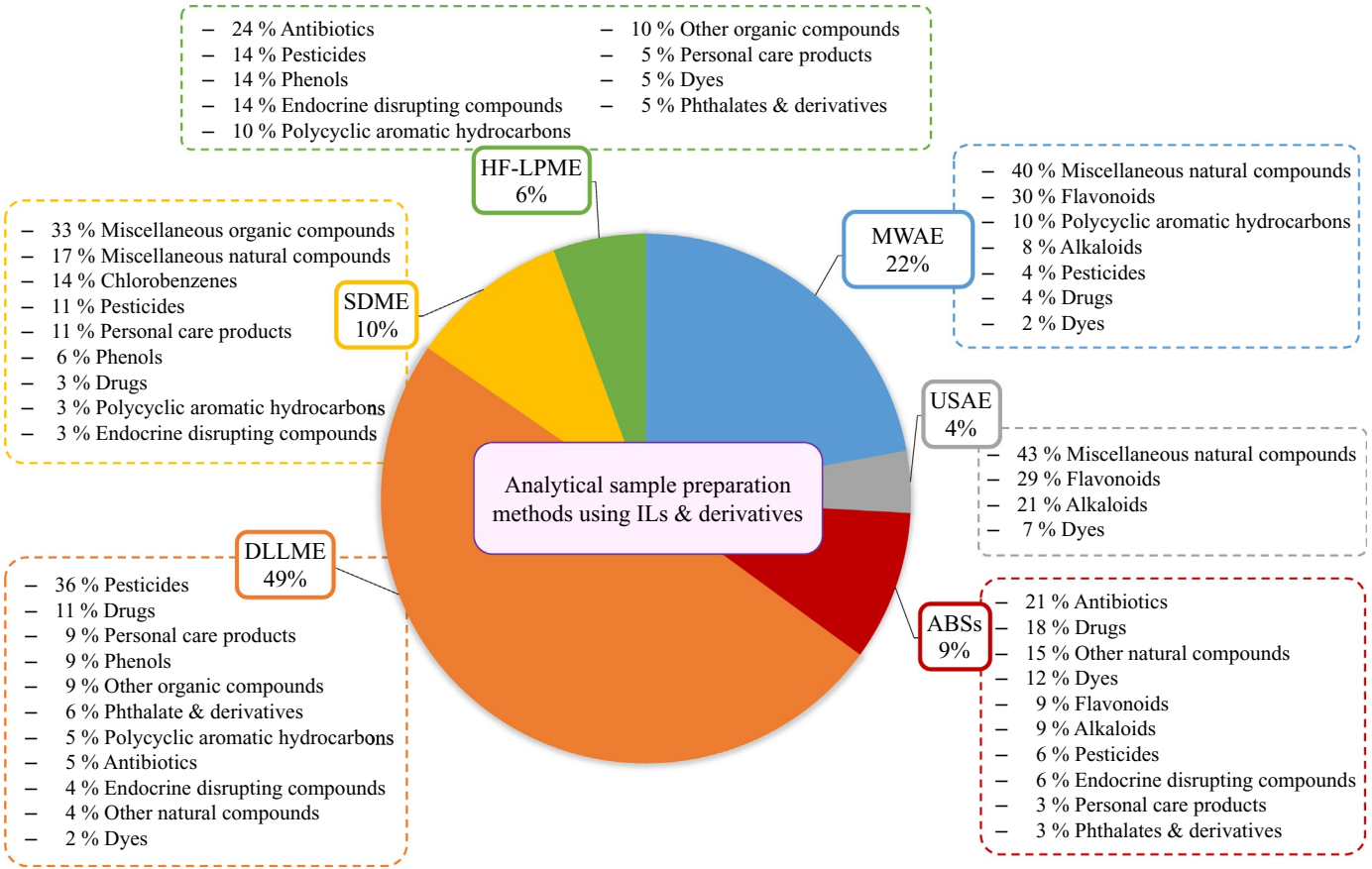

Fig. 17.2 Summary of applications of ILs and derivatives in liquid-phase extraction methods for organic compounds.

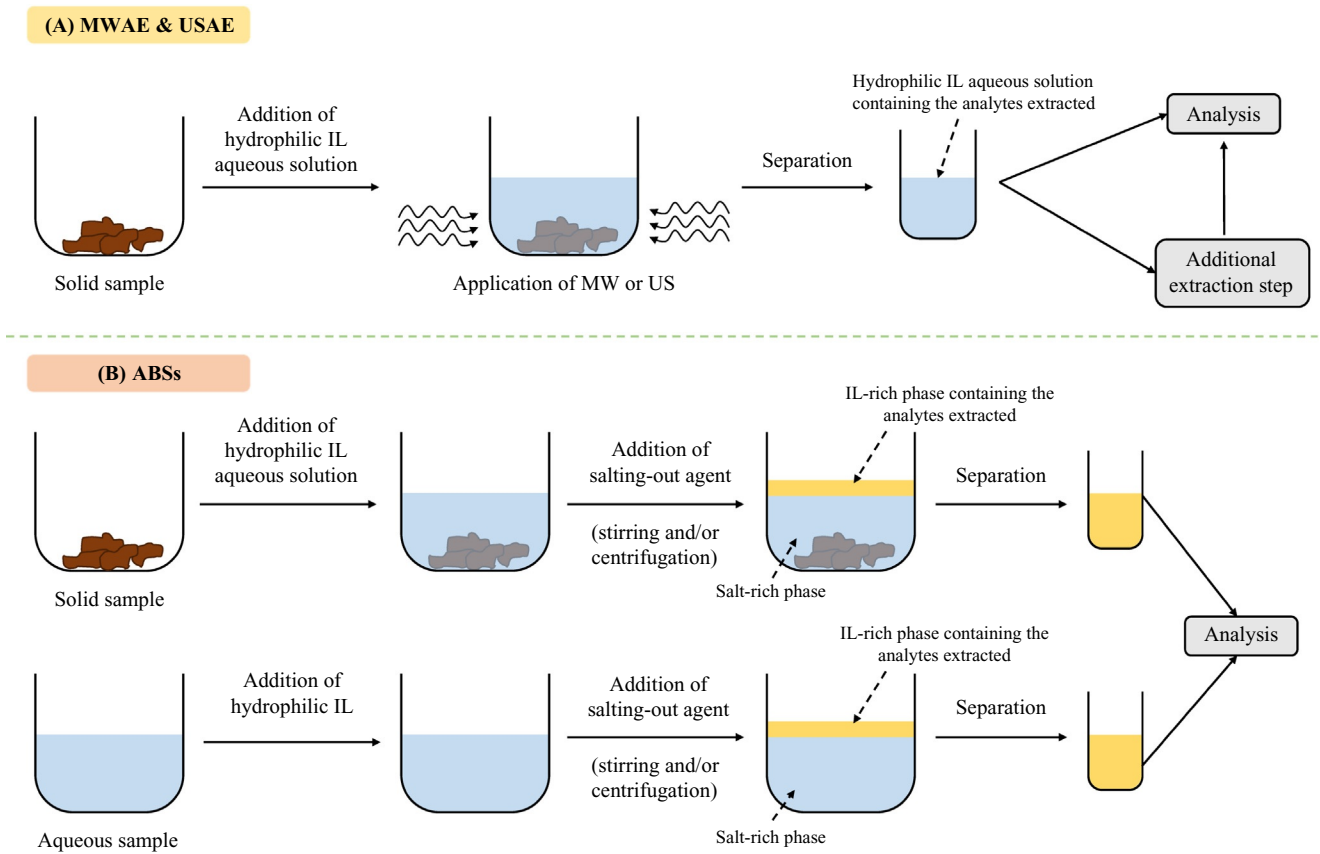

Fig. 17.3 Schemes of the extraction procedure when using ILs in (A) MWAE, USAE, and (B) ABSs.

[25–27] and 1-octyl-3-methylimidazolium ($[C_8C_1Im^+]$) [28, 29]. These cations are paired with tetrafluoroborate ($[BF_4^-]$) or halide anions, which allow the preparation of hydrophilic ILs. The Brönsted acidic IL 1-(4-sulfonylbutyl)-3-methyl imidazolium hydrogensulfate ($[HSO_3C_4C_1Im^+][HSO_4^-]$) was successfully used in MWAE to simultaneously hydrolyze and extract polyphenols from plants [30].

IL-based surfactants have also been used in MWAE [31–35]. These ILs with surface-active properties self-aggregate in water above their critical micelle concentration (CMC), which is lower than that of conventional surfactants with similar structures [36–38]. These aggregates are formed in water requiring low amounts of ILs and can enhance the solubility of hydrophobic compounds providing higher extraction efficiency. 1-Dodecyl-3-methylimidazolium ($[C_{12}C_1Im^+]$) [2, 34], 1-hexadecyl -3-methylimidazolium ($[C_{16}C_1Im^+]$) [31], and 1-hexadecyl-3-butylimidazolium ($[C_{16}C_4Im^+]$) [33, 35] cations with the $[Br^-]$ anion, are the IL-based surfactants mostly used in MWAE.

Most of the applications of ILs in MWAE focus on the isolation of natural compounds from plants, such as flavonoids [18, 23, 25, 28, 32], alkaloids [17, 26, 27], phenols [20, 29, 30], glycosides [19, 21, 22], and other natural compounds [39]. For the extraction of oxygen-sensitive natural compounds with low thermal stability, vacuum MWAE using ILs was described [19, 25]. It should be mentioned that in only a few of the earlier studies (with natural components) the extraction methods were validated for quantification purposes [20, 21, 23, 26–29]. Regarding the development of monitoring methods not related to the field of natural bioactive compounds, MWAE using ILs was used for the determination of polycyclic aromatic hydrocarbons (PAHs) in marine sediments [31] and cereals [35] and herbicides in soils [24]. All these quantification methods are coupled to LC with ultraviolet detection (UV) or fluorescence detection (FD) to accomplish the analytic determination. Table 17.1 [17, 19, 25, 33, 35, 40–47] includes some characteristics of representative examples of MWAE methods using ILs, together with their use in other analytic sample preparation approaches.

It is interesting to mention the development of methods that combine MWAE with a further preconcentration technique, taking advantage of the nature of the ILs present in the extraction solution. The ultimate goal of this preconcentration step is to have a more sensitive analytic method. Thus in situ IL-DLLME was performed after MWAE [33, 34]. In this strategy a hydrophilic IL is transformed into a hydrophobic IL containing the analytes, which is easily separated for further analysis. An aqueous biphasic system was utilized after the MWAE step to concentrate and separate the compounds from the aqueous phase [22]. With these approaches, limits of detection (LODs) at the part-per-billion levels are achieved for the determination of dyes [34], PAHs [33], and natural compounds [22] in complex matrices.

TABLE 17.1 Representative Applications of ILs as Extraction Solvents in Liquid-Phase Extraction Methods (Nonminiaturized) for the Determination of Organic Compounds

Method[a]	IL[b]	Type of IL	Additive	Analytes[c] (Number)	Sample	Additional Step[a]	Analytic Technique[d]	LOD[e]	Ref.
MWAE	$[C_6C_1Im^+][BF_4^-]$	Hydrophilic	–	Flavonoids (3)	Plant	–	LC-UV	–	[25]
MWAE	$[C_4C_1Im^+][Br^-]$	Hydrophilic	–	Alkaloids (4)	Plant	–	LC-UV	–	[17]
MWAE	$[C_4C_1Im^+][BF_4^-]$	Hydrophilic	–	Glycosides (3)	Plant	–	LC-UV	–	[19]
MWAE	$[C_{16}C_4Im^+][Br^-]$	Surfactant	–	PAHs (16)	Cereals	–	LC-FD	3–1037 μg kg^{-1}	[35]
MWAE	$[C_{16}C_4Im^+][Br^-]$	Surfactant	–	PAHs (16)	Cereals	In situ IL-DLLME (with $LiNTf_2$/anion exchange)	LC-FD	0.03–83 μg kg^{-1}	[33]
USAE	$[C_4C_1Im^+][Br^-]$	Hydrophilic	–	Phenyl propanoids (5)	Plant	ABS (with Na_2CO_3/salting-out)	LC-UV	–	[40]
USAE	$[C_2C_1Im^+][BF_4^-]$	Hydrophilic	–	Flavonoids (4)	Plant	–	LC-UV	–	[41]
USAE	$[C_4C_1Im^+][BF_4^-]$	Hydrophilic	–	Alkaloids (2)	Plant	–	LC-UV	11–15 μg L^{-1}	[42]
MWUSAE	$[C_8C_1Im^+][Br^-]$	Hydrophilic	–	Benzoxazinoids (2)	Seeds	–	LC-UV	–	[43]

ABS	$[C_4C_1Im^+]$ $[BF_4^-]$	Hydrophilic	$C_6H_5Na_3O_7$/ salting-out	Antibiotics (6)	Milk	–	LC-UV	2.04–2.84 μg L^{-1}	[44]
ABS	$[N^+_{4,4,4,4}]$ $[Br^-]$	Hydrophilic	K_3PO_4/ salting-out	Drugs (1)	Water	–	LC-UV	–	[45]
ABS	$[C_4C_1Im^+]$ $[N(CN)_2^-]$	Hydrophilic	$(NH_4)_2SO_4$/ salting-out	Flavonoids (total amount)	Plant	Back extraction (n-butane)	UV	–	[46]
ABS	$[C_4C_1Im^+]$ $[Br^-]$	Hydrophilic	K_2HPO_4/ salting-out	Colorants (5)	Food	–	LC-UV	51–74 ng L^{-1}	[47]

[a] Method abbreviations: *ABS* for aqueous biphasic system, *IL-DLLME* for ionic liquid-based dispersive liquid-liquid microextraction, *MWAE* for microwave-assisted extraction, *MWUSAE* for microwave-/ultrasound-assisted extraction, and USAE for ultrasound-assisted extraction.
[b] Ionic liquid abbreviations: $[BF_4^-]$ for tetrafluoroborate, $[Br^-]$ for bromide, $[C_2C_1Im^+]$ for 1-ethyl-3-methylimidazolium, $[C_4C_1Im^+]$ for 1-butyl-3-methylimidazolium, $[C_6C_1Im^+]$ for 1-hexyl-3-methylimidazolium, $[C_8C_1Im^+]$ for 1-octyl-3-methylimidazolium, $[C_{16}C_4Im^+]$ for 1-hexadecyl-3-butylimidazolium, $[N^+_{4,4,4,4}]$ for tetrabutylammonium, and $[N(CN)_2^-]$ for dicyanamide.
[c] Analytes abbreviations: *PAHs* for polycyclic aromatic hydrocarbons.
[d] Analytical technique abbreviations: *FD* for fluorescence detection, *LC* for liquid chromatography, and *UV* for ultraviolet detection.
[e] Limit of detection.

17.2.2 Ultrasound-assisted extraction

USAE methods take advantage of the cavitation phenomenon occurring when an extraction solvent in contact with a sample is subjected to ultrasounds [15]. Such application of ultrasounds leads to the formation cavitation bubbles throughout the solvent (in contact with a sample) that collapse, causing pressure and temperature changes and, therefore, enhancing the rate of mass transfer of analytes to the solvent. The operational procedure of USAE is similar to MWAE (Fig. 17.3A), but in this case the vessel is placed in an ultrasonic bath to perform the extraction. There are no reported applications for ILs employing a sonication probe. Despite MWAE being faster (average times of 10 min for MWAE and 40 min for USAE) and more efficient than USAE methods, the application of ultrasounds is preferred for the extraction of unstable compounds being less aggressive than microwaves [48] and also because a simple sonication water bath is commonly available in most analytic laboratories. In fact the use of ultrasounds to improve the dispersion and efficiency of any extraction (or desorption) solvent, in a number of liquid-phase and solid-phase extraction methods, is quite common [15], thus simplifying the entire process while reducing the extraction times.

As in MWAE, hydrophilic IL aqueous solutions (instead of neat ILs) are used as extraction solvents. $[R_1R_2Im^+]$ cations in combination with $[BF_4^-]$ and $[Br^-]$ anions are the most commonly used ILs, with the $[C_4C_1Im^+]$ cation the most popular [40, 42, 46, 49, 50]. This cation was combined with a dicyanamide anion ($[N(CN)_2^-]$) to prepare a more stable and less viscous IL that facilitates its manipulation in USAE [46].

Despite the fact that USAE is more widely used than MWAE for sample preparation, USAE with ILs is not as widely reported as MWAE with ILs, as shown in Fig. 17.2. The applications of USAE with ILs are mainly aimed at the isolation of bioactive compounds from plants for qualitative analysis, such as flavonoids [41, 46, 50] and alkaloids [42, 51]. USAE was reported as a preliminary step for aqueous biphasic systems (ABS) [40, 46, 49], using the ABS to recover the IL containing the target compounds followed by LC-UV analysis. Table 17.1 lists some representative USAE applications using aqueous solutions of ILs as the extraction solvent.

Microwaves and ultrasounds can be simultaneously applied using a specific device, leading to fast and effective methods by combining the advantages of the MWAE and USAE [15]. $[R_1R_2Im^+][Br^-]$ ILs have been used in this synergetic method for the extraction of a diversity of natural compounds from plants for qualitative analysis [43, 52–54].

17.2.3 Aqueous biphasic systems

Aqueous two-phase systems (ABSs) consist of ternary systems formed by two water-soluble components that can separate in two coexisting phases at a certain concentration.

Conventional ABSs comprise two polymers or a mixture of a polymer and a salting-out inducing salt. The small differences in polarity for these two components limit the applications of ABSs in separation science, leading to a search for alternatives to polymer-rich phases. ILs are suitable candidates to replace polymers in ABSs mainly due to their tunability, low viscosity, and low toxicity [55]. Thus hydrophilic ILs combined with aqueous solutions of inorganic salts were used as ABSs for the development of sustainable extraction methods, thanks to the absence of organic solvents in the entire procedure.

Most applications of ABSs for extraction are devoted to the isolation of biomolecules, such as amino acids and proteins [55, 56]. However, their incorporation in the field of organic compound separation has significantly increased in the recent years [56]. In these methods a hydrophilic IL aqueous solution and a salting-out agent are added to the solid or aqueous sample. The added amounts ensure that the resulting concentrations of the components (IL and salt, in water) lead to the formation of the immiscible phases. In some cases the mass transfer of the analytes from the sample to the IL-rich phase is improved by agitation such as vortex mixing [57–60] or ultrasound disruption [44, 61–63]. The two phases separate when equilibrium is reached. This step can be accelerated by centrifugation [44, 46, 47, 57, 60–63]. Finally the IL-rich phase and the salt-rich phase are collected and taken for analysis by LC-UV, to determine the extracted and the nonextracted compounds, respectively. Fig. 17.3B presents a scheme outlining the operational procedure for ABS extraction methods.

A variety of hydrophilic ILs have been explored in ABSs for the extraction of organic compounds, the most popular being the $[R_1C_1Im^+]$ cations with short alkyl chains (with 2 and 6 carbon atoms) in combination with halide, $[BF_4^-]$, and $[N(CN)_2^-]$ anions [22, 44, 46, 47, 57, 61–64]. In addition, tetrabutylammonium-based ILs ($[N^+_{4,4,4,4}]$) with halide anions were commonly reported [45, 60, 65]. The design and utilization of more biocompatible ILs is a hot topic in ABS formulation due to the recent concern about the toxicity of some ILs, especially toward aquatic ecosystems [66]. In this sense, ions originating from natural sources have been used for the preparation of more benign ILs, such as cholinium cations with amino acid-based anions [59] or guanidinium cations ($[(R_1R_2)(R_3R_4)(R_5R_6)Gu^+]$) [67, 68]. Also of interest is the synthesis of hydrophilic metal-free MIL based on a radical anion for ABS, which facilitates the collection of the IL-rich phase using a magnet once the ABS is formed [67]. For the preparation of IL-based ABSs, high-charge density inorganic salts are typically used, being the most popular dipotassium phosphate [22, 47, 57, 62] and ammonium sulfate [46, 61, 63].

In general, large amounts of ILs are used compared with the initial volume of aqueous phase (10%–70% w/w of IL), which provides low enrichment factors despite the high extraction capability of ABSs. In this sense, most recent studies have focused on the miniaturization of the method by employing smaller amounts of ILs,

less than 1.5% (w/w) [60, 68]. However, the long equilibration time, which ranges from 2 to 24 h [45, 60, 65], remains the principal drawback of ABSs, thus limiting their expansion within the separation and extraction field, despite their high extraction yields and sustainability [11].

As it happens with MWAE and USAE, most applications of ILs in ABSs are focused on the extraction of organic compounds from a complex matrix, rather than their quantitative determination. Typical applications include the extraction of drugs from environmental waters [45, 60, 64, 65] or flavonoids and alkaloids from plants [46, 59]. Nevertheless, it is true that there are several studies that perform the validation of the entire analytic method for the quantification of natural compounds extracted from plants [22, 61, 62], reaching a sensitivity at the part-per-billion level with good precision. It is also worth mentioning the recycle and reuse of the IL with the $[C_2C_1Im^+]$ cation and triflate anion after performing the ABS-based extraction reported by Almeida et al. [64]. After the analysis the analytes present in the IL-rich phase are precipitated by means of pH adjustment, and the IL is subsequently used in the ABS without losing its extraction efficiency after four cycles.

Regarding the monitoring of organic compounds using IL-based ABSs, the determination of antibiotics in water [67], blood [57], and milk [44] was reported in combination with LC-UV, proving the applicability of this extraction method for the analysis of complex matrixes. Quite low LODs and high extraction efficiencies were also obtained for the quantification of colorants in food samples using IL-based ABSs [47, 63]. The analytic characteristics of some representative examples of IL-based ABS for the extraction of organic compounds are summarized in Table 17.1.

17.3 Ionic Liquids in Liquid-Phase Microextraction Methods

DLLME, SDME, and HF-LPME are among the main strategies for the determination of organic compounds that utilize ILs in miniaturized liquid-based extraction methods and are described in this section.

17.3.1 Dispersive liquid-liquid microextraction

DLLME implies the addition of a mixture of water-insoluble extraction solvent and dispersive solvent (the latter being miscible with the extraction solvent and with the sample) to an aqueous sample containing the target analytes. In this way, once the added mixture contacts water, microdroplets of the extraction solvent are dispersed throughout the sample with the aid of the dispersive solvent, enhancing the partitioning of the analytes into the extraction solvent. After centrifugation the microdroplets

containing analytes are collected for further analysis [69]. This method is characterized as simple, quite fast, and low cost and also provides high enrichment factors due to the favorable phase ratio of the aqueous sample (between 5 and 25 mL) to the extraction solvent (up to ~100 μL).

Since its introduction by Rezaee et al. in 2006 [70], an increasing number of applications have been reported and a number of modifications proposed. Among the advances in this microextraction technique, the search for new extraction solvents with safer toxicological profiles and better analytic performance than conventional halogenated organic solvents is of particular interest. In this sense the success of ILs is evident as extraction solvents in DLLME (IL-DLLME) to accomplish these requirements [71]. As shown in Fig. 17.2, almost half of the studies reported using ILs in liquid-phase extraction for the determination of organic compounds are DLLME applications. Indeed, several strategies for DLLME using exclusively ILs with different characteristics have been described, such as in situ IL-DLLME, magnetic-assisted IL-DLLME, and MIL-DLLME, as shown in Fig. 17.4 [72].

17.3.1.1 Conventional IL-DLLME

The conventional IL-DLLME mode, in its more classical approach, follows the same procedure as the initial approach described for DLLME (Fig. 17.4A). Thus a water-insoluble IL is added to the aqueous sample along with a dispersive solvent to form fine droplets of IL that are dispersed throughout the sample with the aid of a stirring method, such as vortex mixing, US, or MW. After centrifugation the microdroplet of IL containing the analytes settles at the bottom of the tube and is collected for analysis [71, 72]. In some applications the mixture is cooled before the centrifugation step to ensure complete insolubilization of the IL and to avoid losses of the extraction solvent during the procedure.

Volumes ranging between 20 and 280 μL of hydrophobic ILs, prepared with $[R_1C_1Im^+]$ cations paired with hexafluorophosphate ($[PF_6^-]$) [73–92] or bis [(trifluoromethyl)sulfonyl]imide ($[NTf_2^-]$) [93, 94] anions are typically used. Imidazolium cations with normally short alkyl chains, $[C_4C_1Im^+]$ [83, 87, 90, 94], $[C_6C_1Im^+]$ [73–76, 80, 84, 88, 89, 92, 93, 95, 96], and $[C_8C_1Im^+]$ [77–79, 81, 82, 85, 86] are the most widely used.

Regarding the nature of the dispersive solvent, methanol [74, 75, 79, 81–84, 90, 93, 95], acetone [86, 94], and acetonitrile [73, 78] were employed. Some surfactants, such as Triton X-114 [87, 89] and Triton X-100 [80], were shown to be useful as dispersive solvent and antisticking agents, to avoid the hydrophobic IL coating the walls of the sample container. $[R_1C_1Im^+]$ hydrophilic ILs as a dispersive solvent was also reported [76, 85], leading to the formation of larger volumes of microdroplets due to aggregation of both the extraction and dispersive ILs after the centrifugation step.

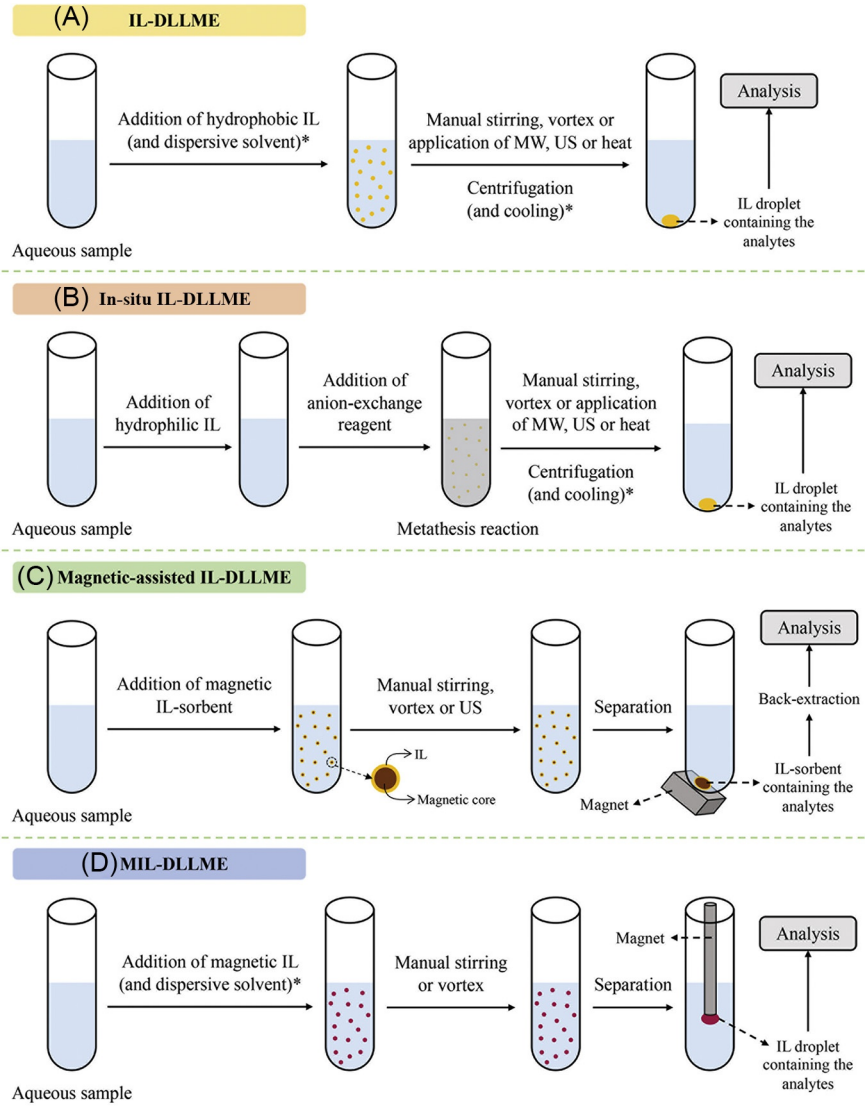

Fig. 17.4 Schemes of the main modes of DLLME using ILs as extraction solvents: (A) conventional DLLME, (B) in situ DLLME, (C) magnetic-assisted DLLME, and (D) DLLME using magnetic ILs.

The IL-DLLME method can also be performed in the absence of a dispersive solvent in the so-called temperature-controlled IL-DLLME [86, 87, 91, 92]. In this case the sample is heated after the addition of the hydrophobic IL to force its solubilization and to improve the migration of analytes from the sample to the extraction phase.

Afterward the IL is insolubilized by cooling the solution, followed by centrifugation to isolate it from the aqueous sample.

IL-DLLME has also been assisted by either US [75–78, 82, 88, 95] or MW [83, 84, 88] to facilitate the dispersion of the IL and ensure adequate formation of fine droplets. The application of these additional mixing steps speeds up the extraction and provides shorter extraction times together with high extraction efficiencies. In some cases, it is possible to avoid the use of organic dispersive solvents, because the US or MW power is enough to ensure the proper dispersion of the hydrophobic IL into the aqueous sample [77, 88].

Of interest is the in-syringe setup of Cruz-Vera et al. for the IL-DLLME method [90]. In this approach the extraction is entirely performed using only a syringe as shown in Fig. 17.5. It avoids the time-consuming centrifugation step and facilitates the collection of the final IL microdroplets. This method can be automated and coupled online with liquid chromatography [73].

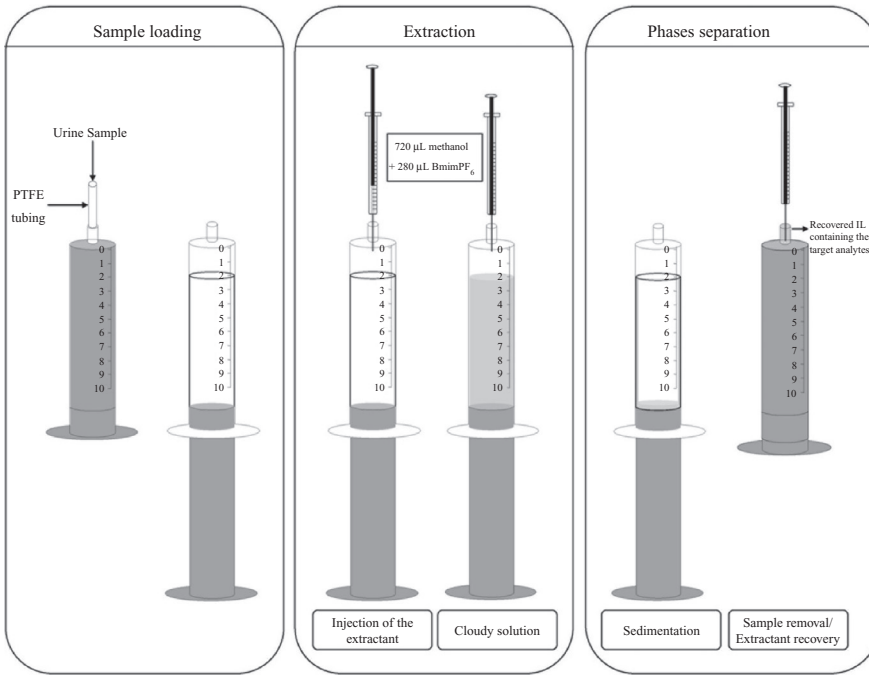

Fig. 17.5 Experimental procedure for the IL-DLLME method performed in a syringe as proposed by Cruz-Vera et al. [90], which has also been used for the in situ IL-DLLME [97, 98]. *(Reprinted from Cruz-Vera M, Lucena R, Cárdenas S, Valcárcel M. One-step in-syringe ionic liquid-based dispersive liquid–liquid microextraction. J Chromatogr A 2009;1216:6459–6465. Copyright (2009), with permission from Elsevier.)*

Most applications of IL-DLLME describe the determination of different classes of pesticides [77, 85, 91, 92], mainly herbicides [74, 88, 89] in environmental waters [77, 85, 91, 92]. Applications in food samples require a pretreatment of the sample to ensure adequate performance of the IL-DLLME in aqueous food extracts [74, 88, 89]. The determination of drugs [75, 78, 81, 90] and antibiotics [80, 84, 87] either in biological samples, such as urine [75, 90] and plasma [79, 81, 84], or in water [78, 87] are also quite common. The remaining studies include environmental monitoring, specifically the analysis of water samples for endocrine-disrupting compounds, including phenols [76, 86] and UV filters [73, 82, 95].

In general, these methods are coupled with LC-UV, requiring dilution of the IL phase with an organic solvent to ensure compatibility of the extract with the mobile phase. However, given the high thermal stability of ILs, the thermal desorption of analytes from the IL phase has been accomplished by placing a microvial insert containing the IL in a thermal desorption unit connected to a gas chromatograph (GC) system [93]. Taking into account the compatibility of ILs with both LC and GC systems, the wide range of organic compounds determined and the variety of samples analyzed is illustrated in Table 17.2 [77, 78, 81, 83, 85, 86, 88, 89, 93, 97, 99–115].

17.3.1.2 In-Situ IL-DLLME

In the in situ IL-DLLME method, unlike the conventional approach, a hydrophilic IL is initially used as extraction solvent. Then an anion-exchange reagent is added to the sample to accomplish a metathesis reaction, thus forming in situ the hydrophobic IL. This strategy yields fine droplets of the IL, enhances the dispersion, and, therefore, avoids the use of an organic solvent as dispersion agent [71]. In general the procedure requires the application of a stirring method after the addition of the anion-exchange reagent to improve the kinetics of the reaction and/or the cooling of the solution to ensure the insolubilization of the IL. Finally the mixture is centrifuged, and the resulting hydrophobic IL phase with extracted compounds is collected for analysis. A scheme outlining this procedure is shown in Fig. 17.4B.

Yao and Anderson were the first to report the development of the in situ IL-DLLME for the extraction of organic compounds using the hydrophilic $[C_4C_1Im^+][Cl^-]$ IL and lithium bis[(trifluoromethyl)sulfonyl]imide (Li-NTf_2) as anion-exchange reagent [116]. This method was compared with the conventional IL-DLLME using the hydrophobic analogue $[C_4C_1Im^+][NTf_2^-]$. The in situ approach provided higher enrichment factors.

Since this first application, many studies report the use of the in situ IL-DLLME for the extraction of organic compounds. Hydrophilic ILs with $[C_4C_1Im^+]$ cations and $[Br^-]$ or $[Cl^-]$ anions are typically used [99–101, 117–123], while tetraalkylammonium-based ILs ($[N^+_{R1,R2,R3,R4}]$) are rarely employed [97, 98]. With the aim of using ILs with reduced toxicity, monoalkylguanidinium chloride ($[R_1Gu^+][Cl^-]$) ILs with surface-active properties were suggested as extraction solvents [102, 124].

TABLE 17.2 Representative Applications of ILs as Extraction Solvents in Liquid-Phase Microextraction Methods for the Determination of Organic Compounds

Method[a]	IL[b]	Type of IL	IL vol. (μL)	Additive and/or Special Configuration[c]	Analytes[d] (Number)	Sample	Analytic Technique[e]	LOD[f]	Ref.
IL-DLLME	$[C_6C_1Im^+][NTf_2^-]$	Hydrophobic	50	MeOH-dispersive solvent	Parabens (5)	Waters	TD-GC-MS	4.3–8.1 ng L^{-1}[g]	[93]
IL-DLLME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	20	MeOH-dispersive solvent	Drugs (3)	Plasma	LC-UV	0.17–0.43 μg L^{-1}	[81]
IL-DLLME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	~85	ACN- dispersive solvent and US to disperse	Drugs (9)	Waters	LC-MS/MS	0.2–60 ng L^{-1}	[78]
IL-DLLME	$[C_4C_1Im^+][PF_6^-]$	Hydrophobic	100	MeOH-dispersive solvent and MW to disperse	Phthalates (5)	Waters	LC-UV	0.71–1.94 μg L^{-1}	[83]
IL-DLLME	$[C_6C_1Im^+][PF_6^-]$	Hydrophobic	60	US and MW to disperse	Herbicides (7)	Milk	LC-UV	0.46–1.96 μg L^{-1}	[88]
IL-DLLME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	40	US to disperse	Fungicides (4)	Waters	LC-UV	0.73–2.2 μg L^{-1}	[77]
IL-DLLME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	35	Temperature-controlled and acetone-dispersive solvent	Phenols (2)	Waters	LC-UV	0.58–0.86 μg L^{-1}	[86]

Continued

TABLE 17.2 Representative Applications of ILs as Extraction Solvents in Liquid-Phase Microextraction Methods for the Determination of Organic Compounds—cont'd

Method[a]	IL[b]	Type of IL	IL vol. (μL)	Additive and/or Special Configuration[c]	Analytes[d] (Number)	Sample	Analytic Technique[e]	LOD[f]	Ref.
IL-DLLME	$[C_6C_1Im^+][PF_6^-]$	Hydrophobic	175	Triton X-114-dispersive solvent	Herbicides (5)	Honey	LC-UV	5.31–8.59 μg kg^{-1}	[89]
IL-DLLME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	50	$[C_4C_1Im^+][BF_4^-]$-dispersive solvent	Bactericides (2)	Waters	LC-UV	0.23–0.35 μg L^{-1}	[85]
In situ IL-DLLME	$[C_4C_1Im^+][Cl^-]$	Hydrophilic	38	$LiNTf_2$- anion-exchange reagent	Phenols (6)	Waters	LC-UV	10–87 μg L^{-1}	[99]
In situ IL-DLLME	$[C_8C_1Im^+][Cl^-]$	Hydrophilic	~50	KPF_6- anion-exchange reagent	Chlorophenols (10)	Waters	TD-GC-MS	0.06–0.44 μg L^{-1}	[100]
In situ IL-DLLME	$[N^+_{4,4,4,4}][Cl^-]$	Hydrophilic	~10	KPF_6- anion-exchange reagent and in-syringe	Insecticides (4)	Honey	LC-UV	0.21–0.42 μg L^{-1}	[97]
In situ IL-DLLME	$[C_4C_1Im^+][Cl^-]$	Hydrophilic	~35	$LiNTf_2$- anion-exchange reagent	Pesticides (9)	Waters	TD-GC-MS	5–16 ng L^{-1}	[101]
In situ IL-DLLME	$[C_{10}Gu^+][Cl^-]$	Surfactant	20	$NaClO_4$- anion-exchange reagent	OHPAHs (4)	Urine	LC-FD	1–2 ng L^{-1}	[102]

Magnetic IL-DLLME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	70	Ferrite MNPs	Insecticides (5)	Waters	LC-UV	0.05–0.15 μg L^{-1}	[103]
Magnetic IL-DLLME	$[C_{16}C_1Im^+][Br^-]$	Surfactant	~2	Ferrite MNPs and GO	Antibiotics (5)	Urine	LC-UV	0.6–1.9 μg L^{-1}	[104]
Magnetic IL-DLLME	$[C_6C_1Im^+][NTf_2^-]$	Hydrophobic	60	Magnetic effervescent tablet	Fungicides (4)	Waters	LC-UV	0.02–0.1 μg L^{-1}	[105]
MIL-DLLME	$[P^+_{6,6,6,14}]_2[MnCl_4^-]$	Magnetic	30	ACN- dispersive solvent	Organic pollutants (13)	Waters	LC-UV	0.25–1 μg L^{-1}	[106]
MIL-DLLME	$[P^+_{6,6,6,14}][Ni(hfacac)_3^-]$	Magnetic	25	Magnetic stir bar	UV filters (8)	Waters	TD-GC-MS	9.9–26.7 ng L^{-1}	[107]
HS-SDME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	2	Suspended with a syringe	BTEX (5)	Waters	TD-GC-MS	20–91 ng L^{-1}	[108]
HS-SDME	$[C_6C_1Im^+][PF_6^-]$	Hydrophobic	5	Suspended with a syringe	Chlorobenzenes (10)	Waters	TD-GC-MS	1–4 ng L^{-1}	[109]
DI-SDME	$[P^+_{6,6,6,14}][FAP^-]$	Hydrophobic	10	Suspended with a syringe	PAHs (13)	Waters	LC-UV	0.03–265 μg L^{-1}	[110]
DI-SDME	$[C_4C_1Im^+][PF_6^-]$	Hydrophobic	0.0024	Suspended with a syringe	Phenols (3)	Waters	CE-UV	5–80 μg·L^{-1}	
HS-SDME	$[P^+_{6,6,6,14}]_2[MnCl_4^-]$	Magnetic	~20	Suspended with a magnet	Aromatic compounds (12)	Waters	LC-UV	0.04–1 μg L^{-1}	[111]
Two-phases HF-LPME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	Extraction and acceptor phase[h]	Directly immersed in the sample	OPPs (4)	Waters	LC-UV	15–26 ng L^{-1}	[112]

Continued

TABLE 17.2 Representative Applications of ILs as Extraction Solvents in Liquid-Phase Microextraction Methods for the Determination of Organic Compounds—cont'd

Method[a]	IL[b]	Type of IL	IL vol. (μL)	Additive and/or Special Configuration[c]	Analytes[d] (Number)	Sample	Analytic Technique[e]	LOD[f]	Ref.
Two-phases HF-LPME	$[C_8C_1Im^+][PF_6^-]$	Hydrophobic	Extraction and acceptor phase[h]	Stainless steel wire for magnetic stirring	Hormones (8)	Cosmetics	LC-UV	1 μg L^{-1}	[113]
Three-phases HF-LPME	$[C_6C_1Im^+][FAP^-]$	Hydrophobic	Extraction phase[h]	NaOH solution-Acceptor phase. Immersion in the sample with a syringe	Chloro-phenols (3)	Waters	LC-UV	0.3–0.5 μg L^{-1}	[114]
Three-phases HF-LPME	$[C_4C_1Im^+][PF_6^-]$	Hydrophobic	25 As acceptor phase	Nonanol-extraction phase. Directly immersed in the sample	PAEs (3)	Tea	LC-UV	0.7–1.7 μg L^{-1}	[115]

[a] Methods abbreviations: *DI* for direct immersion, *HF-LPME* for hollow-fiber liquid-phase microextraction, *HS* for headspace, *IL-DLLME* for ionic liquid-based dispersive liquid-liquid microextraction, *MIL-DLLME* for magnetic ionic liquid-based dispersive liquid-liquid microextraction, and *SDME* for single-drop microextraction.
[b] Ionic liquids abbreviations: $[C_4C_1Im^+][Cl^-]$ for 1-butyl-3-methylimidazolium chloride, $[C_4C_1Im^+][PF_6^-]$ for 1-butyl-3-methylimidazolium hexafluorophosphate, $[C_6C_1Im^+][FAP^-]$ for 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate, $[C_6C_1Im^+][NTf_2^-]$ for 1-hexyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide, $[C_6C_1Im^+][PF_6^-]$ for 1-hexyl-3-methylimidazolium hexafluorophosphate, $[C_8C_1Im^+][Cl^-]$ for 1-octyl-3-methylimidazolium chloride, $[C_8C_1Im^+][PF_6^-]$ for 1-octyl-3-methylimidazolium hexafluorophosphate, $[C_{10}Gu^+][Cl^-]$ for decylguanidinium chloride, $[C_{16}C_1Im^+][Br^-]$ for 1-hexadecyl-3-methylimidazolium chloride, $[N^+_{4,4,4,4}][Cl^-]$ for tetrabutylammonium chloride, $[P^+_{6,6,6,14}][FAP^-]$ for trihexyltetradecylphosphonium tris(pentafluoroethyl)trifluorophosphate, $[P^+_{6,6,6,14}]_2[MnCl_4^-]$ for trihexyltetradecylphosphonium tetrachloromanganate (II), and $[P^+_{6,6,6,14}][Ni(hfacac)_3^-]$ for trihexyltetradecylphosphonium tris(hexafluoroacetylaceto)nickelate (II).
[c] Additives abbreviations: *ACN* for acetonitrile, $[C_4C_1Im^+][BF_4^-]$ for 1-butyl-3-methylimidazolium tetrafluoroborate, *GO* for graphene oxide, $LiNTf_2$ for lithium bis[(trifluoromethyl)sulfonyl] imide, MeOH for methanol, *MNPs* for magnetic nanoparticles, *MW* for microwaves, and *US* for ultrasound.
[d] Analytes abbreviations: *BTEX* for benzene, toluene, ethylbenzene, and xylene; *OHPAHs* for monohydroxylated polycyclic aromatic hydrocarbons; *OPPs* for organophosphorus pesticides; *PAEs* for phthalate esters; *PAHs* for polycyclic aromatic hydrocarbons; and *UV* for ultraviolet.
[e] Analytical technique abbreviations: *CE* for capillary electrophoresis, *FD* for fluorescence detection, *GC* for gas chromatography, *LC* for liquid chromatography, *MS* for mass spectrometry, *TD* for thermal desorption, and *UV* for ultraviolet detection.
[f] Limit of detection.
[g] Limit of quantification.
[h] The hollow fiber is totally immersed in the pure IL to impregnate the pores.

Concerning the anion-exchange reagent to promote the metathesis reaction, Li-NTf_2 [99, 101, 116–124] and potassium hexafluorophosphate (KPF_6) [97, 98, 100] are the preferred salts. These reagents provide high yields of the reaction when a 1:1 mol ratio with the hydrophilic IL is used. Recently the so-called salt-induced IL-DLLME method has been developed. This approach utilizes sodium perchlorate to promote the insolubilization of the IL, avoiding the addition of the highly toxic fluorinated salts commonly used in the in situ IL-DLLME method [102].

The in situ IL-DLLME method was combined with the in-syringe setup to facilitate the handling of the microdroplet dispersion [97, 98]. Full automation of the in situ DLLME method using a solid-phase extraction workstation is possible [121]. In this strategy the hydrophobic IL dispersion is retained on the sorbent of the SPE column and then desorbed with the aid of an organic solvent.

Applications of this IL-DLLME mode are focused on the determination of organic contaminants in environmental waters. Some representative examples are shown in Table 17.2. The analytes determined range from pesticides [98, 101, 121–123] to phenols [99, 100, 120]. Moreover the analysis of complex samples has been accomplished, for example, the determination of insecticides in honey [97] and monohydroxylated PAHs in urine [102]. The quantification of the analytes has been performed either by LC (with prior dilution of the IL phase with a compatible solvent) [97–99, 102, 116, 121–124] or by GC-mass spectrometry (MS), in this case using a thermal desorption unit [100, 101, 120] or headspace analysis [117, 119]. In those cases where the in situ DLLME method is combined with GC, it is important to point out the absence of organic solvents in the entire analytic procedure, thus improving the environmental friendliness of the methodology.

17.3.1.3 Magnetic-Assisted IL-DLLME

Despite the success of ILs in DLLME, the extraction procedure involves a time-consuming and tedious centrifugation step together with some difficulties regarding the handling of the IL microdroplets. Therefore the incorporation of magnetic particles in the process has emerged as an alternative to facilitate the separation and shorten the extraction time [14, 72]. For magnetic-assisted IL-DLLME, magnetic particles are added to the sample together with the IL forming a composite that is dispersed throughout the sample. A strong external magnet placed outside the walls of the tube is used to ensure separation of the sorbent-containing analytes from the sample. The target compounds are then desorbed using a small volume of organic solvent. A general scheme for this procedure is shown in Fig. 17.4C.

The majority of magnetic-assisted IL-DLLME methods report the use of hydrophobic ILs composed of [$C_6C_1Im^+$] cations paired with [PF_6^-] [103, 125] or [NTf_2^-] [126, 127] anions. Regarding the preparation of IL-based magnetic sorbents, ILs were typically combined with ferrite magnetic nanoparticles [103, 125–127]. The hydrophobic IL covers the negatively charged surface of the magnetic particles

by electrostatic interactions, thus forming the composite. Some applications utilize an effervescent tablet containing magnetic material, the IL, and the effervescence precursors, for magnetic-assisted IL-DLLME [105, 128], as shown in Fig. 17.6. Effervescence enables proper dispersion of the sorbent without any additional stirring. The in situ IL-DLLME method using imidazolium-based hydrophilic ILs has also been assisted by magnetic materials [118, 128]. The ferrite nanoparticles are added after the metathesis reaction to interact with the hydrophobic IL formed, in this case to facilitate phase separation.

The IL-based surfactants $[C_{16}C_1Im^+][Br^-]$ [104, 129] and 1,3-didodecylimidazolium bromide ($[C_{12}C_{12}Im^+][Br^-]$) [129] were also explored for magnetic-assisted IL-DLLME. In this case, despite the hydrophilicity of the IL, it interacts with the negative surface of the magnetic nanoparticles forming hemimicelles (monolayers) or admicelles (bilayers) at concentrations lower than the CMC [37], thus taking advantage of the surface-active properties of these ILs using small amounts as extraction solvent.

Regarding the analytic applications (Table 17.2), pesticides are the most commonly determined analytes in food [126, 127] and water samples [103, 118]. The quantification of endocrine-disrupting compounds, such as personal care products [125] and phenols [129] in water, is also an interesting application of this method. All applications use LC-UV [103, 104, 126, 127, 129] or MS detection [125], simply requiring the direct injection of the extract.

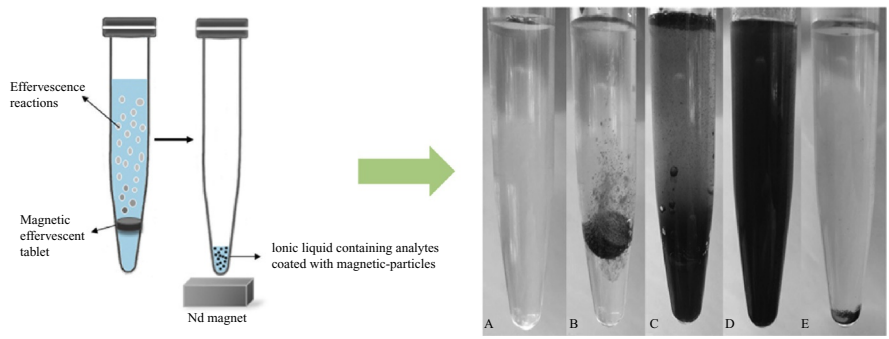

Fig. 17.6 General procedure for the effervescent magnetic-assisted IL-DLLME method developed by Yang et al., including the different sequential steps: (A) aqueous sample with the analytes, (B) addition of the effervescent table containing the magnetic particles and the IL, (C) and (D) dispersion of the magnetic sorbent due to the effervescence, and (E) separation of the IL-based magnetic sorbent from the aqueous sample using an external magnet. *(Reprinted from Yang M, Wu X, Jia Y, Xi X, Yang X, Lu R, et al. Use of magnetic effervescent tablet-assisted ionic liquid dispersive liquid-liquid microextraction to extract fungicides from environmental waters with the aid of experimental design methodology. Anal Chim Acta 2016;906:118–127. Copyright (2016), with permission from Elsevier.)*

17.3.1.4 MIL-DLLME

Recently, MILs have attracted much attention in sample preparation techniques, particularly in IL-DLLME. The paramagnetic behavior of the IL itself allows the magnetic retrieval of the IL microdroplets without any additional magnetic particles, which usually lack stability and tend to aggregate [10, 14]. The experimental procedure for MIL-DLLME is similar to conventional IL-DLLME, as shown in Fig. 17.4D. The hydrophobic MIL together with the dispersive solvent are added to the aqueous sample, which is then stirred to disperse the MIL as fine microdroplets for the extraction. The MIL droplets are collected and separated from the sample with the aid of a magnet and subjected to analysis.

The MILs used in MIL-DLLME are prepared with $[N^{+}_{R1,R2,R3,R4}]$ or tetraalkylphosphonium ($[P^{+}_{R1,R2,R3,R4}]$) cations together with a metal-containing anion, which is the moiety responsible of the paramagnetic properties of the IL. Tetrachloromanganate (II) ($[MnCl_4^{2-}]$) [106, 111, 130] and bromotrichloroferrate (III) ($[FeCl_3Br^{-}]$) anions [131] were used originally for this purpose. The tris(hexafluoroacetylaceto) ligand combined with nickel or dysprosium metal centers to prepare the $[Ni(hfacac)_3^{-}]$ [107, 132] and $[Dy(hfacac)_4^{-}]$ [133] anions, respectively. These anions yielded less viscous MILs that were successfully used in MIL-DLLME. Nevertheless, because of the relatively high viscosity of the MILs a small volume of dispersion solvent, ranging between 5 [106, 130] and 500 μL [131] is typically required.

Chisvert et al. proposed a stir bar MIL-DLLME method that combines the advantages of DLLME with those of stir bar sorptive microextraction [107, 132]. In this approach the MIL coats a magnetic stir bar that is added to the sample. At high stirring rates the MIL is dispersed into the aqueous sample but remains adsorbed on the stir bar at low speeds (or when the stirring stops). After the extraction the MIL-coated magnetic bar is placed in a thermal desorption unit to desorb the analytes for quantification by GC-MS. A schematic outline of the procedure is shown in Fig. 17.7.

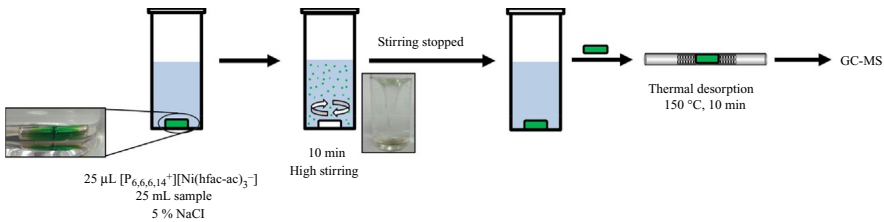

Fig. 17.7 Schematic procedure of the developed stir bar MIL-DLLME using a magnetic ionic liquid as extraction solvent developed by Chisvert et al. [107, 132]. *(Reprinted from Benedé JL, Anderson JL, Chisvert A. Trace determination of volatile polycyclic aromatic hydrocarbons in natural waters by magnetic ionic liquid-based stir bar dispersive liquid microextraction. Talanta 2018;176:253–261. Copyright (2018), with permission from Elsevier.)*

This way the method does not require organic solvent neither in the sample preparation process nor in the separation and determination step, compared with other MIL-DLLME methods combined with LC.

The majority of analytic applications of MIL-DLLME are devoted to the analysis of environmental waters, for PAHs [106, 111, 131, 132] and emerging organic pollutants, such as UV filters [107], and antibiotics [133]. The first study on the analysis of complex biological samples by MIL-DLLME was reported for the determination of estrogens in urine [130]. Given the current interest in MILs, more applications to complex samples are expected.

17.3.2 Single-drop microextraction

SDME is a preconcentration method using a small volume of extraction solvent suspended from the tip of a syringe needle to extract analytes [134]. There are two main modes of operation as shown in Fig. 17.8A: headspace mode (HS-SDME) in which the extraction solvent droplet is exposed to the headspace above the sample, and the direct immersion mode (DI-SDME), where the drop is directly introduced into the aqueous sample. Afterward the drop is withdrawn into the syringe and injected into the analytic system for determination of the extracted compounds. This method has become quite popular due to its simplicity, low cost, high extraction capacity, the possibility of determining nonvolatile and volatile compounds, and the low solvent volumes employed (around 1–15 μL). However, drop instability is the main drawback. The traditional organic solvents volatilize at high temperatures or low pressures leading to losses of the extraction solvent during the process [14, 134]. Here the low vapor pressure of ILs together with their high viscosity makes them attractive for use as extraction solvent in SDME, with improved drop stability [11, 14].

Hydrophobic ILs are used as extraction solvent in both HS-SDME [108, 109, 135–141] and DI-SDME [110, 142–145]. ILs containing $[R_1C_1Im^+]$ cations and $[PF_6^-]$ [109, 140–142, 144, 145] or $[NTf_2^-]$ anions [138] are typically used, with $[C_8C_1Im^+][PF_6^-]$ IL the most common in this microextraction method [108, 135–137, 139, 143]. Nevertheless, $[PF_6^-]$-based ILs partially dissolve in aqueous solutions after a relatively short time (~30 min). To overcome this problem an ultrahydrophobic IL containing a trihexyltetradecylphosphonium cation ($[P^+_{6,6,6,14}]$) and tris(pentafluoroethyl)trifluorophosphate ($[FAP^-]$) anion was used for DI-SDME [110].

More recently, MILs have been explored for HS-SDME [111, 146, 147]. In this case the MIL used as extraction solvent is suspended in the headspace of the sample with the aid of a rod magnet as shown in Fig. 17.8A. Hydrophobic MILs containing $[P^+_{6,6,6,14}]$ cations and $[MnCl_4^{2-}]$ [111] or $[Mn(hfacac)_3^-]$ anions [147] and hydrophilic MILs with 1-ethyl-3-methylimidazolium cation and tetraisothiocyanatocobaltate (II) anion ($[C_2C_1Im^+][Co(NCS)_4^{2-}]$) have been used [146]. The use of MILs not only

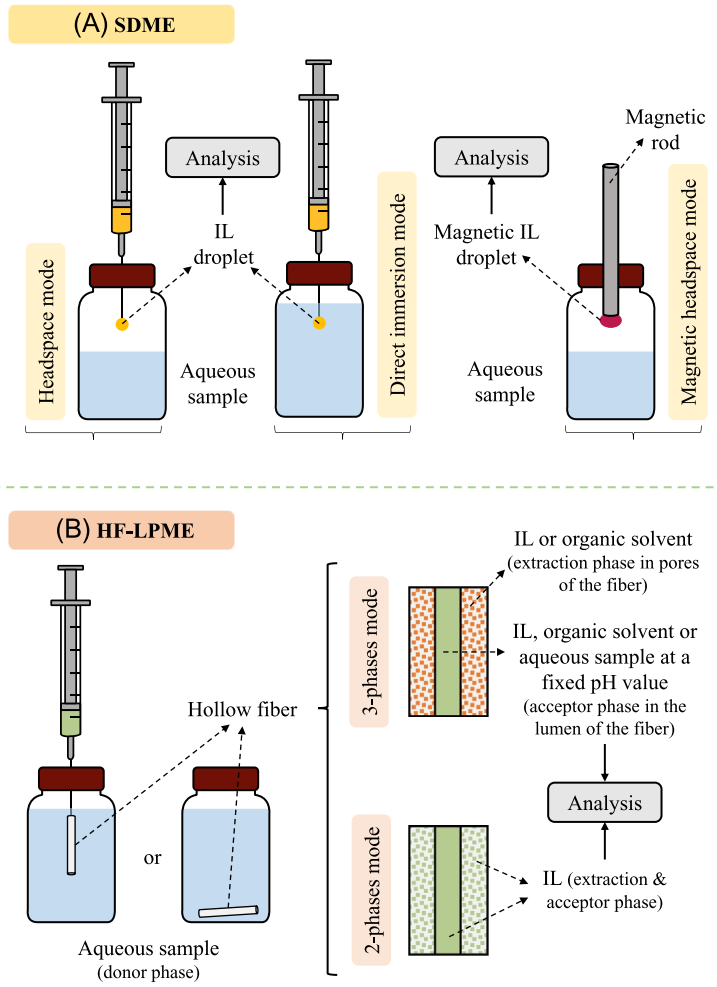

Fig. 17.8 Schemes of (A) SDME and (B) HF-LPME using ILs as extraction solvents.

facilitates the collection of the extraction solvent after the HS-SDME but also improves drop stability during the entire process, since the utilization of a magnet to suspend the MIL prevents the droplet from falling. Indeed the use of MILs as extraction solvent allows the use of reduced pressure conditions during the extraction process, which was not possible previously in SDME due to the detaching of the solvent drop during the air evacuation step [147]. This vacuum-assisted MIL-HS-SDME method only required modification of the cap to provide a leak-tight seal and exhibited high extraction efficiency while decreasing the extraction time compared with the MIL-HS-SDME method at atmospheric pressure.

The vast majority of applications of IL in SDME are intended for the analysis of water samples for the determination of a wide variety of organic compounds as shown in Fig. 17.2, except for those studies focused on the extraction of natural compounds from plant materials [135, 142]. The analytes extracted and quantified using SDME include chlorobenzenes [109, 139, 141, 146], aromatic compounds [108, 110, 111], and personal care products [136, 137, 144]. Some representative examples are summarized in Table 17.2.

The IL or MIL microdrop containing extracted analytes is usually injected directly in an LC system (or previously diluted with an organic solvent) [110, 111, 141, 143, 144] or in a GC system [136, 137]. The coupling of IL-based SDME with GC has been fully automated using an autosampler [136]. Thermal desorption of the extracted compounds for GC analysis is the preferred approach [108, 109, 135, 138–140, 146, 147]. Aguilera-Herrador et al. were the first to develop a removable interface device before the inlet of the GC for thermal desorption avoiding introduction of the IL into the separation column [140]. Since then, different approaches have been proposed for thermal desorption: the use of a commercial thermal desorption unit [109]; the injection of the IL droplet in the GC but using a homemade liner with silica wool to avoid the IL entering the column [139]; and the use of headspace thermal desorption, by placing the droplet in a small vial followed by injection of the headspace after heating [147]. Fig. 17.9 illustrates the most common thermal desorption configurations for SDME using ILs. The SDME with ILs has also been coupled online with capillary electrophoresis, allowing the trace determination of organic compounds from only 2.4 nL of IL [142, 144]. These methods and those used in combination with GC are characterized as solvent-free and are promising environmentally friendly analytic sample preparation techniques.

17.3.3 Hollow-fiber liquid-phase microextraction

HF-LPME involves the use of a hollow fiber as extraction device, typically of polypropylene, containing the extraction solvent, which is then placed in contact with the aqueous sample [148]. This way the extraction solvent is protected from the large particles possibly present in the aqueous sample (donor phase) while applying high stirring ratios. Furthermore the contact area between the extraction solvent and the sample increases in HF-LPME compared with SDME. In the three-phase mode, the pores of the hollow fiber are impregnated with a few microliters of the extraction solvent, while an acceptor phase (immiscible with the extraction phase) is placed in the lumen of the hollow fiber. When the acceptor phase is the same as the extraction solvent, the method is termed two-phase HF-LPME. The operational mode implies that the hollow fiber is in contact with the aqueous sample under stirring, and after an optimum extraction time, the acceptor phase is removed for analysis. In general an organic solvent is employed as the extraction/acceptor phase in the

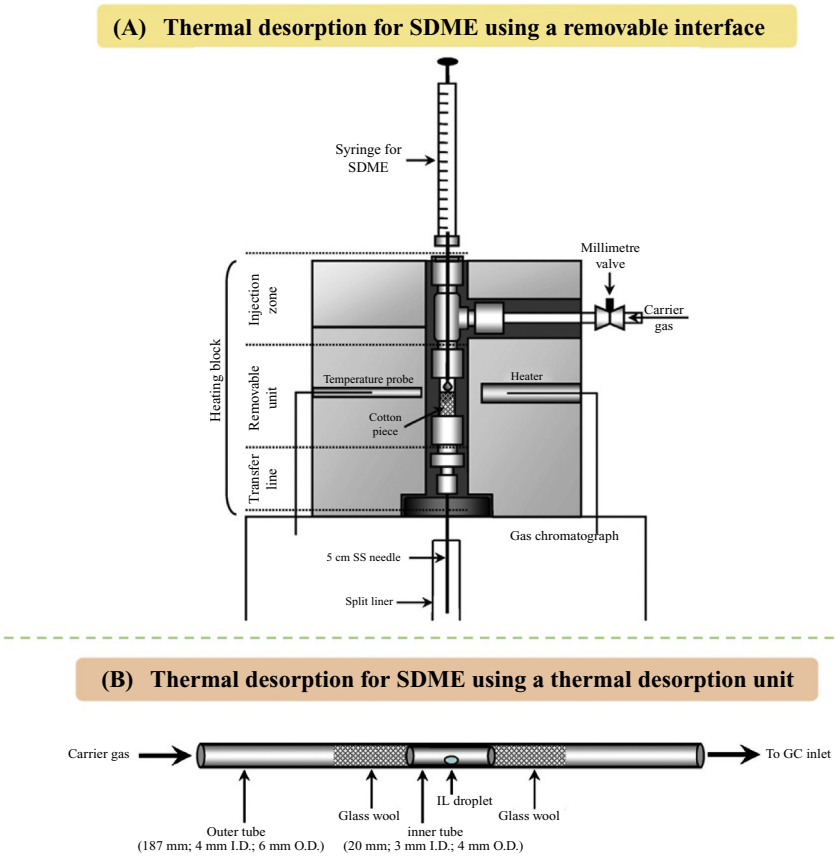

Fig. 17.9 Most common strategies for the thermal desorption of the analytes after performing SDME using ILs: (A) removable interface connected to the GC inlet and (B) commercial thermal desorption unit connected to the GC inlet. *(Panel (A): Reprinted from Aguilera-Herrador E, Lucena R, Cárdenas S, Valcárcel M. Ionic liquid-based single-drop microextraction/gas chromatographic/mass spectrometric determination of benzene, toluene, ethylbenzene and xylene isomers in waters. J Chromatogr A 2008,1201:106–111. Copyright (2008), with permission from Elsevier. Panel (B): Reprinted from Chisvert A, Román IP, Vidal L, Canals A. Simple and commercial readily-available approach for the direct use of ionic liquid-based single-drop microextraction prior to gas chromatography. Determination of chlorobenzenes in real water samples as model analytical application J Chromatogr A 2009;1216:1290–1295. Copyright (2009), with permission from Elsevier.)*

two-phase HF-LPME, whereas aqueous solutions at a pH different to that of the sample are selected as the acceptor phase in the three-phase HF-LPME.

ILs have also been explored in this LPME technique [11, 14]. Fig. 17.8B illustrates the main operational configurations of HF-LPME using ILs. Neat ILs were utilized as extraction phases in two-phase HF-LPME [112, 113, 149, 150] and for three-phase HF-LPME [114, 151–154]. Neat hydrophobic ILs [115] and aqueous solutions of hydrophilic ILs [155] have been used as the acceptor phase in three-phase HF-LPME. In these applications, both ends of the hollow fiber are sealed, followed by its immersion in the sample [112, 115, 153, 155], and in other cases the hollow fiber is introduced in the aqueous sample with the aid of a syringe [114, 150]. In several cases the acceptor phase is collected once the analytes partition properly into it and then injected into an LC [114, 150, 151, 153, 155]. In other cases the analytes are desorbed from the hollow fiber by immersing the device in a few microliters of an organic solvent, followed by the chromatographic determination [112, 113, 115, 149, 152]. There is a specific setup in which a stainless steel wire is inserted into the hollow fiber, with the IL acting as extraction solvent impregnating the pores of the fiber. In this case, there is no IL or solvent in the lumen of the fiber (because the wire occupies it). This device resembles a stir bar sorptive microextraction design, since it allows the magnetic stirring of the hollow fiber. Analytes enriched in the IL are subsequently desorbed by placing the device in contact with an organic solvent [113, 149].

Application of an electric field to HF-LPME to enhance the efficiency of the mass transfer, similar to the electromembrane isolation concept proposed by Pedersen-Bjergaard and Rasmussen [156], termed electromembrane extraction (EME), can also be used. Despite the success of this strategy for the determination of multiple analytes in complex matrixes [157], the use of ILs in EME is scarce [152, 154] due to their high viscosity, which slows down the mass transfer rate leading to longer extraction times. When using ILs in EME, the IL acts as the extraction phase impregnating the pores of the hollow fiber and is typically also the acceptor phase. The system also requires platinum wires acting as negative or positive electrodes. Depending on the charge of the analytes, one electrode is placed in the sample, while the second electrode is introduced into the lumen of the hollow fiber. The charged analytes migrate, quite fast, with the aid of the applied voltage, and are preconcentrated in the acceptor phase.

Hydrophobic ILs are typically used in HF-LPME. In general, they are composed of $[R_1C_1Im^+]$ cations and $[PF_6^-]$ anions, with $[C_8C_1Im^+][PF_6^-]$ the IL most commonly reported [112, 113, 149, 152]. Ultrahydrophobic ILs containing $[FAP^-]$ anions have also been used in HF-LPME to avoid losses during extraction [114, 150]. Hydrophilic ILs with $[Cl^-]$ anions have been employed in this extraction method, requiring low amounts when used as additives in the extraction phase [151] or in the acceptor phase of the three-phase mode [155].

The preparation of the extraction devices for the two-phase HF-LPME with ILs requires the use of hollow fibers with sizes from 1 to 10 cm. These fibers are totally immersed in the IL for a fixed time to ensure adequate impregnation in the fiber pores. If the IL is also required in the lumen, volumes from 4 to 25 μL are required to fill it, depending on the fiber size. For three-phase HF-LPME, the fiber pores can be impregnated with the IL or with an organic solvent (in the latter case with a solution of the IL in the lumen of the fiber).

As shown in Fig. 17.2, HF-LPME with ILs has been used for the extraction of numerous organic compounds including antibiotics [149, 151]; different types of pesticides [112, 152, 154]; and a wide variety of endocrine-disrupting compounds, such as phenols [114, 153], hormones [113], and UV filters [150]. Most methods were developed for the extraction of environmental water samples [112, 114, 150, 153, 155]. HF-LPME is also suitable for the analysis of highly complex samples, such as milk [151], butter [149], urine [154], and cosmetics [113]. These samples did not require a previous extraction to remove the main matrix interferences, and in fact, they are only diluted with water prior to the HF-LPME. Some representative applications are summarized in Table 17.2.

17.4 Concluding Remarks

The search for alternatives to conventional organic solvents in sample preparation methods is a demanding area within analytical chemistry research. ILs have emerged as the most promising candidates given their relative low toxicity and impressive tunability. These solvents are particularly advantageous in miniaturized methods in which small amounts of IL are used in the extraction process, thus enhancing the environmental sustainability of the methods.

The synthetic versatility of ILs also plays an important role in their success, due to the possibility of preparing ILs with defined characteristics for a specific application. Thus MILs are being evaluated in different extraction methods so that the tedious centrifugation and handling steps can be avoided to speed up the entire extraction process. Furthermore the preparation of biodegradable ILs using ions obtained from natural sources is beginning to be explored for the development of sample preparation methods with truly environment-friendly solvents.

Given the outstanding features of ILs, their high extraction capability toward organic compounds with different characteristics, and the incoming advances in the synthesis of ILs with particular (and targeted) properties…, it can be foreseen that new IL-based extraction methods will be developed with improved analytic characteristics in terms of simplicity, greenness, sensitivity, and selectivity.

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Chapter 18

Metal Ion Extraction With Ionic Liquids

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Chapter Outline

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18.1 Introduction

This chapter is intended to provide an overview of the present state of ionic liquids (ILs) as media for metal ion extraction and to offer guidance as to the factors that must be considered in any effort to devise an IL-based system for application in this field. It should be noted that it is not aimed at a comprehensive review of all aspects of ILs. Rather the intent is to build upon several books and recent reviews that offer detailed accounts of the properties of ILs [1–4] by describing those aspects of IL structure and properties having a significant influence on metal ion separations. For background on metal ion solvent extraction into molecular liquids, the reader is referred to an earlier chapter in this book.

While the history of the development of ILs is rather circuitous, some highlights are worth noting here in the context of IL solvent design. More than a hundred years ago, when Paul Walden first reported the deliberate preparation and characterization of an ambient temperature liquid salt (i.e., ethylammonium nitrate) [5], few noticed his work. In fact, nearly 40 years passed before the next systematic investigation of ILs was published by Hurley et al. [6], a study that spurred several decades of research on chloroaluminate salts. In the 1980s ethylammonium nitrate reemerged in the literature, with the recognition that it is a nonaqueous, hydrogen-bonding solvent [7]. This inspired efforts to apply quaternary ammonium and phosphonium salts as gas chromatographic stationary phases [8, 9], providing a useful platform through which their solvent properties could be investigated [10]. Meanwhile, Wilkes and coworkers had been examining chloroaluminate melts as electrolytes for thermoelectric generators and had begun using 1,3-dialkylimidazolium cations to improve the electrochemical window and transport properties of their salts [11]. By the 1990s this group had developed the first ILs combining 1,3-dialkylimidazolium salts with air- and water-stable anions [12], ushering in what many consider the modern era of ILs.

Among the most common ILs are low-melting salts comprising an aprotic, bulky, and asymmetrical organic cation paired with any of a wide range of organic or inorganic anions [13]. For properly chosen combinations of anion and cation, charge dispersion, together with charge separation enhanced by appropriate substituents, disrupts crystal packing, reducing the lattice energy. The result is a substance with a melting point well below that seen for any common organic or inorganic salt. By arbitrary definition, ILs undergo their solid-liquid transition below the boiling point of water ($t_m < 100°C$). For obvious practical reasons, many of the ILs reported in the literature are also liquids at room temperature. Most of these ILs are synthesized by two steps: (i) quaternization, usually by alkylation of a base with a haloalkane or an organosulfate to form the cation, and (ii) anion exchange, either by reacting the onium salt with a Lewis acid or by metathesis with a Brønsted acid or salt of the desired anion. A set of cations and anions that constitute common ILs, along with

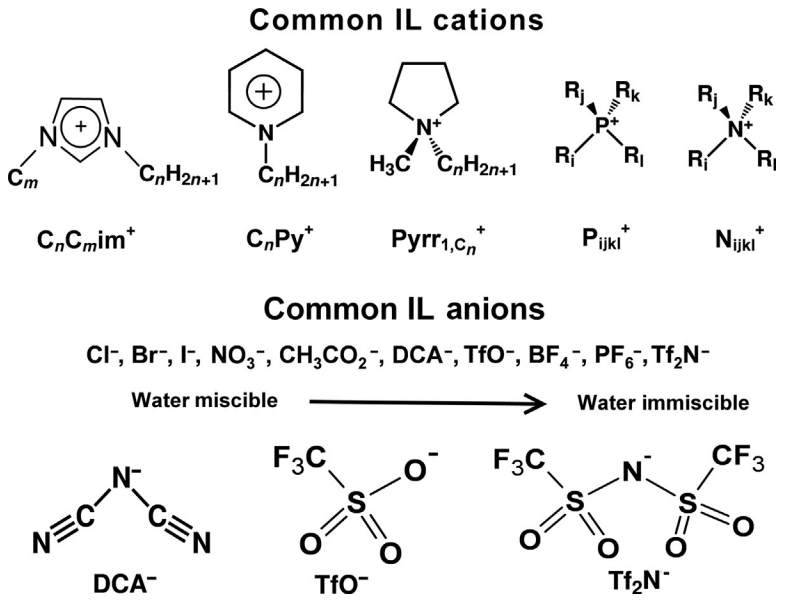

Fig. 18.1 Representative examples of common ionic liquid cations and anions.

their generalized abbreviations, are given in Fig. 18.1, where the ranges of water solubility based on the anion are shown. In the early 2000s commercial availability of low-cost organic halide and sulfate salts with reasonable purity led to a veritable explosion of new ILs and applications. Since then, myriad studies and inventions using ILs have been reported. Currently, >33,000 papers and nearly 5000 patents are returned by a query of the Chemical Abstracts Service using the term "ionic liquids." There are indications, however, of a "saturation point" in the literature, with some suggesting that the rate of production may be leveling off at c. 3500 published papers annually [4]. This same general trend is also evident in publication statistics for papers dealing with metal ion extraction by ILs.

Adding to the structural diversity of these solvents are several other categories of ILs. In addition to "conventional" aprotic ILs, proton transfer from an acid to a base can form protic ILs in which the melting point and stability of the compound are proportional to ΔpK_a [14–17]. "Solvate" ILs incorporate a complex cation that imparts either the necessary bulk or charge delocalization [18–23]. Less common are inorganic ILs, which take advantage of the same disparities in ion structure to achieve low melting points [24–27]. Finally, task-specific ionic liquids (TSILs) incorporate a cation or anion to which a specific functional group is appended [28, 29].

Roughly a million binary ion combinations are estimated to yield ILs [30], providing an immense range of options in selecting suitable candidates for an intended application. Although this vast range complicates efforts to offer generalizations

concerning IL characteristics and behavior, it is now widely recognized that these solvents often exhibit several unique and useful properties. For example, many demonstrate significant electric conductivity [31], making them useful as electrolytes for batteries and as the basis for various other electrochemical devices [32–35]. Also, it has been known for some time that certain ILs display thermal stability and conductivity far surpassing that observed for conventional molecular liquids [36–38], and this knowledge has been exploited in new types of gas chromatographic stationary phases [8, 9, 39–41] and to design new heat transfer fluids [42–45]. Other ILs exhibit a wide liquidus range and offer distinctive solvation behavior, rendering them well suited as media for organic and polymer synthesis [46–48] and for the fabrication of novel materials [49–51]. The most notable characteristic of ILs, however, remains the relative ease with which their physicochemical properties can be modified by, for example, structural variations of the constituent ions, mixing different binary ILs [52], or relatively small changes in temperature [53]. As a better understanding of IL structure-property relationships has developed, formative guidelines for the design of ILs are slowly emerging. Yet because of the vast number of possible IL structures and multifarious intermolecular interactions, general rules for choosing an IL solvent suitable for metal ion extraction remain elusive.

18.2 Solvent Properties of ILs

Interest in the application of ILs in liquid-liquid extraction (LLE) has been motivated by the unique combination of physicochemical properties that distinguish ILs from molecular solvents. In either conventional or IL-based extraction systems, the transfer of a metal ion from one phase to another distributes it between the phases, an equilibrium process that is governed in large part by the thermodynamics of complexes established in the feed phase (i.e., aqueous for extraction) and the extracting phase. That is, the partitioning of a metal is defined by the various solvent-solvent, solute-solvent, and solute-solute interactions in the two liquid phases. The balance between the charged, polar, and apolar fragments of its constituent ions governs these interactions and thus determines the properties of the IL (e.g., melting point, viscosity, density, polarity, hydrophilicity, and solubility) and its suitability as an extraction solvent. Here the properties of ILs most relevant to LLE are discussed in terms of their impacts on the design of solvent extraction systems for metal ion separations.

18.2.1 Viscosity and Density

As a family, ILs are, with few exceptions, significantly more viscous than typical molecular solvents. Although reported IL viscosities span a wide range (from around 10 cP to well over 1000 cP), values on the order of 40–80 cP are more typical, higher

than those of common molecular solvents employed for extraction such as dodecane (1.4 cP) or *n*-octanol (7.4 cP). For ILs, the cohesive forces and thus viscosity generally increase with the charge of the constituent ions and their coordinating ability and decrease with added solutes that disrupt interionic interactions, especially water [54, 55]. Their densities, however, change modestly with dissolved water. A change in 0–10 mol% water results in approximately 0.20% reduction in the density of typical ILs [54]. In contrast the same change in water content can lower viscosity of an IL by roughly 10%–30%. While viscosity and density data for ILs vary greatly with structure (and even between measurements by different researchers), these properties limit their applicability to industrial-scale metal ion solvent extraction for several reasons. First, higher viscosities often translate to more difficult dispersion and slower phase transfer kinetics. However, as long as the IL does not form an emulsion in turbulent contact with an aqueous phase, greater cohesive forces will result in faster coalescence and phase disengagement. Secondly, equipment for solvent extraction has been designed for low-viscosity molecular solvents and those with densities less than water. Therefore, to utilize most ILs for large-scale metal ion separations, higher pressures would require more powerful pumps, more durable components, and significant increases in energy use. Considering the vast catalog of ILs, it is possible that some of these limitations could be overcome by structural variations. Some exceptions to high IL viscosity exist, such as those based on the tetraisothiocyanatocobaltate(II) anion. For example, $[C_2C_1im]_2[Co(NCS)_4]$ exhibits an astonishingly low viscosity of only 0.07 cP [56]. Accurate prediction of their physical properties based on structure would represent a major step toward the realization of the potential of ILs as designer solvents. Recent advances in molecular dynamics and in the computation of structure-property relationships may soon bring this goal within reach [57, 58].

18.2.2 Polarity

The chemical properties of solvents most important to the partitioning of solutes in LLE are those that underlie solubility and solvation, and polarity/polarizability and electron pair-donating/electron pair-accepting abilities are central to these processes. Perhaps the most ubiquitous measure of solvent polarity is the dielectric constant (ε_r), values of which for molecular solvents are listed in numerous references. It is important, however, to consider solvent structure when attempting to define polarity. For ILs the convenience of this parameter is outweighed by its ineffectiveness as a means to describe the sum of the intermolecular interactions that provide the basis of polarity. That is, many models that consider ε_r for molecular liquids rely on the assumption that they are continuous dielectric media. This is not the case for ILs, however, where the interdependence of separate species (i.e., cation and anion) with both polar and nonpolar moieties (for most ILs) results in mesoscopic ordering that tends

toward long-range self-assembly of charged/polar and nonpolar nanodomains, not ion pairs [59–61]. For aprotic ILs, aggregation into nanodomains has been described as micelle-like, where the charged head groups assemble, expelling the hydrophobic alkyl chains and forming semispherical reversed micelles [62, 63]. In any case, distinct ordering in ILs plays a role in phase behavior and influences solvation and therefore affects metal ion partitioning mechanisms and both extractant and metal complex solubility.

Although several studies have reported dielectric constants (ε_r) for ILs and these generally follow the expected trends in cation/anion hydrophobicity, they are significantly fewer in number than the catalog of ε_r values for molecular solvents. The most notable scales for ILs are based on chromatographic solvation parameters [10] and the behavior of test solutes [64]. Studies of the solvatochromic behavior of ILs and octanol-water partition coefficients of molecular solutes have reported IL polarities that are in a range from those of short-chain alcohols to polar, aprotic solvents such as acetonitrile (i.e., $\varepsilon_r \approx 10$–40) [32, 64–68]. Best practice, however, is not to rely on a single probe to evaluate bulk properties of these complex liquids; rather, useful information can be derived from a combination of techniques.

18.2.3 Solubility and solvation

The unique liquid structures of ILs complicate efforts to deduce their bulk properties from first principles. In molecular dynamic simulations the liquid state of ILs has displayed distinct polar and apolar nanodomains in dynamic equilibrium [61, 69, 70]. These theoretical results confirm considerable experimental evidence of amphiphile self-assembly [71–74]. As a consequence of this unusual liquid structure, ILs display a dual solvating behavior toward molecular species and ions [75, 76]. Moreover, on account of the delicate interplay among cations, anions, and water molecules, the nanodomains of wet ILs have been shown to sustain very high water contents [61, 70, 77]. The state of water in aprotic ILs is quite unusual [78], as water tends to solvate the anion preferentially [70, 73], and this has been implicated in the solvation of metal ion complexes in solvent extraction [79]. This is also confirmed in the solvation of multivalent metal ions, as Eu(II) can be stabilized in ILs [80], while it is very sensitive to water in molecular solvents.

Mutual solubility of the two immiscible phases is a critical parameter in solvent extraction. Practically speaking, IL water solubility can be adjusted from completely miscible to sparingly soluble by changing the anion from a highly coordinating, soluble halide to a weakly coordinating, hydrophobic anion such as bis(trifluoromethylsulfonyl)imide $[Tf_2N]^-$. To a significant degree the hydrophobicity of the IL can also be modified by the extent and functionality of cation substitutions. Several studies have shown that the water solubility of a variety of hydrophobic ILs is directly proportional to the IL cation-anion interactions and

linearly correlated with the hydrophobicity of the cation (i.e., size, alkyl chain length, and polarity of substituents) for a given anion [81, 82]. In general a combination of relatively low polarity and comparatively weak coordination between constituent ions renders many ILs immiscible with water and solvents of lower polarity (e.g., alkanes, ethers, and some aromatic hydrocarbons). When mixed with water, many aprotic ILs will behave as a liquid precipitate, like insoluble solid salts. In that respect, ILs are suitable for LLE of metal ions from aqueous solutions. However, their polarity can make ion-pair dissolution in water quite favorable [83]. Dissolution of significant amounts of the IL in the aqueous phase contaminates the feed in subsequent stages and results in the loss of valuable solvent. Likewise the IL cations and anions are prone to ion-exchange processes, which can transfer significant quantities of the IL ions to the aqueous phase (see Section 18.3). Given favorable conditions (e.g., ions with large, negative hydration enthalpy will exchange for those with lower ΔH_{hyd}), ion exchange can dominate phase partitioning behavior. Furthermore, dissolved ILs are difficult to remove and recover from water [84], and this poses a challenge to early and persistent views that ILs are innately "green" solvents. Therefore limiting water solubility is an important parameter in the design of an IL, if it is to be employed in solvent extraction from aqueous media. This can be accomplished by considering the hydrophobicity of the constituent ions.

On the other hand, large fractions of dissolved or entrained water in the IL phase can affect its bulk properties and even change its volume and density to an extent that inverts the phases upon coalescence. Phase inversion is obviously unacceptable in an automated solvent extraction system. Moreover, even small amounts of dissolved water in the organic phase can result in only partially dehydrated complexes, which limit the solubility of many metal ion complexes in nonpolar solvents [85]. Nonetheless, as the historical use of halometallate-based ILs demonstrates, charged, hydrated metal ion complexes may be quite soluble in ILs, albeit to a lesser extent in more hydrophobic ILs.

Innovation in transition metal catalysis has been a major driver in the study of metal ion complex solubility in ILs. Understanding metal speciation in ILs is also of central importance to those interested in utilizing the unique properties of ILs in solvent extraction. In general, metal salts are poorly soluble in ILs, but dissolution of metal ions can be improved by complexation and interactions in the outer-sphere solvation environment of suitable ligands. From the earliest work on low-melting chloroaluminate salts by Hurley and Weir [6] in the 1940s and 1950s and chlorocuprate salts by Yoke in the early 1960s [86], it was clear that Lewis acid-base chemistry could be utilized in directly solvating metals in eutectic melts comprising coordinating anions. Aqueous conditions (e.g., acidity and the presence of anionic ligands) and the nature of the solvent (e.g., polarity/hydrophobicity, hydrogen-bonding ability, and π-charge interactions) can be adjusted to maximize the stability and solubility of a given complex. In traditional solvent extraction systems, the application of

mineral acids and salts is a common way to adjust conditions to and from soluble metal ion complexes for extraction and recovery. Similarly, in IL-based systems, the judicious choice of acid and salts can promote the salting-out of the IL from the aqueous phase. This follows the same principle as the metathesis of a halide salt to a hydrophobic IL, which precipitates from an aqueous solution as a liquid phase. Predictably the effects of acids and salts on IL solvent behavior follow a Hofmeister trend [87]. In fact, there are numerous reports of IL/aqueous biphasic systems comprising relatively hydrophilic ILs with a high ionic strength aqueous phase [88]. Additional factors, however, are involved with the presence of significant quantities of salts in IL-based systems that do not often appear in those with nonionic solvents. Namely, ionic components in the aqueous phase can influence the extent of ion exchange and protonic speciation of extractants and protic ILs. Therefore these components can lead to complex equilibria that must be understood to effectively utilize them and avoid solvent loss.

Solvent extraction is a very sensitive technique to investigate solution thermodynamics, as a relatively small change in free energy (~0.2 kJ/mol) corresponds to a readily measureable (10%) change in the solute distribution ratio, D. This sensitivity lends itself to exploration of outer-sphere coordination effects in LLE. Although explicitly considering activity and ionic strength corrections has proved to be very successful in molecular solvents by the use of the SIT approach [89, 90], in view of the significant solubility of molecular and ionic species in ILs and the numerous theoretical unknowns that arise, various assumptions must be made to model these systems. For example, the use of conditional equilibrium constants in modeling has been shown to work reasonably well [91].

18.3 ILs as Diluents

18.3.1 Cation exchange and ion-pair extraction

Arguably the first report on LLE of a metal ion into an IL was in 1999, when Dai et al. [92] observed unexpectedly high distribution values for the extraction of Sr(II) from water to hydrophobic 1,3-dialkylimidazolium-based ILs containing dicyclohexano-18-crown-6 ether (a neutral extractant hereafter referred to as DCH18C6). The highest D_{Sr} values exceeded those in similar systems comprising even the most efficient conventional organic solvents (i.e., *n*-alcohols) by as much as two orders of magnitude, suggesting that an IL could be a direct replacement for a molecular solvent. Subsequent studies by Rogers and others using similar extracting phases across a range of acidities indicated that the trends in extraction were more complicated than those observed with molecular diluents [93, 94]. During these experiments, it was also found that ILs of the fluorinated anions PF_6^- and BF_4^- hydrolyze in acidic media to form HF [95], which severely limits their utility in metal ion extraction systems. Several systematic studies

by Dietz et al. [96, 97] identified another cause for concern: that the extraction behavior observed by Dai [92] and Rogers [93] is attributable to ion exchange between the cationic component of the IL and a cationic 1:1 metal ion-DCH18C6 complex. Subsequent work by Dietz [98] and others has demonstrated that such cation exchange processes will predominate when a neutral extractant is employed and coextraction of the aqueous anion to promote neutral ion-pair extraction is unfavorable. Cation exchange as a mode of extraction is clearly undesirable for practical solvent extraction systems, as the optimization of extraction efficiency results in concomitant loss of the solvent and contamination of the aqueous phase. Therefore considerable effort was undertaken to discern the factors that influence the balance between metal ion extraction pathways using IL solvents.

Analysis of the trends observed in Fig. 18.2 indicates that cation exchange (decreasing D_M with increasing [HNO_3]) is favored by the use of relatively hydrophilic IL cations (e.g., $C_nC_1$im, where $n \leq 6$), low acid concentrations, relatively hydrophobic IL anions, and acids or salts with relatively hydrophilic anions (e.g., HCl) [99–103]. In addition to Sr^{2+}, the original paper [103] reports similar acid

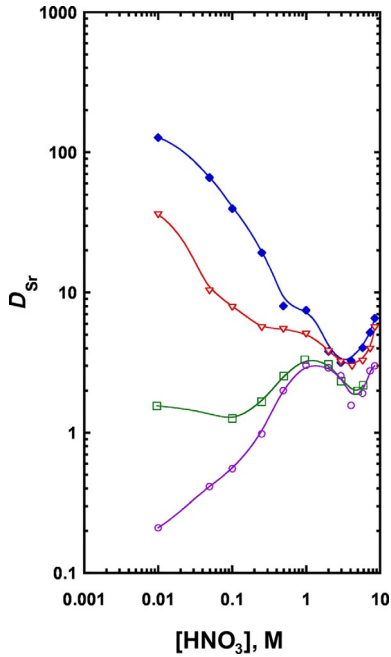

Fig. 18.2 Effect of nitric acid concentration on the extraction of Sr^{2+} by DCH18C6 (0.10 M) in [C_5C_1im][Tf_2N] (*solid diamonds*), [C_6C_1im][Tf_2N] (*open triangles*), [C_8C_1im][Tf_2N] (*open squares*), and [$C_{10}C_1$im][Tf_2N] (*open circles*). *(Reproduced from Hawkins CA, Garvey SL, Dietz ML. Structural variations in room-temperature ionic liquids: influence on metal ion partitioning modes and extraction selectivity. Sep Purif Technol 2012;89:31–38, with permission from Elsevier. Copyright (2012).)*

dependence for extraction of Ca^{2+} and Ba^{2+}, where comparison amongst the three demonstrates trends toward cation-exchange with more polarizable (i.e., lower Lewis acidity) metal ions.

The resulting equilibrium model for 1:1 metal ion extraction into an IL by a neutral extractant (L) includes neutral complex/ion-pair extraction (NCE/IP, Eq. 18.1), where X^- is the coextracted aqueous anion, and cation exchange (IX-1, Eq. 18.2), where C^+ is the IL cation that participates and A^- is the IL anion. Note that those species that exist in the aqueous phase are without designation:

$$M^{n+} + nX + L_{org} \rightleftharpoons M(X)_n \cdot L_{org} \tag{18.1}$$

$$M^{n+} + L_{IL} + n[C^+][A^-]_{IL} \rightleftharpoons [M^{n+}(L)][A^-]_{n\,IL} + n[C^+] \tag{18.2}$$

Cation exchange (IX-1) is a frequently observed partitioning mechanism in IL-based metal ion extraction systems in which the IL is used as a solvent for the extractant. In many cases, authors simply report that IX-1 is present and do not quantify its contribution to D_M or suggest ways to mitigate it. To avoid decomposition of the IL through the IX-1 pathway, alkyl chain lengths of the IL cations can be increased, rendering the cations increasingly hydrophobic and more difficult to transport into aqueous phases [98]. However, this approach alone leads to a substantial reduction in extraction efficiency, most notably at HNO_3 concentrations of practical significance (i.e., 1 and 3 M), as can be seen in the $[C_{10}C_1im][Tf_2N]$ trace (open circles) in Fig. 18.2. Moreover, due to their bulk, cations of greater hydrophobicity inevitably lead to less favorable physical properties, particularly higher viscosity and melting point. One noteworthy effort to suppress the IX-1 mechanism and to promote NCE/IP, described by Janssen et al. [104], exploits differences in the relative hydrophilicity of anions. Specifically a system comprising 18-crown-6 in $[C_nC_1im][Tf_2N]$ ($n = 4$, 6, and 8) has been shown to extract Sr^{2+} from aqueous solutions of $SrCl_2$ to which significant quantities of $LiTf_2N$ are added. In this case, instead of IX-1, strontium extraction occurs via coextraction of Tf_2N^- (leaving Cl^- as a spectator ion). Strontium loadings of up to 80% are achievable, even at neutral pH. Although this approach requires an extra reagent, it does demonstrate that neutral ligands can be employed for extraction into an IL without significant loss of the solvent.

The model specific to extractants having an affinity for acid also includes a third pathway for metal extraction, referred to as "extractant-mediated" cation exchange (Eq. 18.3, IX-2). This mode arises from the fact that DCH18C6 in an organic phase forms acid complexes. That is, IX-2 presents as the IL phase containing the crown ether is conditioned with acid prior to extraction of the metal ion. It differs from acid extraction competition observed in molecular solvents. Namely, in systems comprising DCH18C6 in *n*-octanol, this conditioning step forms neutral nitric acid adducts, whereas in an IL solvent, a charged hydronium-crown ether complex is observed [105]. While acid extraction is not unique to DCH18C6, it is defined here specifically for a crown ether. To maintain electroneutrality in partitioning of a multivalent ion,

Eq. (18.3) suggests that at least two $H_3O^+(CE)$ complexes must be decomposed for every metal ion extracted. This process could be thermodynamically unfavorable for multivalent metal ions, as it would generate free extractant molecules:

$$M^{n+} + nH_3O^+(CE)_{IL} \rightleftharpoons [M(CE)]^{n+}{}_{IL} + nH_3O^+ + (n-1)CE_{IL} \tag{18.3}$$

To whatever extent it plays a role in the balance of pathways, current understanding of the IX-2 equilibrium does not assert a definite mechanism or preclude the participation of aqueous anions to form extracted complexes of lower charge. Indeed, models by Billard and others [106] have indicated that the speciation of the metal ion complex (i.e., cationic complexes with the aqueous anion) could account for the trends observed in Fig. 18.2.

18.3.2 Anion exchange

Formation of cationic metal ion complexes in the IL phase, driven by cation exchange of IL^+, is well established as a general phenomenon. Therefore, it does not come as a surprise that certain conditions can promote the extraction of *anionic* complexes via anion exchange. A general equilibrium for the formation of an anionic metal complex in the IL in 1:1 exchange for an IL anion is written in Eq. (18.4), where L is an anionic extractant:

$$M^{n+} + (n+1)L_{IL}^{m-} + [C^+][A^-]_{IL} \rightleftharpoons [C^+][M(L_{n+1})^{m-}]_{IL} + [A^-] \tag{18.4}$$

The tendency of anions to exchange in an aqueous/organic biphasic system typically favors partitioning of the most hydrophobic anions (i.e., lowest hydration enthalpy) toward the IL phase. A general prediction for the tendency to promote anion exchange in both IL synthesis and metal ion extraction follows a Hofmeister series: $SO_4^{2-} < Cl^- < Br^- < NO_3^- < I^- < ClO_4^- < SCN^- < BF_4^- < PF_6^- < Tf_2N^-$ [87, 107]. Anion exchange for metathesis of one IL to another anionic form is, therefore, influenced largely by hydration and mass action. However, for metal ion extraction, this propensity is also influenced by the free energy of anionic metal ion complex formation and solvation. This case was clearly made by Jensen et al. [108] in the extraction of trivalent lanthanides (Ln(III)) by the fluorinated acidic extractant 2-thenoyltrifluoroacetone (Htta) into $[C_4C_1im][Tf_2N]$, where the ion-pair complex $[C_4C_1im][Ln(tta)_4]$, exchanged for the relatively hydrophilic Tf_2N^-, is supported by a fourth-power pH dependence and complete dehydration of the inner coordination sphere. Although this process incurs the loss of IL anion to the aqueous phase, because of the steep acid dependence, the Ln(III) and the IL are, in theory, both recoverable by treatment of the postextraction IL phase with the conjugate acid HTf_2N. Interestingly, by changing the IL anion to nonafluoro-1-butanesulfonate, the extracted Ln(III) complexes are 1:2 and 1:3 with increasing Htta, and they are completely hydrated at low Htta concentration. In the hydrated and 1:2 complex, the dominant partitioning mechanism tends to be IX-1 [109].

In contrast to most molecular solvents, an IL can solubilize significant quantities of acid, which will affect the extent of ion exchange. For example, a 1:1 volume contact of pure $C_4C_1imTf_2N$ with an initial aqueous HNO_3 concentration of 7.4 M was found to dissolve 1.6 M HNO_3 in the IL phase [91]. Billard et al. found that the U-shaped curve in the extraction of U(VI) into this IL containing TBP extractant with varying nitric acid concentration could be explained by a threefold mechanistic model. The model includes cation exchange of $[UO_2(TBP)_2]^{2+}$ for H^+ and $C_4C_1im^+$, neutral extraction of $UO_2(NO_3)_2(TBP)_2$, and anion exchange of $[UO_2(NO_3)_3(TBP)_2]^-$, as HNO_3 concentration increases. The role of aqueous acid and ionic species on the IL phase behavior was explored, and it was found that TBP does not extract the acid. Instead, H^+ is dissolved in the IL, and another acid cation exchange mode (i.e., distinct from IX-2) was established, involving the transfer of a cationic complex for dissolved H^+. This work emphasizes the importance of considering the role of all species in a system through not just partitioning experiments but also spectroscopic data and computational modeling, if necessary.

18.3.3 Consideration of extractant solubility

Because simple metal salts are poorly soluble in noncoordinating ILs, in systems where hydrophobic ILs are employed as diluents, the solubility of the extractant is a major factor limiting capacity. The solubility of both the extractant and its metal ion complex(es) can be increased by rendering them more lipophilic. That is, given their amphiphilic nature, hydrophobic IL diluents can screen the polarity and charge of donor atoms and ions while solvating the metal ion complex in a water-immiscible environment. However, hydrophobic ILs tend to self-assemble into polar, charged domains and hydrophobic domains, and solubility in these phases can be difficult to predict [61]. This is especially the case when the water content of the IL phase is appreciable, as the structure may become even more segregated. In some cases, in fact, approaches to extraction based on the cloud point, which take advantage of the amphiphilic behavior of the IL [87], may be worth consideration. The solubility of an extractant in an IL is also influenced by the molar volume of both the extractant and the diluent. In fact, with increasing molecular size and concentration of extractant, the definition of solvent and solute can become ambiguous, limiting the extractant solubility [109a].

18.4 ILs as Extractants

As ILs typically incorporate noncoordinating anions, it was initially assumed that significant extraction into an IL would not be possible without the addition of a ligand. However, data from Dai et al. [92] in the extraction of Sr^{2+} by DCH18C6

in $C_4C_1imPF_6$ indicated that extraction could be as high as $D_{Sr} = 0.89$ ($E_{Sr} = 47\%$) without the extractant present. Given the structural diversity of ILs (already noted), such nonnegligible extraction into an aprotic IL suggests that many pure, unmodified ILs could act as both solvent and extractant. In fact, one of the most commonly employed liquid anion exchangers, Aliquat 336, is now recognized as an IL [110]. Thus, ILs have actually been unknowingly employed as the basis for solvent extraction for many decades [111, 112], albeit as extractants in molecular solvents. Aliquat 336 is sold commercially as a mixture of trioctylmethyl- and tridecylmethylammonium chloride or nitrate. In this context, it is worth surveying the influence of halometallate chemistry on solvent extraction in IL-based media.

18.4.1 Liquid anion exchangers

Although the composition and stability of many 3d metal halometallate complexes under the widely varying conditions of IL-based solvent extraction are somewhat uncertain, halo- and pseudohalometallate complexes of 4/5d and 4/5f elements can be readily formed in pure ILs [113–116]. Recently a variety of reagents, not unlike Aliquat 336 but specifically designed to be ILs, have been investigated for use in solid-supported LLE, namely, as the basis for extraction chromatographic (EXC) stationary phases. Of these, trihexyl(tetradecyl)phosphonium chloride (available commercially as "Cyphos 101") has undoubtedly been the most widely employed, due in large part to the type of reaction responsible for extraction. That is, Cyphos 101 (and many other ILs) typically extracts metals from halide media as their halometallate complexes. This stands in contrast to neutral organophosphorus reagents, which tend to extract metal ions from halide media as either anionic halide complexes or solvated ion pairs [117]. Guibal, Navarro, and coworkers have described the retention behavior of a series of metal ions (e.g., Hg^{2+}, Zn^{2+}, Fe^{3+}, and Au^{3+}) on EXC resins prepared from Cyphos 101 and Amberlite XAD-7 [118–125]. In these systems in which metal ion uptake was examined via batch uptake experiments, sorption (i.e., extraction) was dominated by the exchange of an anionic metal chlorocomplex for an equivalent of chloride ions transferred to the aqueous phase. This extraction processes can be expressed by the general reaction in Eq. (18.5):

$$MCl_x^{n-x} + y[C^+][Cl^-]_{IL} \rightleftharpoons [C^+]_y\left[MCl_z^{n-x}\right]_{IL} + w\,Cl^- \tag{18.5}$$

where MCl_x^{n-x} (n = charge on metal ion) is the predominant species in aqueous solution, C^+Cl^- is the IL, $[C^+]_yMCl_z^{n-x}$ is the adsorbed species, and the number of exchanged chlorides (w) is equivalent to the charge of the extracted complex.

The variety of metal ions that can be efficiently extracted using this anion exchange mechanism demonstrates the applicability of this chemistry to a wide range of metals. In fact, reaction (18.5) is the basis for several other extraction

and separation systems not utilizing halides. In the system comprising high concentrations of HNO_3 and $[C_4C_1im][Tf_2N]$ or $[C_8C_1im][Tf_2N]$, for example, slope analysis indicates that the separation of Pu(IV) from Am(III) and U(VI) is enabled by more favorable formation of $[Pu(NO_3)_5]^-$ and $[Pu(NO_3)_6]^{2-}$ in exchange for Tf_2N^- between 3 and 5 M nitric acid [126]. As a function of D_{Pu} versus $[Tf_2N^-]$ at 3 M HNO_3, the bilogarithmic slope of -1.4 suggests partitioning of a combination of these species into $[C_8C_1im][Tf_2N]$. However, these results do not preclude the presence of other competitive mechanisms, such as one involving the exchange of dissolved H^+.

18.4.2 Task-specific ILs

Task-specific ionic liquids (TSILs, Fig. 18.3) represent an increasingly important subset of ILs comprising a conventional IL to which is attached a functional group chosen to introduce specific physicochemical, catalytic, or solute-binding properties into the solvent [28]. First described by Davis et al. more than two decades ago [29], TSILs now encompass a large and ever-expanding family of solvents with demonstrated utility in synthesis [127], catalysis [128], and chemical separations [129], among other areas.

As applied to the separation of metal ions, the use of TSILs most often involves LLE. As noted elsewhere, the use of ILs, either conventional or task-specific, represents an effort to avoid adverse environmental impact associated with the use of conventional organic extraction solvents [130]. While all ILs offer decreased fugitive emissions (due to their near absence of vapor pressure), task-specific ILs offer the additional possibility of reducing solubilization losses of the extractant and/or IL constituents to the aqueous phase. In addition, by relying on an extractant *bound to* rather than *dissolved in* the IL, TSILs offer a means of eliminating the problem of inadequate extractant solubility that plagues some conventional ILs [131].

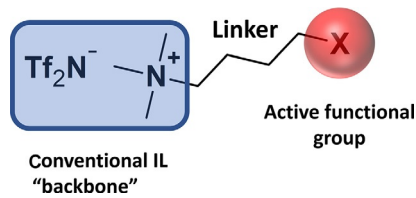

Fig. 18.3 Generalized structure of a task-specific IL, comprising the cationic and anionic constituents of the IL "backbone," the functional group responsible for carrying out the desired "task" (e.g., extraction of a metal ion), and a linker joining this group to the IL backbone.

Although in principle, either the TSIL cation or anion may incorporate the functional group, the vast majority of TSILs incorporate functionalized cations, a consequence of the comparative ease with which active groups can be incorporated into the cation. To date, task-specific ILs involving attachment of a functional group to all of the most widely known families of IL cations, including pyridinium [132], piperidinium [133], pyrrolidinium [133], and quaternary ammonium ions [134], have been described, but TSILs based on imidazolium cations remain the most common [135]. In fact the first report of a TSIL incorporating a metal ion-complexing moiety, a 2001 study by Visser et al. [136], involved an imidazolium cation to which a urea, thiourea, or thioether functional group was appended. A combination of the resultant cations (examples of which are shown in Fig. 18.4) and hexafluorophosphate anion (PF_6^-) yielded viscous liquids capable of serving as both a water-immiscible solvent and an efficient extractant for Hg^{2+} and Cd^{2+} ions. Since this first report, a number of studies have appeared describing the synthesis, characterization, and application of TSILs based on imidazolium or other IL cations to which any of a wide variety of functional groups capable of interacting with metal ions have been appended [135]. Curiously, despite the substantial effort in this area, relatively few studies have specifically considered the fundamental mechanistic aspects of metal ion extraction by TSILs, and studies directly comparing TSILs with their conventional counterparts (i.e., IL as diluent) are unexpectedly sparse.

As already noted, the mechanism by which a given metal ion is transferred into a typical IL phase in the presence of an extractant often differs markedly from that observed for a molecular solvent under the same conditions. As described in Section 18.3.1, extraction into an IL is often a composite of both neutral complex/ion-pair extraction (NCE/IP) and one or more ion-exchange (IX) processes, frequently involving constituents of the IL. The relative contribution of these processes to the overall partitioning is determined by characteristics of the metal ion [103] and the extractant, the composition of the aqueous phase [102], and the nature of the IL itself [98, 137, 138]. Given that in a TSIL the extractant molecule is tethered to the IL "backbone," it is not unreasonable to expect that the relative importance of the possible partitioning modes (i.e., NCE/IP and IX) might be affected. In addition,

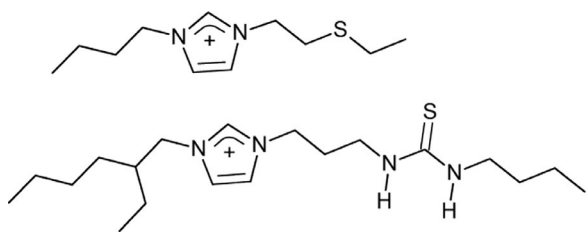

Fig. 18.4 Thioether-, urea-, and thiourea-functionalized imidazolium cations.

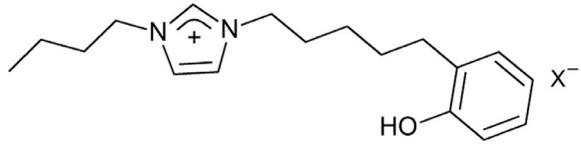

Fig. 18.5 1-Butyl-3-[3-(2-hydroxybenzoamino)propyl]-3*H*-imidazol-1-ium TSILs (with X as either hexafluorophosphate (PF_6^-) or *bis*[(trifluoromethylsulfonyl)]imide (Tf_2N^-)).

extraction pathways unlike those seen for conventional ILs might emerge. In considering what has actually been observed, it is important to point out that with few exceptions, TSILs are often extremely viscous [135], so much so, in fact, that they are nearly always diluted with a conventional IL prior to use. Accordingly, studies of metal ion extraction into undiluted TSILs are quite limited.

In 2006, Ouadi et al. [139] published one of only a handful of fundamental studies of the LLE of a metal ion by a task-specific IL in which the TSIL was employed in undiluted form. Specifically the extraction of Am(III) with an IL incorporating an imidazolium cation onto which a hydroxybenzyl-amine moiety had been grafted (Fig. 18.5) was examined. Modeling of the extraction data suggested that Am partitioning occurs via an anion exchange process involving an anionic Am complex and the $[Tf_2N]^-$ component of the IL, an observation having obvious negative implications for the application of the TSIL in LLE.

Along these same lines, Egorov et al. [140] prepared trioctylmethylammonium salicylate (TOMAS), a TSIL in which one component comprised the anionic form of salicylic acid (Sal), which is known to form extractable complexes with various transition metal ions. While highly viscous, the TSIL was nonetheless employed in undiluted form to extract Fe^{3+} and Cu^{2+} from aqueous solution. A detailed investigation of the mechanism of iron partitioning found that it involves ion-pair extraction of a cationic 1:1 iron salicylate complex with bisulfate ion, eventually yielding $[TOMA^+][FeSal_2]^-$ in the IL phase. While interesting from a fundamental point of view, from a practical perspective, this result is most notable for the absence of ion exchange-induced losses of the IL components, with the overall extraction process being represented by the following:

$$\begin{aligned} Fe^{3+} + 2[TOMA^+][HSal^-]_{IL} + HSO_4^- \rightleftharpoons [TOMA^+][FeSal_2]_{IL}^- \\ + [TOMA^+]\left[HSO_4^-\right]_{IL} + H^+ \end{aligned} \tag{18.6}$$

More recently, Biswas et al. [141], employing a related derivative of Aliquat 336 (designated TOMAHP, for trioctylmethylammonium hydrogen phthalate) in which hydrogen phthalate anion served as both an IL constituent and the active functional group, examined the extraction of U(VI) and Th(IV), along with Fe(III) and several

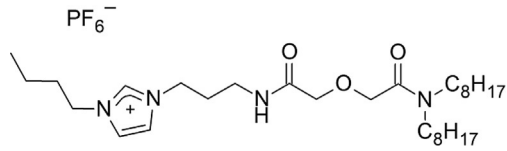

Fig. 18.6 Diglycolamide-functionalized imidazolium-based ionic liquid (DGA-TSIL).

trivalent lanthanides, from aqueous mineral acid solutions (e.g., HNO_3 and H_2SO_4). The uranium partitioning data were shown to be consistent with a mechanism involving exchange of the uranyl cation (UO_2^{2+}) for the hydrogen ion of the IL anion:

$$UO_2^{2+} + 2[C^+][HA^-]_{IL} \rightleftharpoons [C^+]_2[UO_2(A)_2]_{IL} + 2H^+ \qquad (18.7)$$

where HIL represents the TOMAHP. Although extraction also occurs on the basis of an ion-exchange process in this system, catastrophic loss of the IL components (and the concomitant destruction of the TSIL) is again avoided.

Less encouraging are the vast majority of other LLE studies employing TSILs, which address the issue of their high viscosity by diluting the TSIL with an analogous IL. Recent work by Sengupta et al. [142], which examined the application of TSILs based on diglycolamide-functionalized dialkylimidazolium cations (DGA-TSIL, Fig. 18.6) in the LLE of UO_2^{2+}, NpO_2^{2+}, PuO_2^{2+}, Np^{4+}, and Pu^{4+}, is illustrative of the nature of the results obtained. Because of the high viscosity of the DGA-TSIL and the accompanying slow extraction kinetics, it was dissolved in any of several analogous dialkylimidazolium-based ILs incorporating the bis(trifluoromethylsulfonyl)imide anion, $[C_n mim^+][Tf_2N^-]$.

In all cases the extraction was found to proceed by an IX-1 mechanism, in which transfer of the metal ion into the IL phase is accompanied by the loss of the cationic component of the diluent. Further investigation revealed the extracted species to be a cationic 1:1 metal/DGA-TSIL complex, regardless of metal ion. In this respect then, these systems are similar to those involving the extraction of alkali and alkaline earth cations into conventional ILs by macrocyclic polyethers (described earlier), where the formation and transfer of cationic 1:1 metal-extractant complexes underlie the propensity of the systems toward ion exchange [96]. When considered together with dozens of related studies of the LLE of metal ions by TSILs incorporating any of a variety of extracting moieties into an IL cation [135], it is clear that TSIL-based extraction systems, like their conventional analogs, are plagued by undesirable ion-exchange processes. Although this might be acceptable if TSILs were to provide greatly improved extraction efficiency or selectivity, the superiority of TSILs over systems in which an extractant is simply dissolved in a conventional IL has yet to be consistently demonstrated.

18.5 Conclusions

It is clear from the foregoing that certain of the physical properties of ILs, in particular their high viscosity, pose significant challenges to the design of workable LLE schemes. Similarly, the propensity of ILs to participate in various ion-exchange processes often renders IL-based LLE systems susceptible to substantial solvent losses to the aqueous raffinate. Taken together, these and other difficulties (e.g., complications in stripping/recovering extracted metal ions) [143] have meant that efforts to replace conventional organic diluents with ILs in LLE have, thus far, proved largely unsuccessful. Going forward then, it is important to ask what, if anything, can be done to change this.

In part the answer is readily apparent. That is, without an improved understanding of the fundamental aspects of the metal ion transfer process between aqueous solution and ILs, including such areas as metal ion speciation in ILs, the thermodynamics of metal complex formation, surface and interfacial phenomena in IL-based LLE systems, and the kinetics of metal ion transfer, progress in the application of ILs to metal ion separations is certain to be slow. The greater challenge, however, may lie in devising creative approaches to exploiting this improved understanding. That is, the use of ILs to directly replace molecular organic diluents in an established LLE process, which has long been the primary focus of efforts involving ILs and LLE, represents only one (and by far, the least imaginative) means of applying these solvents. In fact, this approach generally fails to exploit most of the properties that distinguish ILs from conventional solvents. For example, a number of ILs are immiscible not only with water but also with various organic solvents. This raises the possibility of employing an IL not as a substitute for a conventional organic phase, but rather as a replacement for the *aqueous* phase. Yet, aside from a few reports, most notably that of Shkrob et al. [144] in which an organic immiscible TSIL was used as a replacement for the aqueous phase in a LLE system designed to mimic the well-established TALSPEAK process, little effort has been devoted to ILs as aqueous phase substitutes. Along these same lines, perhaps the propensity of ILs for ion exchange should be embraced, not avoided. With this in mind, it has been suggested [145] that ILs be employed *in combination with* (rather than as a replacement for) conventional organic diluents, with the objective of simultaneously overcoming some of the limitations of ILs (e.g., high viscosity) and exploiting IX to enhance metal ion extraction efficiency and selectivity. In fact, certain preliminary results [146] indicate that this seemingly heretical notion has merit. Continuing in the same vein, some TSILs can apparently serve as stripping agents [147], perhaps offering a means to overcome the flat [148] or "U-shaped" [149] acid dependencies that characterize the partitioning of many metal ions into ILs and complicate the use of "acid swings" to effect extraction and stripping. More extensive investigation of such

reagents seems well worth pursuing. Finally, some ILs exhibit interesting thermotropic behavior, which may provide a path to addressing the issue of slow mass transfer into and out of viscous IL phases. In work by Nockemann et al. [150], for example, choline bistriflimide was found to form a single phase with water at elevated temperatures ($\geq$72°C), but a biphasic system at room temperature. As applied to separations, this suggests the possibility of using temperature swings to control the distribution of metal ions and thus their separation. Potentially novel approaches to exploiting the capabilities of ILs to improve the LLE of metal ions are not limited to the suggestions outlined here. Rather, they are limited only by the imagination and ingenuity of the separation practitioner.

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Chapter 19

Preanalytical Treatments: Extraction With Deep Eutectic Solvents

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Chapter Outline

19.1 Introduction

In the past decade, deep eutectic solvents (DES) have been recognized as interesting solvents for a variety of applications, together with ionic liquids (IL), such as solvent for chemical or enzymatic reactions, to extract bioactive compounds from various

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materials, and to dissolve macromolecules such as cellulose and lignin [1]. The advantages, associated with green technology, are the high selectivity that can be achieved by engineering the most suited solvent for an application; nonvolatile and nonflammable liquids. All reasons to consider this as green solvents, although they have some drawbacks, like high costs and toxicity of some of their components. Abbott et al. [2] reported a series of deep eutectic solvents based on some carboxylic acids, urea, and quaternary ammonium salts (choline chloride). Based on extensive ^{1}H NMR-based metabolomics studies of plant extracts, Choi et al. [3] found that they always contain large amounts of acids, such as malic acid, and also bases such as choline, usually in about equal molar amounts, which raised the question if nature may rely on ionic liquids for various functions since early stages of life. The first experiment of mixing equimolar amounts of choline chloride and malic acid indeed resulted in an ionic liquid. Also, other common compounds found in all kinds of cells like sugars, polyalcohols, and amino acids had similar molar levels as the mentioned organic acids and bases. Similar observations were made in the ^{1}H NMR metabolomics of microorganisms and mammalians cells. A number of combinations of these compounds were made, and many were found to form liquids in certain molar ratios (Table 19.1).

Based on these findings the following hypothesis was proposed: natural deep eutectic solvents (NADES) act as a third liquid phase in all living cells and organisms [3]. The occurrence of NADES as a third liquid phase can explain many biological phenomena, like the biosynthesis of complex molecules that are neither water- nor lipid-soluble, like the secondary metabolites (e.g., paclitaxel, terpenoids, and flavonoids), or macromolecules, like lignin and cellulose. Indeed, a series of studies on the solubility of secondary metabolites and macromolecules showed that these compounds could be dissolved in NADES. Potentially the NADES might play a role in drought and cold resistance, senescence of metabolism in seeds, resurrection plants, desert plants, and various other organisms. Clear evidence for a role of NADES in plants was provided by direct NMR measurement of nectar. The main ingredients of nectar were found to be sugar-based NADES, which avoids evaporation to dryness and thus protects the flowers from drying up of the nectar.

TABLE 19.1 Classes of Natural Deep Eutectic Solvents Obtained by Mixing Different Classes of Common Primary Metabolites Found in all Kinds of Cells and Organisms

Acid and base, ionic liquids
Polyalcohols, including sugars with organic acids
Polyalcohols, including sugars with organic bases
Polyalcohols including sugars with amino acids
Mixtures of polyalcohols and sugars

In addition to the application as extraction or dissolution solvents, stability of labile metabolites like phenolics and proteins was shown in a number of NADES [3, 4]. NMR studies showed that the NADES are based on strong hydrogen bonding between the molecules, among others, resulting in clear shifts of the proton signals if compared with the individual spectra of the components of NADES in water. By dilution with water the intermolecular interactions gradually decrease, and over 50% water the ^{1}HNMR is like that of an aqueous solution of the NADES components [5]. The combination of water and NADES from a physicochemical perspective is very interesting. For example, when a NADES is prepared by freeze-drying the aqueous solution of the NADES components, usually about 5% (w/w) of water remains, which when converted to molar concentration turns out to be about equimolar to the NADES compounds. This remaining water is impossible to evaporate. Water in NADES could also play a practical role. Most of NADES are highly viscous, but the addition of small amounts of water immediately reduces the viscosity [5] (see later). Some NADES are hygroscopic [6]. Based on all these properties, we consider NADES more as a natural concept to have tailor-made solutions for various biological functions, rather than only consider them as strictly defined deep eutectic mixtures.

Future research on NADES will with no doubt lead to a number of new insights into the role of these mixtures in cells and whole organisms. At the same time, it is obvious that we can learn new things from nature that can be turned into new applications. One of these is the use as green solvents in analytical chemistry. In analytical chemistry, organic solvents are widely used for the extraction of compounds from various matrices. These solvents have a number of limitations, such as high costs, toxicity, sustainability, flammability, and the costly disposal. Here, we will review the possibilities for NADES as a more sustainable approach for extraction.

Here, we review the applications of NADES as preanalytic solvents (extraction, preparation, and/or derivatization). So far, over 200 combinations of natural products have been described as NADES, all with very different physicochemical characteristics. So NADES cannot be considered as universal solvents; in fact, they are highly selective solvents. Therefore, for each method, one has to optimize the method from extract to the final analytic detection. There are some interesting reviews of NADES as extraction solvents published by de los Angeles Fernandez et al. [7], Cunha & Fernandes [8], Espino et al. [9], and Vanda et al. [10].

The goal of this chapter is to give a concise general overview of the potential of NADES as preanalytic extraction solvent for specific analytes and to point out what limitations there are.

Any analytic method starts with the sample collection and sample preparation. In this preanalytic part the extraction is the key factor for a reliable and reproducible analysis. There is a series of requirements for an optimal extraction method. These include the following:

- Extract should be compatible with analysis method
- High and reproducible yield of target compound(s)
  - High selectivity in case of a targeted analysis
  - Low selectivity in case of a metabolomics analysis
- High sensitivity
- Low risks of artifact formation and degradation of compounds
- Stability of extract
- Greenness of the process

Based on these requirements, we will discuss the performance of NADES reported in the past few years. The specific properties of NADES and their advantages and disadvantages will be compared with the classical solvents such as water, alcohols, and other organic solvents like ethers, esters, ketones, dichloromethane, and chloroform.

19.2 Compatibility

Considering the compatibility of NADES with the methods of analysis, there are some limitations that relate to the viscosity and nonvolatility of the NADES. The high viscosity of NADES can be an obstacle in applying the extract on a column in LC and GC or on a TLC plate. The viscosity of some pure NADES is high as can be seen in Table 19.2 [6]. The viscosity of NADES can be reduced by temperature and water content. An increase of the temperature slightly decreases the viscosity of NADES. (Fig. 19.1). A greater effect could be achieved by the addition of water; already a small amount considerably reduces the viscosity (Fig. 19.1), whereas the solubility parameters are not much affected [6]. In fact, to achieve the maximum solubility for a compound, water content and the NADES ingredients themselves should be optimized. For example, a higher water content improved the extraction of rutin (choline chloride-glycerol [1:1] 20% water and choline chloride-1,4-butanediol [1:4] 20% water), quercetin (L-proline-glycerol [2:5] 10% water), vanillin (lactic acid-fructose [5:1] 25% water), amentoflavone (choline chloride-1,4-butanediol [1:5] 35% water), apigenin (choline chloride-1,6-hexadienol [1:7] 30% water; choline chloride-betaine hydrochloride-ethylene glycol [1:12] 20% water), and hyperin (choline chloride-1,4-butanediol [1:4] 30% water) [11–18]. The viscosity problem is in general laboratory practice solved by diluting the NADES extract by the addition of a certain amount of water. Dai et al. [19] used NADES extracts modified with different amounts of water (0%, 10%, 25%, 50%, and 75%) for the extraction of phenolic compounds from *Carthamus tinctorius*. For cartormin and hydroxysaffor yellow A, the highest extraction yield was achieved with 50–100% (v/v) water in sucrose-choline chloride, 25%–50% water in proline-malic acid, and with no addition of water in lactic acid-glucose. For carthamin, the

TABLE 19.2 Viscosity of Some NADES at 40 °C as Reported by Dai et al. [6]

NADES	Composition (Mole Ratio)	Viscosity (40°C) Mm^2/s
MCH	Malic acid-choline chloride-water (1:1:2)	445.9
GlyCH	Glycerol-choline chloride-water (2:1:1)	51.3
MAH	Malic acid-β-alanine-water (1:1:3)	174.6
PMH	Proline-malic acid-water (1:1:3)	251
FCH	Fructose-choline chloride-water (2:5:5)	280.8
XCH	Xylose-choline chloride-water (1:2:2)	308.3
SCH	Sucrose-choline chloride-water (1:4:4)	581
FGSH	Fructose-glucose-sucrose-water(1:1:1:11)	720
GCH	Glucose-choline chloride-water (2:5:5)	397.4
PCH	1,2-Propanediol-choline chloride-water (1:1:1)	33
LGH	Lactic acid-glucose-water (5:1:3)	37
SoCH	Sorbitol-choline chloride-water (2:5:6)	138.4
XoCH	Xylitol-choline chloride-water (1:2:3)	86.1
H_2O	Water	=1

highest extraction yield was reached with 25% (v/v) water in sucrose-choline chloride, 10% water in proline-malic acid, and no water in lactic acid-glucose. Sucrose/choline chloride-based NADES are not only excellent solvents but also the most viscous (Table 19.2). However, dilution with water makes it easier to handle.

In the case of GC sampling, NADES extract would be difficult to handle due to the solvent characteristics (high viscosity and polarity and virtually zero vapor pressure) and the high polarity of the NADES-extracted compounds. To overcome these problems a liquid-liquid partitioning, for example, n-hexane fractionation from NADES extract and a headspace-solvent microextraction (HS-SME) coupled with GC was developed for the analysis of terpenoids, in *Chamaecyparis obtuse* plant material [20]. Farajzadeh et al. [21] reported a liquid-liquid partition approach to get rid of DES-based residues. In this research, some pesticides were used as model compounds. Acetonitrile (disperser solvent) was mixed with DES (choline chloride-4-chlorophenol [1:2]) as the extraction solvent, and the mixture was rapidly injected into an aqueous phase containing the pesticides. A cloudy solution was formed. In order to separate the extractant from the aqueous phase, the cloudy solution was centrifuged, and the sedimented phase was injected into the GC system for analysis. The results showed that this method is rapid, sensitive, and repeatable and can be used for the determination of analytes in different samples.

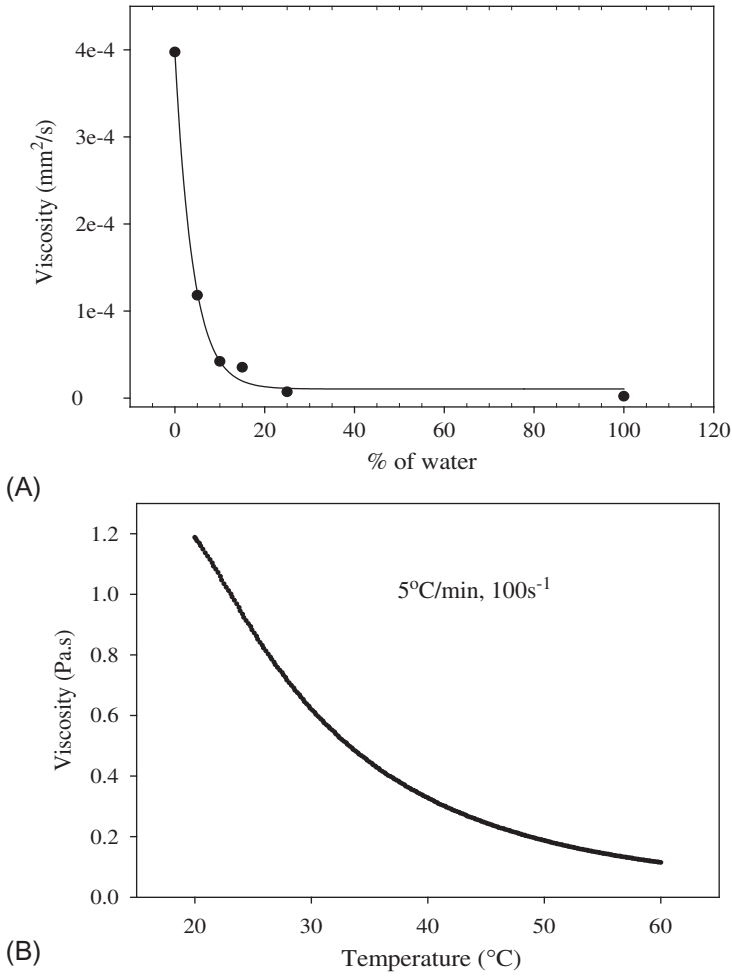

Fig. 19.1 Example of effects of adding water and temperature on viscosity of NADES. Glucose-choline chloride-water (2:5:5). (A) Effect percentage water on viscosity of NADES. (B) Effect of temperature on NADES. *(From Dai Y. Natural deep eutectic solvents and their application in natural products research and development. PhD thesis, Leiden University, Institute of Biology, 2013, 73 p., with permission of the publisher.)*

Solid-phase extraction (SPE) methods have been applied to recover the target compound(s) from NADES. This step could also be used as a way to improve sensitivity by enriching and concentrating the analytes in the extract for the final determinant step, that is, to improve the sensitivity and selectivity of the method [14, 22].

Choi et al. [23] used high-performance thin-layer chromatography (HPTLC) to analyze the efficiency of a diverse set of NADES for the extraction of ginkgolides and ginsenosides from *Ginkgo biloba* and *Panax ginseng*. They first analyzed the NADES extracts directly, without any prepurification steps; the NADES extract was applied onto the HPTLC silica plate and developed with a mobile phase; but the NADES caused severe tailing of spots in all of the systems. To overcome this problem an SPE purification step was developed. Oasis HLB cartridges were used for the isolation of ginkgolides and ginsenosides from the NADES extracts. The NADES components were removed by an initial elution with water after which the compounds of interest were eluted with ethanol from the adsorbent and analyzed by HPTLC. SPE has also been used to recover the NADES. By simply running the threefold water diluted NADES extract of ginseng powder over an SPE column (Waters, Oasis HLB), followed by elution with water, the aqueous eluent can be collected and freeze-dried to yield fresh NADES for extraction [24]. The ginsenosides were isolated from the adsorbent in the next step: elution with ethanol.

A targeted SPE approach obviously does not match with a holistic type of analysis that aims at measuring as many metabolites as possible. For example, for a metabolomics analysis of a broad range of analytes, the direct analysis of NADES extracts by LC and TLC is the preferred approach.

19.3 High Reproducible Yields

Among the advantages of NADES is the high solubility of medium polar compounds that are poorly soluble in both lipids and water. In particular, the secondary metabolites found in most organisms, fall into this category. From studies on extraction, it becomes clear that the extraction conditions for each individual compound needs to be optimized and that none of the NADES are a real universal solvent (see Table 19.3). Though some efforts have been made to set general rules for designing tailor-made solvents for certain targets [31, 36, 37, 39], still a prediction method for a NADES suited for a given target compound is challenging. Numerous factors should be optimized for the prediction, type of solvents, water content, possible hydrogen bonding locations, and influence of external factors (e.g., temperature and pH). Apparently not only polarity is determining solubility, as medium polar compounds not soluble in water are quite soluble in NADES with a similar polarity as water, methanol, and ethanol. The possibility to form hydrogen bonds is thought to play a major role. However, in a system where there is strong hydrogen bonding between the NADES components, the solute must find is place in the molecular network of the NADES. That NADES are quite selective extraction solvent was shown by Dai et al. [19], who tested a large number of NADES for the extraction of *C. tinctorius* flowers.

Due to the difficulties in the prediction of the most suited NADES, identifying the best NADES for an extraction is usually done by screening experiments in which the solubilization of pure target compounds is measured. However, the solubility data do not fully match the best extraction system, as was shown in the case of vanillin [14]. There might be a matrix effect that inhibits the full extraction of some compounds (unpublished results) and also large effect from coextracted compounds. It seems that particularly phenolic compounds can be efficiently extracted by NADES compared with classical organic solvents (see Table 19.3). Recovery of target compounds can be achieved that match or are even better than obtained with classical extractions used as a control [30, 33, 40, 41]. Reproducibility was shown to be good for NADES extraction [25, 27, 28].

To improve the extraction efficiency, microwave- or ultrasonic sound-assisted methods [29, 32, 34, 35, 38, 42] can be used, but high temperatures should be avoided, among others, to prevent caramelization of sugars and reactions between NADES components and compounds in the matrix.

19.4 Sensitivity

Sensitivity is one of the most important issues in analytical chemistry. It depends on several factors, the most important of course being the detection method. If there is an improvement in the extraction step, it is possible to avoid all the problems that might cause lower sensitivity, such as overlapping signals. A preconcentration step is often applied to improve the sensitivity. When using NADES extraction, one needs to include a solid phase (see earlier SPE) or liquid-liquid phase type of preconcentration step, because evaporation of the solvent is not possible. The classical SPE columns, such as C18, various resins, and ion exchangers, are used for this purpose. For example, in the extraction of isoflavones from soy products, the target compounds extracted by NADES were concentrated by SPE and subsequently recovered elution with water (17 mL), ethanol (11 mL), and methanol (1 mL) [40]. For the concentration of phenolic compounds from *C. tinctorius*, a Diaion resin column was used from which the phenolics were eluted by water (66.5 mL), ethanol (325 mL), and methanol (1.5 mL) [19]. In the case of *Ficus carica*, a microporous resin was used for concentration of the furanocoumarines, which were then eluted with water (250 mL) and ethanol (250 mL) [43]. For the recovery flavonoids from NADES extract of *Scutellaria baicalensis* Georgi (Radix scutellariae), a similar method was applied [26]. It is important to mention that Gan et al. [44] used SPE cartridges packed with octadecylsiloxane-bonded silica (ODS), anion-exchange resin (AER), and anion-exchange resin modified with choline chloride-glycerol (1:2) for the extraction of cleistanthol from *Phyllanthus flexuosus* extracts. The results demonstrated that the anion-exchange resin modified with choline

chloride-glycerol (1:2) not only offered higher adsorption capacity for cleistanthol but also exhibited better extraction efficiency of cleistanthol than ODS and AER. This is the first application of a deep eutectic solvent for the modification of AER. Another challenging approach is to combine supercritical fluid technology either in preparation or solvent recovery steps, but it has to be further explored.

19.5 Stability of Extracts

The formation of artifacts is one of the major problems of classical solvent extraction methods [45–47]. This is due to two processes; one based on reaction with the solvents themselves or contaminations in the solvent. Particularly, aldehydes and ketones are known to react with amines and alcohols. The occurrences of peroxides in ethers and phosgene in chloroform are examples of contaminants. Some solvents are even quite reactive, for example, dichloromethane that easily reacts with amines such as in alkaloids. Also, with NADES ingredients such as sugars, amines, and amino acids, there is a possibility of artifact formation with compounds present in the biomaterial. Thus, high temperatures should always be avoided, a precaution that is important for any extraction method. The other cause of artifact formation is oxidation by the air, often in combination with light.

Various experiments showed that certain NADES could stabilize the dissolved compounds, as shown in Table 19.4 [6]. The high viscosity and low oxygen solubility in these NADES could be involved in their conservation properties. Some NADES are able to conserve various biomaterials such as flowers or leaves of a plant and salmon tissue, whereas others (partly) dissolve such materials. In terms of developing a NADES-based extraction, similar to the development of any classical extraction method, stability tests are required, because of the different stability profiles for different NADES. Using a NADES that stabilizes the target compounds allows long-term storage without alteration of the chemical profile of the extract. For developing the preanalytic steps of extraction, a stabilizing NADES for the target compounds is thus important. At least, this is a major advantage compared with classical organic solvents such as chloroform, dichloromethane, and ethyl acetate in which often poor stability of analytes is found. These extracts are taken to dryness for storage and thus require solubilization to make the sample ready for the analysis, which may affect the recovery of some compounds, particularly when a solvent is used that is different from the extraction solvent, for example, the use of the LC mobile phase for dissolving a dry extract.

An interesting aspect of the NADES is that they will extract both metabolites and macromolecules such as proteins, including enzymes. As most enzymes seem not to be active in a pure NADES [3], it means that the NADES can be used to quench enzyme reactions. For example, in the extraction of plants with water, glycosidases

TABLE 19.3 NADES and DES as Extraction Solvents

Chemical Class	NADES and Des Composition	Species	Part of Plant or Sample	Method of Extraction	Instrumental Analysis	Reference
(A) PHENOLIC COMPOUNDS 1. Flavonoids 1.1. Flavones Amentoflavone	Choline chloride: 1,4-butanediol 1:5 (35% water)	*Chamaecyparis obtuse*	Leaves	DES-stirring, heating, and ultrasonic irradiation	HPLC-UV	[15]
Apigenin	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
	Choline chloride: 1,6-hexanediol 1:7 (30% water)	*Cajanus cajan* (pigeon pea)	Roots	DES-microwave-assisted extraction (MAE)	RP-HPLC	[16]
	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]
Apigenin-5-*O*-β-D-glucopyranoside	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]

Apigenin-6,8-Di-C-α-L-arabinoside	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
Apigenin-8-C-α-L-arabinoside	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
Baicalin	Choline chloride: citric acid 1:2	*Scutellaria baicalensis*	Roots	NADES-microwave-assisted extraction (MAE) and direct macroporous resin adsorption (ME-2) and desorption process	HPLC	[26]
Baicalein	Choline chloride: lactic acid 1:2; 3:1; 2:1; 1:1; 1:3; 1:4	*Scutellaria baicalensis*	Roots	NADES-microwave-assisted extraction (MAE) and direct macroporous resin adsorption (ME-2) and desorption process.	HPLC	[26]
Genkwanin	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]
Genkwanin-5-*O*-β-D-glucopyranoside	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]

Continued

TABLE 19.3 NADES and DES as Extraction Solvents—cont'd

Chemical Class	NADES and Des Composition	Species	Part of Plant or Sample	Method of Extraction	Instrumental Analysis	Reference
Hinokiflavone	Choline chloride: laevulinic acid 1:2	*Platycladus orientalis* (Platycladi cacumen)	Herbal material bought on a local market	DES-ultrasound-assisted extraction (UAE) combined with macroporous resin (LX-38)	HPLC-UV	[27]
Luteolin	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]
Luteolin-7-*O*-β-D-glucopyranoside	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]
Myricitrin	Choline chloride: laevulinic acid 1:2	*Platycladus orientalis* (Platycladi cacumen)	Herbal material bought on a local market	DES-ultrasound-assisted extraction (UAE) combined with macroporous resin (LX-38)	HPLC-UV	[27]

Orientin	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
Vitexin	Choline chloride: glucose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
Wogonin	Choline chloride: glucose 1:2 Choline chloride: sorbitol 1:2 Choline chloride: maltose 1:2 Citric acid: sucrose 1:2 Citric acid: glucose 1:2 Lactic acid: sucrose 1:2; 2:4	*Scutellaria baicalensis*	Roots	NADES-microwave-assisted extraction (MAE) and direct macroporous resin adsorption (ME-2) and desorption process	HPLC	[26]
Wogononin	Choline chloride: lactic acid 1:2 (20% water)	*Scutellaria baicalensis*	Roots	NADES-microwave-assisted extraction (MAE) and direct macroporous resin adsorption (ME-2) and desorption process	HPLC	[26]
Wogonoside	Choline chloride: malic acid 1:2 Choline chloride: lactic acid 1:2 (20% water)	*Scutellaria baicalensis*	Roots	NADES-microwave-assisted extraction (MAE) and direct macroporous resin adsorption (ME-2) and desorption process	HPLC	[26]
1.2. Flavonols						
Hyperin	Choline chloride: 1,4-butanediol 1:4 (30% water)	*Pyrola incarnata*	Herbal material collected from forest	DES-microwave-assisted extraction (MAE)	HPLC-UV	[18]

Continued

TABLE 19.3 NADES and DES as Extraction Solvents—cont'd

Chemical Class	NADES and Des Composition	Species	Part of Plant or Sample	Method of Extraction	Instrumental Analysis	Reference
Isorhamnetin	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
	Choline chloride: D-(+)-glucose 1:1 L-proline: D-(+)-glucose 5:3 Citric acid: D-(+)-glucose 1:1 Citric acid: adonitol 1:1 Betaine: DL-malic acid 1:1 L-proline: glycerol 2:5 (10% water)	*Sophora japonica* (Flos sophorae)	Herbal material bought on a local market	DES-ultrasound-assisted extraction (UAE)	LC-UV	[13]
Kaempferol	Choline chloride: Xylitol 5:2	*Sophora japonica* (Flos sophorae)	Herbal material bought on a local market	DES-ultrasound-assisted extraction (UAE)	LC-UV	[13]
Kaempferol-3-*O*-β-D-rutinoside-7-*O*-β-D-glucopyranoside	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]
Myricetin	Choline chloride: 1,4-butanediol 1:5 (35% water)	*Chamaecyparis obtuse*	Leaves	DES-stirring, heating, and ultrasonic irradiation	HPLC-UV	[15]

Quercetin	Citric acid: glucose 1:1	*Allium cepa*	Bulb	NADES-ultrasonic extraction	HPLC	[28]
	L-Proline: glycerol 2:5 (10% water)	*Sophora japonica* (Flos sophorae)	Herbal material bought on a local market	DES-ultrasound-assisted extraction (UAE)	LC-UV	[13]
	Choline chloride: 1,4-butanediol 1:4 (30% water)	*Pyrola incarnata*	Herbal material collected from forest	NADES-microwave-assisted extraction (MAE)	HPLC-UV	[18]
Quercetin-*O*-rhamnoside	Choline chloride: 1,4-butanediol 1:4 (30% water)	*Pyrola incarnata*	Herbal material collected from forest	NADES-microwave-assisted extraction (MAE)	HPLC-UV	[18]
Quercetin-3-*O*-β-D-glucopyranoside	Choline chloride: betaine hydrochloride: ethylene glycol 1:1:2 (20% water)	*Equisetum palustre*	Dry plant	NADES-negative-pressure cavitation-assisted extraction (NPCE) combined with macroporous resin enrichment (HPD-826)	HPLC	[17]
Quercitrin	Choline chloride: Laevulinic acid 1:2	*Platycladus orientalis* (Platycladi cacumen)	Herbal material bought on a local market	NADES-ultrasound-assisted extraction (UAE) combined with macroporous resin (LX-38)	HPLC-UV	[27]
Rutin	Choline chloride: glycerol 1:1 (20% water)	*Fagopyrum tataricum*	Tartary buckwheat hull Buds	NADES-ultrasound-assisted extraction (UAE)	RP-HPLC	[11]
	Choline chloride: 1,4-butanediol 1:4 (20%			DES-stirring and	HPLC	[12]

Continued

TABLE 19.3 NADES and DES as Extraction Solvents—cont'd

Chemical Class	NADES and Des Composition	Species	Part of Plant or Sample	Method of Extraction	Instrumental Analysis	Reference
	water) Choline chloride: citric acid 1:1 (20% water) Choline chloride: D-sorbitol 1:1 (20% water) Choline chloride: ethylene glycol 1:2 (20% water) Choline chloride: fructose 5:2 (20% water) Choline chloride: glucose 5:2 (20% water) Choline chloride: malic acid 1:1 (20% water)	*Sophora japonica*		macroporous resin (AB-8) for recover target compounds in NADES extraction solution		
1.3. Flavanones						
Naringenin	Lactic acid: glucose Citric acid: glucose Fructose: citric acid	*Allium cepa* *Olea europea* *Solanum lycopersicum* *Pyrus communis*	Olive cake, the by-product from onion seed production, tomato, and pear (peels, seeds, and fruits)	NADES-ultrasound-assisted extraction (UAE)	HPLC-DAD	[29]
Pinostrobin	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]

1.4. Isoflavonoids Formononetin	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
Genistin	Choline chloride: 1,6-hexanediol 1:7 (30% water)	*Cajanus cajan* (Pigeon pea)	Roots	DES-microwave-assisted extraction (MAE)	RP-HPLC	[16]
Genistein	Choline chloride: 1,6-hexanediol 1:7 (30% water)	*Cajanus cajan* (Pigeon pea)	Roots	DES-microwave-assisted extraction (MAE)	RP-HPLC	[16]
1.5. Flavanols-Catechins Epicatechin	Choline chloride: ethylene glycol Choline chloride: xylitol Choline chloride: phenol Choline chloride: formic acid Choline chloride: citric acid Choline chloride: oxalic acid Choline chloride: malonic acid	*Trachycarpus fortunei* (palm)	Leaves	DES-reflux extraction and SPE for recovered the target compounds from DES solution	HPLC-MS	[30]
Epigallocatechin-3-gallate	Betaine: glycerol: D-glucose 4:20:1 (19% water)	*Camellia sinensis*	Leaves	NADES-ultrasound-assisted extraction (UAE)	LC-UV	[31]
1.6 Anthocyanidins Delphinidin-3-*O*-glucoside			Grape skin	NADES-microwave-	HPLC	[32]

Continued

TABLE 19.3 NADES and DES as Extraction Solvents—cont'd

Chemical Class	NADES and Des Composition	Species	Part of Plant or Sample	Method of Extraction	Instrumental Analysis	Reference
	Choline chloride: oxalic acid 1:1	*Vitis vinifera* cv. Plavac mali		assisted extraction and ultrasound-assisted extraction (MAE; UAE)		
Cyanidin-3-*O*-glucoside	Choline chloride: oxalic acid 1:1	*Vitis vinifera* cv. Plavac mali	Grape skin	NADES-microwave-assisted extraction and ultrasound-assisted extraction (MAE; UAE)	HPLC	[32]
Petunidin-3-*O*-glucoside	Choline chloride: oxalic acid 1:1	*Vitis vinifera* cv. Plavac mali	Grape skin	NADES-microwave-assisted extraction and ultrasound-assisted extraction (MAE; UAE)	HPLC	[32]
Peonidin-3-*O*-glucoside	Choline chloride: oxalic acid 1:1	*Vitis vinifera* cv. Plavac mali	Grape skin	NADES-microwave-assisted extraction and ultrasound-assisted extraction (MAE; UAE)	HPLC	[32]
Malvidin-3-*O*-glucoside	Choline chloride: oxalic acid 1:1	*Vitis vinifera* cv. Plavac mali	Grape skin	NADES-microwave-assisted extraction and ultrasound-assisted extraction (MAE; UAE)	HPLC	[32]
2. Phenolic acids Caffeic acid	Choline chloride: 1,3-butanediol 1:6 (10% water)	*Lonicera japonica*	Dried flowers	DES-microwave-assisted extraction (MAE)	RP-HPLC	[33]
Cinnamic acid	Choline chloride: ethylene glycol 1:2	*Olea europea* *Prunus dulcis* *Sesamum indicum* *Cinnamomum verum*	Oils	NADES-ultrasonic-assisted liquid-liquid microextraction (UALLME)	HPLC-UV	[34]

Chlorogenic acid	Choline chloride: 1,3-butanediol 1:6 (10% water)	*Lonicera japonica*	Dried flowers	DES-microwave assisted extraction (MAE)	RP-HPLC	[33]
Ferulic acid	Choline chloride-ethylene glycol 1:2	*Olea europea* *Prunus dulcis* *Sesamum indicum* *Cinnamomum verum*	Oils	NADES-ultrasonic-assisted liquid-liquid microextraction (UALLME)	HPLC-UV	[34]
Gallic acid	Lactic acid: glucose Citric acid: glucose Fructose: citric acid	*Allium cepa* *Olea europea* *Solanum lycopersicum* *Pyrus communis*	Olive cake; by-product from onion seed production, tomato and pear (peels, seeds and fruits)	NADES-ultrasound-assisted extraction (UAE)	HPLC-DAD	[29]
Salvionic acid A and Salvionic acid B	Choline chloride: ethylene glycol Choline chloride: 1,2 propanediol Choline chloride: glycerol Choline chloride: 1,4 butadienol Choline chloride: oxalic acid Choline chloride: succinic acid Choline chloride: urea Choline chloride:maltose	*Salvia miltiorrhiza*	Roots	NADES-microwave-assisted extraction (MAE)	HPLC	[35]
Trans-ferulic acid	Lactic acid: glucose Citric acid: glucose Fructose: citric acid	*Allium cepa* *Olea europea* *Solanum lycopersicum* *Pyrus communis*	Olive cake, the by-product from onion seed production, tomato and pear (peels, seeds and fruits)	NADES-ultrasound-assisted extraction (UAE)	HPLC-DAD	[29]

Continued

TABLE 19.3 NADES and DES as Extraction Solvents—cont'd

Chemical Class	NADES and Des Composition	Species	Part of Plant or Sample	Method of Extraction	Instrumental Analysis	Reference
Vanillin	Lactic acid-1, 2-propanodiol 1:1 (25% water)	*Vanilla planifolia*	Vanilla pods	NADES-ultrasonic extraction	HPLC-DAD	[14]
3. Coumarins Cajanuslactone	Choline chloride: maltose	*Cajanus cajan*	Leaves	NADES-microwave-assisted extraction (MAE)	UPLC	[25]
B) TERPENOIDS 1. Terpenes Artemisinin	Methyl trioctyl ammonium chloride:1-butanol 1:4	*Artemisia annua*	Leaves	DES-ultrasound-assisted extraction (UAE) combined with AB-8 macroporous resin	HPLC	[36]
Linalool	Choline chloride: ethylene glycol 1:4	*Chamaecyparis obtusa*	Leaves	NADES-headspace-solvent microextraction (HS-SME)	GC	[20]
α-terpineol	Choline chloride: ethylene glycol 1:4	*Chamaecyparis obtusa*	Leaves	NADES-headspace-solvent microextraction (HS-SME)	GC	[20]
Terpinyl-acetate	Choline chloride: ethylene glycol 1:4	*Chamaecyparis obtusa*	Leaves	NADES-headspace-solvent microextraction (HS-SME)	GC	[20]
2. Terpene lactones Ginkgolides	Malic acid: choline chloride 1:1	*Ginkgo biloba*	Leaves	NADES-Ultrasonic extraction combinated	HPTLC	[23]

	Glycerol: proline: sucrose 9:4:1			with solid-phase extraction (SPE)		
3. Saponins Total saponins	Choline chloride: glycerol Choline chloride: urea Choline chloride: acetic acid Choline chloride: lactic acid Choline chloride: oxalic acid	*Agave sisalana* *Ziziphus joazeiro*	Leaves	NADES-stirring	Spectrophotometry HPTLC	[37]
Ginsenosides	Choline chloride: malonic acid	*Panax ginseng*	Leaves and stems	NADES-Ultrasonic extraction combinated with solid-phase extraction (SPE)		[23]
	Malic acid: choline chloride 1:1 Malic acid: glucose 1:1 Glycerol: L-proline: sucrose 9:4:1	*Panax ginseng*	White ginseng powder	DES-ultrasound-assisted extraction (UAE) combined with SPE using HLB cartridges	LC-UV	[24]
C) ALKALOIDS Caffeine	Lactic acid: sucrose Citric acid: glucose	*Coffea arabica L.*	Coffee beans	NADES-based ultrasonic assisted extraction (UAE)	HPLC	[38]

TABLE 19.4 Stability of Typical Phenolic Compounds in Different NADES

NADES	Compositions (Molar Ratio)	Degradation Percentage in Sunlight at Room Temperature (days 0–15)		
		Carthamin	Hydroxysafflor Yellow A	Cartormin
LGH	Lactic acid-glucose (5:1)	15 (100% degraded)	15 (60% degraded)	15 (60% degraded)
PMH	Proline-malic acid (1:1)	15 (100% degraded)	15 (60% degraded)	15 (60% degraded)
GCH	Glucose-choline chloride (2:5)	15 (50% degraded)	15 (0% degraded)	15 (0% degraded)
SuCH	Sucrose-choline chloride (1:4)	15 (50% degraded)	15 (0% degraded)	15 (0% degraded)
Water		15 (100% degraded)	15 (0% degraded)	15 (10% degraded)
EtOH (40%)	40 v% Ethanol in water	15 (100% degraded)	15 (0% degraded)	15 (10% degraded)

Water and 40% Ethanol Were Taken as Ref. [6].

may hydrolyze glycosides, and thus, the analysis may not give the correct concentrations of certain compounds. Extraction with alcohols also reduces this risk. When harvesting plant material, quenching enzyme activities is usually done by liquid nitrogen, followed by freeze-drying. The NADES offer an alternative in case of a targeted analysis of biomass. Grinding the material in a NADES may quench enzyme activities allowing samples to be stored for prolonged periods. Elgharbawy et al. [48] showed that lipases were more stable in a pure NADES (sucrose-choline chloride) and then in a buffer. When tested in the lipase enzyme assay, some of the lipase solutions in NADES showed a more than threefold increase in activity.

19.6 Green Solvent

There are internationally accepted definitions for green solvents. It is outside the scope of this chapter to discuss this, and we refer to the recent comprehensive review by de los Angeles Fernandez et al. [7] for an extensive discussion on this topic. Rather than entering an academic discussion, it is more important to focus on the new opportunities the NADES offer. New applications can then be compared with existing methods for sustainability. Jeong et al. [24] showed that the NADES can be recovered through an SPE step. After extraction the obtained NADES extract is put on an SPE column, which is then washed with water. This water is freeze-dried to

yield NADES again for extraction. The target analytes are then recovered from the column by extraction with ethanol.

The NADES components are common ingredients of our daily food, and their safety profiles have been well established. However, for the NADES themselves, there are only a few studies. Paiva et al. [49] tested the cytotoxicity of different NADES based on choline chloride, D(+) glucose, D-xylose, citric acid, sucrose, and L(+) tartaric acid, using a cell line of L929 fibroblast-like cells. Their results suggest that the presence of tartaric acid has a detrimental effect on the metabolic activity of the cells. However, these results do not indicate a clear trend concerning the cytotoxic effect of the others constituents of the NADES. Frade et al. [50] reported the cytotoxicity of different magnetic ionic liquids in two human cell lines: normal skin fibroblasts (CRL.1520) and colorectal adenocarcinoma cells (CaCo-2), following the same test as Pavia et al. [49]. They concluded that the viability decreased about 60% at 1.2×10^{-3} M. But in general, the prepared choline-based solvents were not cytotoxic. The disposal of NADES is not a problem as they are nonflammable and nontoxic and could, for example, be fermented to produce novel products or energy.

19.7 Conclusions

NADES research is booming, and novel areas for applications are being explored. One of these is the use of NADES as an extraction solvent in analytical chemistry. Considering the advantage of NADES as highly selective solvents, the major area of applications is the targeted analysis of certain compounds. Gas chromatography is not the first choice of analytic method, but liquid-based chromatography such as LC, TLC, and capillary electrophoresis in combination with different types of detectors can handle NADES extracts. The stabilizing effect of some NADES is of interest for long-term storage of extracts prior to analysis. At least in exploring NADES for various applications, it is clear that such methods will be major tools. However, there is still a long way to go before NADES might eventually replace the classical organic extraction solvents used in preanalytic steps.

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Chapter 20

Environmental Applications

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Chapter Outline

20.1 Introduction

Because contaminants are present in the environment at such low levels, extraction techniques are mandatory for most applications. Of these extraction techniques, liquid-phase extraction is often used to extract contaminants from all sorts of

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environmental matrices although water is the one with the highest number of applications.

Current trends in analytical chemistry toward automation and the development of environmentally friendly methods have made it necessary to develop numerous miniaturized techniques, one of which is dispersive liquid-liquid microextraction (DLLME). This technique is used to extract both inorganic and organic contaminants from environmental waters. However, its application to saline water samples still presents some limitations.

For some applications, especially those that focus on solid samples such as sediments, soil, or sludge, various liquid-phase extraction techniques are often combined not only to extract contaminants but also to clean up the matrix. In fact, the complexity of solid matrices usually requires a laborious multistep treatment before the instrumental analysis.

Air is still usually analyzed by thermal desorption (TD) because it can be easily coupled to gas chromatography. Nonetheless, liquid-phase extraction techniques have been effectively applied to this matrix. Unlike water samples, instrumental extraction techniques such as pressurized liquid extraction (PLE) or microwave-assisted extraction (MAE) are usually used, although the classical Soxhlet extraction is still used in some methods.

As far as contaminants are concerned, most inorganic analysis applications focus on metals and, in some cases, on speciation studies, most of them in water samples. On the other hand, organic contaminants include an extensive list of compounds that are present in all environmental matrices. Polycyclic aromatic hydrocarbons are one of the classical families of contaminants that have been studied mainly not only in air but also in water. However, current studies tend to focus on emerging contaminants such as pharmaceuticals, personal care products, and musk fragrances.

This chapter discusses the most representative applications of liquid-phase extraction techniques to environmental samples since 2007. The first section focuses on inorganic contaminants, and the applications are classified in terms of the extraction technique used. The second section is devoted to organic contaminants and is divided into three subsections, one for each matrix: air, solid samples, and water. Examples have been selected to be representative of recent trends and to illustrate new and innovative applications as regard techniques and/or contaminants.

20.2 Inorganic Contaminants

One of the main aims of analytical chemistry regarding inorganic compounds is to reliably quantify metals at (ultra) trace levels in environmental matrices. Trace metals are challenging above all because they do not decompose in the environment. Therefore they can accumulate. In the approaches that have been reported for

determining these kinds of contaminants in environmental matrices, separation and preconcentration procedures are often required before determination. The techniques most commonly used for determining these metals are atomic absorption spectrometry equipped with flame (FAAS) or graphite furnace (GFAAS) and inductively coupled plasma emission spectrometry (ICP) [1, 2]. However, in some cases, these techniques are not sensitive enough to directly determine trace metals in environmental samples. Therefore a sample preparation step is needed. In the past, metals were usually extracted from environmental matrices by using liquid-liquid extraction (LLE). However, this is a time-consuming and tedious technique, which uses large amounts of potentially toxic organic solvents. Therefore, in recent years, a great deal of effort has been directed toward the use of environmentally friendly sample extraction strategies. In the following subsections, the main strategies are presented and examples are given in each case. In particular, we focus on liquid-phase microextraction (LPME) and cloud-point extraction (CPE) techniques because they are the approaches that have been most reported since 2007. Table 20.1 shows selected examples of the application of different liquid-phase extraction techniques applied to the determination of metals in environmental matrices.

20.2.1 LPME APPROACHES

In recent years, analytical chemistry has evolved toward miniaturization and automation and adopted the principles of green analytical chemistry, and this has led to new extraction procedures based on the principles of LPME being successfully used for inorganic analysis. LPME techniques such as single-drop microextraction (SDME), hollow-fiber LPME (HF-LPME), and DLLME were used to determine inorganic compounds in environmental matrices. All these techniques have undergone important modifications involving different solvents (ionic liquids (ILs), magnetic ionic liquids (MIL), surfactants, etc.), dispersion modes (ultrasound, vortex, etc.), energy and radiation (microwave, ultrasound, etc.), or automated procedures [2, 24–28].

20.2.1.1 Single-Drop Microextraction

The first report on SDME in the inorganic field was published in 2003 [29]. Since then, the number of publications using this technique to extract/preconcentrate inorganic analytes has increased considerably, even though in recent years and due to the lack of stability of the droplet, other LPME strategies have been mostly used. Of the approaches that use SDME, direct-SDME (DI-SDME) [3, 30, 31] and headspace-SDME (HS-SDME) [4, 32, 33] are the operational modes that have been most widely used.

One of the developments in SDME is that ILs are now used as extraction solvents. This means that the drops are larger and more stable than is the case for

TABLE 20.1 Selected Examples of the Application of Different Liquid-Phase Extraction Techniques Applied to the Determination of Metals in Environmental Matrices

Extraction Technique	Compound	Matrix	Extraction Technique Features	Instrumental Analysis Technique	Enrichment Factor	LODs (μg/L)	Ref
DI-SDME	Hg	River water	Sample volume: 15 mL Extraction solvent: 10-μL drop *m*-xylene Complexing agent: dithizone	ETV-AAS	970	0.01	[3]
HS-SDME	Sb (III) and total Sb	Spring water	Sample volume: 20 mL Extraction solvent: 3-μL drop containing 30 mg/L of $Pb(NO_3)_2$ in 1.5% HNO_3 (w/v)	ETAAS	176	0.025	[4]
IL-DI-SDME	$MeHg^+$, $EtHg^+$, $PhHg^+$, Hg^{2+}	Tap and river water and wastewater	Sample volume: 12 mL Extraction solvent: 4-μL $[C_6MIM][PF_6]$ Complexing agent: dithizone	LC-DAD	3–27	1.0–22.8	[5]
CF-SDME	Co, Hg, Pb	Lake and river water	Sample volume: 1.5 mL Extraction solvent: 2.5-μL $[C_4MIM][PF_6]$ Complexing agent: PAN	ETV-ICP-MS	60–350	1.5–9.8 ng/L	[6]
SFOD-SDME	As(III), As(V)	Tap and well water	Sample volume: 20 mL Extraction solvent: 15-μL 1-undecanol Chelating agent: APDC	ETAAS	1000	9.2 ng/L	[7]

HF-LPME	Cu^{2+}	Tap and river water	Sample volume: 7 mL Extraction solvent: 20-µL 1-octanol (placed in the lumen of the hollow fiber) Complexing agent: 8-hidroxy quinoline	FAAS	551	4	[8]
HF-LPME	Cr(VI), Cr(III)	Tap, river, and industrial water	Sample volume: 100 mL Extraction solvent: 25-µL 1-octanol Enhancement reagent (IL): $[C_4MIM][PF_6]$ Chelating agent: DDTC	FAAS	175	0.7	[9]
SBME	Ni	Seawater	Sample volume: - Extraction solvent: solution 0.87-M DEHPA Acceptor phase: solution 1.86-M HNO_3	GFAAS	10.96	44 ng/L	[10]
EME	Hg	Tap and river water	Sample volume: 5 mL Carrier: 2% DEHP in 1-octanol (v/v) Acceptor phase: 10-µL 0.001-M HCl 70 V	Microvolume UV-Vis spectrophotometry	130–176	0.7	[11]
DLLME	Co	Tap and river water	Sample volume: 10 mL Extraction solvent: 75-mg $[C_6MIM][PF_6]$ dissolved in 500-mL EtOH (disperser solvent) Chelating agent: PAN	FAAS	118	0.1 µg/L	[12]

Continued

TABLE 20.1 Selected Examples of the Application of Different Liquid-Phase Extraction Techniques Applied to the Determination of Metals in Environmental Matrices—cont'd

Extraction Technique	Compound	Matrix	Extraction Technique Features	Instrumental Analysis Technique	Enrichment Factor	LODs (μg/L)	Ref
DLLME-SFOD	Co, Cu, Ni, Pb, Zn	Wastewater	Sample volume: 50 mL Extraction solvent: 100-μ L1-undecanol Disperser solvent: 1.5-mL EtOH Chelating agent: PAN	ICP-MS	240–270	0.77–2.183 ng/mL	[13]
DLLME-SFOD	Cu	Tap, river, and seawater	Sample volume: 10 or 20 mL Extraction solvent: 70-μL1-undecanol Disperser solvent: 0.5-mL EtOH Chelating agent: OVAC	FAAS	10–20	0.03–1.84	[14]
SM-DLLME	Co	Tap, well, and mineral water and wastewater	Sample volume: 5 mL Extraction solvent: 41-mg decanoic acid dispersed in 0.5-mL THF Chelating agent: PAN	FAAS	58	4.2	[15]
SM-DLLME	Cu	Well, sea, and underground water	Sample volume: 500 μL Extraction solvent: 150-μL 1 decanol dispersed in 600-μL THF Chelating agent: ADPC	FAAS	60.3	0.11	[16]
IL-CIAME	Se(IV)	Tap, river, and mineral water and seawater	Sample volume: 25 mL Extraction solvent: 100-μL $[C_4MIM][PF_6]$ Chelating agent: dithizone Heated in a water bath at 50°C, 4 min	Spectrophotometric detection	25	1.5	[17]

IL-CIAME	Ni	Tap, river, and mineral water and seawater	Sample volume: 45 mL Extraction solvent: 120-μL $[C_6MIM][PF_6]$ Chelating agent: TAN Heated in a water bath at 45°C, 5 min	FAAS	90	0.8	[18]
UAE-DLLME	Ag	Tap and river water	Sample volume: 40 mL Extraction solvent: 1.2-mL CCl_4 Chelating agent: dithizone Ultrasound extraction time: 3 min	Spectrophotometric detection	35	0.45	[19]
IL-USA-DLLME	Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}	River and lake water	Sample volume: 15 mL Extraction solvent: 10-mL Cyphos IL104 Chelating agent: APDC Ultrasound: 40 W, 60 s	LC-UV	0.02–0.03	207–211	[20]
SI-DLLME	Cd, Pb	Tap and river water and seawater	Sample volume: 8.1 mL Extraction solvent: 5-μL xylene dispersed in methanol Chelating agent: APDC	ETAAS	34 (Cd) 80 (Pb)	0.002 (Cd) 0.01 (Pb)	[21]
CPE	Co^{2+}, Cu^{2+}, Fe^{3+}, Zn^{2+}	Tap water	Sample volume: 50 mL Extraction solvent: Triton X-114 (0.1%, v/v) Chelating agent: [2-(3-ethylthioureido)benzoic acid]	FAAS	48.82–52.61	0.23–1.5	[22]

Continued

TABLE 20.1 Selected Examples of the Application of Different Liquid-Phase Extraction Techniques Applied to the Determination of Metals in Environmental Matrices—cont'd

Extraction Technique	Compound	Matrix	Extraction Technique Features	Instrumental Analysis Technique	Enrichment Factor	LODs (μg/L)	Ref
RS-CPE, UA-CPE	Se	Tap, lake, river, and bottled water	*RS-CPE*: Sample volume: 40 mL Extraction solvent: Triton X-114 (0.05% v/v) in 1.2-mL octanol Chelating agent: dithizone *UA-CPE*: Sample volume: 40 mL Extraction solvent: Triton X-114 (0.05% v/v) Ultrasound 50°C, 20 min Chelating agent: dithizone	Spectrophotometric detection	UA-CPE: 103 RS-CPE: 124	UA-CPE: 0.3 RS-CPE: 0.2	[23]

$[C_4MIM][BF_4]$, 1-butyl-3-methylimidazolium tetrafluoroborate; *$[C_6MIM][PF_6]$*, 1-hexyl-3-methylimidazolium hexafluorophosphate.; *APDC*, ammonium pyrrolidinedithiocarbamate; *CCl_4*, carbon tetrachloride; *CF-SDME*, cycle-flow single-drop microextraction; *CPE*, cloud-point extraction; *DAD*, diode array detector; *DDTC*, diethyl carbamate; *DEHP*, di(2-ethylhexyl) phosphate; *DEHPA*, Di-2-ethylhexyl phosphoric acid; *DI-SDME*, direct injection single-drop microextraction; *DLLME*, dispersive liquid-liquid microextraction; *EME*, electromembrane extraction; *ETAAS*, electrothermal atomic absorption spectrometry; *ETV*, electrothermal vaporization; *FAAS*, flame atomic absorption spectrometry; *GFAAS*, graphite furnace atomic absorption spectrometry; *HF-LPME*, hollow-fiber liquid-phase microextraction; *HS-SDME*, headspace single-drop microextraction; *ICP*, inductively coupled plasma emission spectrometry; *IL-CIAME*, ionic liquid-based cold-induced aggregation microextraction; *ILs*, ionic liquids; *LC*, liquid chromatography; *LOD*, limit of detection; *MS*, mass spectrometry; *OVAC*, N-o-vanillidine-2-amino-p-cresol; *PAN*, 1-(2-pyridylazo)-2-naphthol; *RS-CPE*, rapidly synergistic cloud-point extraction; *SBME*, solvent bar microextraction; *SDME*, single-drop microextraction; *SFOD*, solidification of the floating organic drop; *SM-DLLME*, supramolecular solvent-based dispersive liquid-liquid microextraction; *TAN*, 1-(2 thiazolylazo)-2-naphthol; *THF*, tetrahydrofuran; *UAE-DLLME*, ultrasound-assisted emulsification dispersive liquid-liquid microextraction; *USA-CPE*, ultrasound-assisted cloud-point extraction.

traditional organic solvents, so extraction is more efficient. Even so, there are some examples of organic solvents: for example, Bagheri et al. [3] used a DI-SDME to extract mercury from river water samples. To do so, the authors used a microdroplet of 10 μL of *m*-xylene containing 0.05-M dithizone as complexing agent immersed in 15-mL water samples with an agitation of 300 rpm. Electrothermal vaporization atomic absorption spectroscopy (ETV-AAS) was used after the extraction for quantification, and a limit of detection (LOD) of 0.01 μg/L was obtained. Ionic liquids are being increasingly used today because of their unique properties. $[C_nMIM][PF_6]$ ($n = 4,6,8$) are by far the most commonly used extractant phases for inorganic species. For example, Pena-Pereira et al. [5] reported a method for the separation and speciation of mercury (Hg^{2+}, $MeHg^{+}$, $EtHg^{+}$, and $PhHg^{+}$) in water samples with a strategy based on IL-DI-SDME using a microdroplet of 1-hexyl-3-methylimidazolium hexafluorophosphate $[C_6MIM][PF_6]$ as an extraction solvent and dithizone as a complexing agent before liquid chromatography-diode array detector (LC-DAD) analysis. Extraction efficiency was best with a drop volume of 4 μL exposed for 20 min to a sample volume of 12 mL and stirring at 900 rpm. The drop volume is restricted due to its stability at the syringe tip. To overcome this drawback, approaches such as cycle-flow SDME (CF-SDME) have been developed. CF-SDME enables the sample to flow freely around the solvent microdrop. In this regard the work of Xia et al. [6] should be highlighted. They developed a method based on CF-SDME with electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS) to determine Hg, Co, and Pb in biological and environmental samples. Using the CF-SDME system the authors extracted the analytes by exposing a 2.5-μL IL droplet to a flowing stream of sample, which has the advantage that mechanical agitation is replaced by continuous flow. 1-butyl-3-methylimidazolium hexafluorophosphate $[C_4MIM][PF_6]$ was used as the extraction solvent and 1-(2-pyridylazo)-2-naphtol (PAN) as both the complexing agent and chemical modifier. The enrichment factors (EFs) obtained were higher than in static conditions due to the continuous contact between the IL phase and the fresh flowing samples.

Solidification of the floating organic drop (SFOD) has also emerged as an interesting solution to the problem of the instability of the solvent drop in SDME. Ghambarian et al. [7] developed a method for the preconcentration and speciation of arsenic in water samples (tap and well waters) based on the combination of SFOD-SDME and ETAAS. The extracting solvent was 15 μL of 1-undecanol, which was solidified after extraction in an ice bath. The chelating agent in this case was ammonium pyrrolidinedithiocarbamate (APDC), and $Pd(NO_3)_2$ was the chemical modifier. The results demonstrated that the technique is highly promising.

20.2.1.2 Hollow-Fiber LPME

One of the main drawbacks of SDME is the instability of the hanging drop. HF-LPME can overcome this problem by immobilizing the extractant phase within a supported liquid porous membrane, so the sample can be stirred vigorously without any loss of the extraction phase. Although HF-LPME is mostly applied in organic analysis, the determination of metals and their speciation has also attracted some interest [24–27, 34]. HF-LPME was used in either a two-phase or three-phase mode to determine inorganic analytes with organic solvents such as 1-octanol, toluene, or carbon tetrachloride, which are the extracting phases of choice for determining inorganic species like selenium, cadmium, vanadium, copper, or arsenic [34–36]. For example, copper was determined in tap and surface water samples with a two-phase HF-LPME strategy in combination with FAAS [8]. Copper was first complexed with 8-hydroxyquinoline and the complex extracted for 30 min into 20 μL of 1-octanol in the lumen of the HF. Peng et al. [37] also used 1-octanol in three-phase HF-LPME prior to ETAAS to determine cadmium in seawater samples. The organic solvent dissolving the mixture of dithizone, which acts as a carrier, and oleic acid, which prevented the loss of the liquid membrane, was immobilized in the pores of the HF (polypropylene) to form a liquid membrane for the extraction of cadmium from high saline samples. The results demonstrated that the extraction device was free of any interference. Despite the potential of HF-LPME and organic solvents, in recent years, ILs have been increasingly used. Some ILs have functional groups that allow them to play a double role in this microextraction technique as extractants and reagents, thus enhancing the extraction efficiency for some applications [38]. Zeng et al. [9] developed a method for chromium speciation (Cr(VI) and Cr(III)) in environmental water samples using IL-HF-LPME followed by FAAS. In this method, 1-octanol was immobilized in the pores of the polypropylene membrane and used as the acceptor solution. Diethylcarbamate (DDTC), as chelating agent, and the IL, 1-butyl-3-methyilimidazolium tetrafluoroborate ($[C_4MIM][BF_4]$), as enhancement reagent, were added to the sample solution. The addition of the IL improved the extraction of Cr (VI) 3.5-fold.

As typically used, HF-LPME presents some limitations for routine analysis. One limitation is the need for a specific support for the hollow fiber. To overcome this drawback, a novel configuration known as solvent bar microextraction (SBME) has emerged [26, 39]. In this technique, the ends of the fiber are thermally sealed so, impregnated with the organic solvent, it can be left free in the sample during the extraction, without any support. The process is thus simplified, and at the same time the transfer of analytes to the extraction solvent increased. SBME is mainly used to extract such inorganic species as Ag, Cd, or Ni in high saline samples [10, 40]. The reported strategies based on SBME miniaturize trace metal extraction from saline samples and considerably improve the analytic features of existing membrane-based

methods. Another important drawback of HF-LPME is the relatively slow passive diffusion, which makes the procedure so time-consuming. One way of speeding up the extraction kinetics is to use electromembrane extraction (EME), which reduces the time the analytes take to move from the donor phase to the membrane [34, 39]. EME has been explored by several researchers in the field of inorganics. For example, Fashi et al. [11] developed a method combining EME with microvolume UV-Vis spectrometric detection to determine mercury in fish and water samples. After parameters such as the applied voltage (70 eV) had been optimized, the results showed that the EME method had some advantages over existing methods. In particular, the analysis time was shorter than that of other strategies based on HF-LPME (10 min instead of 20–40 min).

20.2.1.3 Dispersive Liquid-Liquid Microextraction

Since first introduced, DLLME and its variations have attracted attention for the extraction and preconcentration of inorganic species in environmental samples, mainly water samples, and it is now the most widely used LPME strategy in this field [25–27, 41–43]. However, it has several shortcomings. One of these is the choice of extraction solvent. A suitable extraction solvent should be denser than water. The number of organic solvents that accomplish this condition is relatively small, so hazardous solvents such as halogenated hydrocarbons are frequently used. In recent years, research into extraction solvents has attempted to address this drawback in two different ways: by using new solvents such as ILs and low-density organic solvents [41, 43, 44]. ILs have been successfully applied in the DLLME process for various metals. However, this approach has considerable limitations when it is used for saline samples. The increase in salt enhances the solubility of ILs in the aqueous medium, and the cloudy solution does not form so analytes are not extracted. However, Yousefi et al. [12] introduced an IL-based DLLME strategy for preconcentrating cobalt in aqueous samples with high salt concentrations. The authors use $[C_6MIM][PF_6]$ and ethanol (EtOH) as the extraction solvent and disperser solvent, respectively, and to reduce the solubility of the IL in the saline samples, a common ion (PF_6^-) was added. With this strategy, the method can be applied to aqueous samples containing high salt concentrations (up to 40% w/v). As has been mentioned, using solvents that are less dense than water is an interesting alternative to toxic organic solvents, but it requires new strategies for collecting the enriched organic phase after phase separation, for example, SFOD, which provides a solid drop that can be easily withdrawn after extraction [41–43, 45]. DLLME-SFOD was utilized to determine such metals as Cr, Co, Hg, Ni, or Pb mainly in environmental waters as extractable complexes after adding a chelating agent [13, 14, 45, 46]. For instance, Karadas et al. [14] used DLLME-SFOD in combination with FAAS, with *N*-*o*-vanillidine-2-amino-*p*-cresol as the chelating agent dissolved in EtOH (disperser solvent),

and 70 μL of 1-undecanol as the low-density solvent, to determine copper in various samples such as tap water, river water, and seawater. Supramolecular solvents (SUPRASs), also known as coacervates, have also been used as an alternative to new solvents in DLLME. The resulting strategy is known as supramolecular solvent-based dispersive liquid-liquid microextraction (SM-DLLME) [15, 16, 43]. As SUPRAs have regions with different polarities, they can provide a variety of interactions with analytes, and as they have a high number of binding sites, extraction efficiencies are generally high even for low extraction solvent volumes. For example, Aydin et al. [16] developed a method to determine copper in a small sample volume, 500 μL of water, by creating a SUPRA phase by mixing 600 μL of tetrahydrofuran (THF) and 140 μL of 1-decanol. This method was used to determine copper at trace levels in real samples.

The selection of the disperser solvent is also a key factor in DLLME. The disperser solvent directly affects the formation of the cloudy solution, which enhances the contact between the organic and aqueous phases and improves the extraction rate. However, one important drawback is that DLLME requires relatively large volumes of disperser solvent, and this usually decreases the partition constant of analytes in the extraction solvent. Several strategies have been devised to reduce or even eliminate the need for disperser solvents in DLLME. For example, temperature and ultrasound energy have been used for inorganic analyses [41–43]. A variety of approaches use temperature in combination with ILs as extraction solvents, for example, temperature-controlled ionic liquid-dispersive liquid-liquid microextraction (TC-IL-DLLME) or ionic liquid-based cold-induced aggregation microextraction (IL-CIAME) [17, 18, 47]. Jamali et al. [18] reported a preconcentration procedure based on IL-CIAME in combination with FAAS for the determination of nickel in different environmental water samples. In the optimized procedure, 120 μl of IL, $[C_6MIM][PF_6]$, was added to the sample solution (45 mL) containing Ni previously complexed with 1-(2-thiazolylazo)-2-naphthol (TAN). Subsequently, the solution was heated and the IL dissolved completely. Then, the solution was left in an ice bath, and a cloudy solution was formed because of the decrease in the solubility of the IL. After phase separation by centrifugation, the IL phase (diluted with EtOH to 500 μL) was analyzed with FAAS, and the LOD was at the nanogram per milliliter level. This strategy meant that a disperser solvent did not have to be used. Ultrasound radiation is another strategy that can prevent the need for a disperser solvent. Ultrasound radiation also accelerates the mass-transfer process between the two immiscible liquids, which increases the extraction efficiency and requires shorter times than conventional DLLME [42, 43]. Ultrasound-assisted DLLME (USA-DLLME) or ultrasound-assisted emulsification DLLME (UAE-DLLME) use ultrasounds to induce the dispersion of the extraction solvent and avoid the use of the disperser [19, 20, 48]. Werner [20] developed a method based on IL-USA-DLLME that used the solidification of the aqueous phase (SAP) in combination with LC-UV for the

simultaneous determination of several heavy metals in river and lake waters. The authors demonstrated that the ultrasound energy efficiently dispersed the extractant in a short time (only 60 s) and without using a dispersion solvent.

20.2.1.4 Combination of LPME Techniques With Other Techniques

Some recent reviews have focused on the coupling of LPME techniques with other extraction techniques, since this is often the solution adopted to address some of the limitations of LPME [49, 50]. However, the literature on the analysis of inorganic ions is still very limited in comparison with the numerous publications on organic analytes. The combinations described for determining metal species usually involve either solid-phase extraction (SPE) or a microextraction technique [51, 52]. For example, Shamsipur et al. [51] developed a method coupling SPE with SFOD prior to ETAAS for the determination of As(III) and As(IV) in water samples. In this strategy, As(III) was first preconcentrated using SPE with an octadecylsilica sorbent and the extract subsequently subjected to DLLME with SFOD. Total inorganic arsenic was extracted in a similar way after As(V) had been reduced to As(III). The main advantages of this method are the high preconcentration factor and the low LOD, the quick sample preparation (<15 min), and the consumption of low amounts of toxic organic solvents.

20.2.1.5 Automation for LPME Techniques

In recent years, several researchers have focused on automation, and the number of publications on the automation of microextraction techniques has increased accordingly [53]. For example, Anthemidis' group reported various strategies in which the DLLME procedure was fully automated [21, 54]. In the proposed methods the disperser solvent, extraction solvent, and chelating agent were mixed on line with a stream of aqueous sample using a sequential injection (SI) system. After extraction, the droplets of the extractant were retained in a microcolumn and methyl isobutyl ketone was used as elution solvent. Finally, this was forwarded to a FAAS nebulizer [54] or injected into the graphite tube of an ETAAS [21]. The automated system has considerable potential for the determination of metals such as thallium, lead, and copper in environmental water samples. Fig. 20.1 presents a scheme of the system used for SI-DLLME coupled with FASS.

20.2.2 Cloud-point extraction

CPE, also known as micelle-mediated extraction (MME), is a surfactant-based extraction technique widely used for preconcentrating trace metals before their determination [55, 56]. In fact, much of the development of CPE has dealt with the extraction and preconcentration of inorganic analytes. The CPE procedure

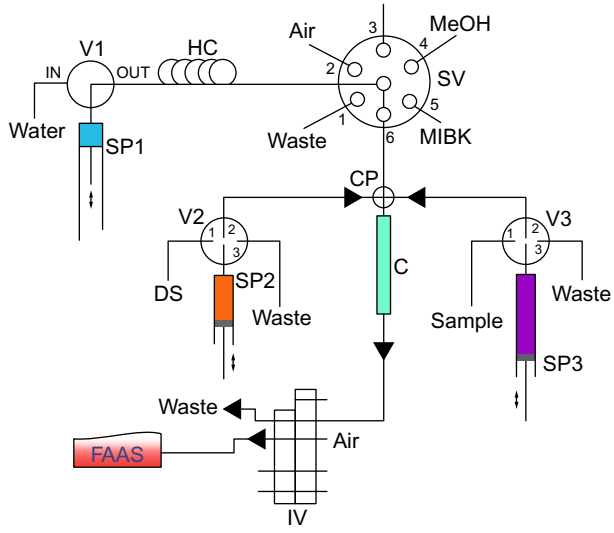

Fig. 20.1 Schematic manifold of SI-DLLME coupled with FAAS.*C*, microcolumn; *CP*, confluent point; *DS*, dispersive solvent containing 6.0% (v/v) [C_6MIM][PF6]; *HC*, holding coil; *IV*, injection valve in "load position"; *SP1*, *SP2*, and *SP3*, syringe pumps; *SV*, selection valve; *V1*, *V2*, and *V3*, valves at the top of syringe pumps; *W*, waste. *(Reprinted with permission of Elsevier Anthemidis AN, Ionnou K-I G. Sequential injection ionic liquid dispersive liquid-liquid microextraction for thallium preconcentration and determination with flame atomic absorption spectrometry. Anal Bioanal Chem 2012;404:685–691.)*

involves forming hydrophobic species by complexation with an organic ligand in a medium containing the surfactant, so the metal complex is extracted by being incorporated into a micelle system. Therefore, several studies have focused on the choice of the surfactant and chelating agent. Chelating agents such as APDC, PAN, TAN, dithizone, or 8-hydroxyquinoline have been used in CPE. Nonionic surfactants are the most usual surfactants in CPE. For example, Triton X-114 was selected in several studies for the determination of metals like Co, Cu, Fe, Ni, or Zn mainly in water samples [22, 56, 57]. Aware of the limitations of conventional CPE, however, many researchers have attempted to develop strategies to improve it. Of these, displacement cloud-point extraction (D-CPE), dual cloud-point extraction (d-CPE), rapidly synergistic cloud-point extraction (RS-CPE), and ultrasound-assisted cloud-point extraction (USA-CPE) are used in the field of inorganic analysis. These strategies have improved the selectivity of CPE and shortened the extraction time [56]. For example, Wen et al. [23] compared two different CPE-based procedures, RS-CPE and USA-CPE, for the spectrophotometric determination of selenium in water samples. While RS-CPE simplified and accelerated the conventional CPE procedure, since the extraction time was only 1 min and heating was not needed, with

USA-CPE, the extraction time was relatively lengthy (20 min), and an equilibration temperature was needed for clouding (50°C). There have been several attempts to automate CPE [56, 58], and despite the promising results, there are some drawbacks (e.g., the dilution of the surfactant-rich phase and the use of organic solvents in the elution step). In an attempt to overcome these limitations, Frizzarin et al. [59, 60] reported several strategies, for example, the use of a multipumping flow system CPE (MPFS-CPE) for the spectrophotometric determination of iron in freshwater samples. The resulting procedure has some advantages over previously proposed methods for flow-based CPE (e.g., an improved sampling rate).

20.3 Organic Contaminants

20.3.1 Air

Air quality is attracting increasing concern nowadays since little is known about the environmental and health effects of many chemical substances that are in daily use in modern life. Researchers and authorities are interested in both indoor and outdoor air. Studies on outdoor air mainly focus on industrial and human emissions and aim to determine temporal or spatial variations, while those on indoor air mainly focus on compounds that can be harmful to human health. Since people in developed countries spend more than 90% of the day in indoor environments, indoor air contaminants can be a serious threat to human health.

Although air is one of the cleanest matrices, it is a heterogeneous mix of gases, liquids, and solid particles, the composition of which can be affected by meteorologic conditions, diffusion, and reactivity. Consequently, sampling is strongly dependent on the purpose of any particular study. Various sampling strategies have been developed [61, 62]: whole-air collection, continuous sampling and on-line analysis, and collection onto sorbents (through active or passive samplers). The last of these options is the most versatile because the analytes retained can be desorbed from sorbents by TD or solvent extraction. Due to the volatility of most of the analytes determined in air and the interest in individual compounds (e.g., to perform risk characterization), TD is the most used option because it can be coupled to gas chromatography (GC), and desorption and analysis can be automated. As well as this advantage and the fact that LODs are usually lower because the analytes are completely transferred into the GC, its main drawbacks are that the equipment is expensive, it cannot be applied to thermally unstable analytes or those with high-boiling points (above 300°C desorption efficiency decreases), and the whole sample is consumed in a single analysis. Solvent extraction overcomes these drawbacks and makes it possible for sorbent beds to be larger that, in turn, enables larger sample volumes to be used. Its main drawback compared with TD is the dilution of the sample.

Because of the low levels of organic contaminants in air samples, the sample needs to be enriched usually by solvent evaporation, which can lead to losses of the most volatile analytes.

Currently, solvent extraction methods have largely been used to determine semi-volatile organic compounds (SVOCs) [63, 64]. Of these compounds, polyaromatic hydrocarbons (PAHs) have been the most widely studied probably because they are the class of SVOCs that cause most concern in industrialized countries. Regarded as hazardous air pollutants under the Clean Air Act published by the US Environmental Protection Agency (USEPA) in 1990, polychlorinated biphenyls (PCBs) have also received considerable attention (see Fig. 20.2.) followed by organochlorine pesticides (OCPs) and organophosphate and brominated flame retardants. Solvent extraction has also been used to determine volatile organic compounds (VOCs) such as those that act as ozone precursors or pose a serious hazard to human health. Occasional studies have focused on contaminants such as drugs of abuse [65], *N*-nitrosamines [66], benzothiazoles, benzotriazoles, and benzene sulfonamides [67].

VOCs have mainly been determined in the gas phase of urban and industrialized outdoor air at levels between ng/m^3 and low μg/m^3 since they are largely produced by activities carried out in those environments. Due to their volatility, their presence in particulate matter has been given less attention. However, SVOCs have been determined in both outdoor and indoor air at levels of pg/m^3 and ng/m^3, respectively. Most SVOCs are very stable and resistant to degradation, which is consistent with their

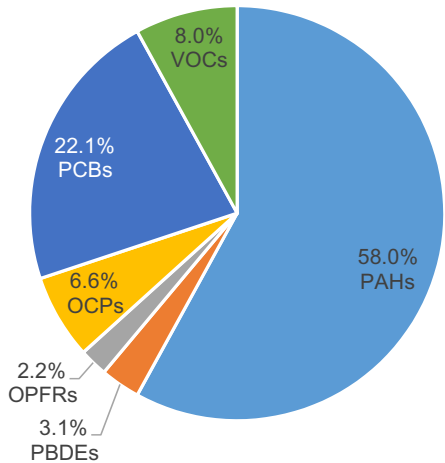

Fig. 20.2 Frequency of application of solvent extraction techniques in air samples according to family of compounds in the period 2007–18. *(Source: Scopus. Date: October 2018. Keywords: air, environmental science, Soxhlet or sonication or MAE or pressurized liquid extraction, PAH, PBDE, OPFR, OCP, PCB, or VOC.)*

presence in indoor air, even though their use was banned some time ago (e.g., the pesticide dichlorodiphenyltrichloroethane [DDT]). In addition, the physicochemical properties of SVOCs determine their capacity to be partitioned between the gas and particulate phases. Thus both phases are usually sampled to determine the extent to which they are present in air. In the case of indoor air, dust samples are sometimes analyzed because particulate matter tends to concentrate in them. Similarly, the physicochemical properties of drugs of abuse, *N*-nitrosamines, etc. favor their presence in the particulate phase. Nevertheless, in this case, unlike SVOCs, particles in outdoor air have mostly been analyzed, and the concentrations found have been between pg/m^3 and low ng/m^3.

Because air is usually sampled onto sorbents, the most commonly used solvent extraction techniques are those that are suitable for extracting compounds from solid matrices. Of these, classical techniques such as Soxhlet extraction [68], which is still part of some official methods, are often use. However, the current trend in analytical chemistry toward environmentally friendly methods has forced the volume of organic solvents to be reduced. In line with this trend, instrumental extraction techniques such as ultrasound-assisted extraction (UAE), MAE, and PLE are commonly used. The popularity of these techniques is reflected by the number of applications developed in the last 10 years (about 50% of all published applications), Fig. 20.3.

Soxhlet extraction has mainly been used to extract PAHs, OCPs, and PCBs in outdoor air. Recently, two OCPs (DDT and hexachlorocyclohexane [HCH]) and their isomers, the use of which was banned or restricted in agriculture in the 1970s, were determined in outdoor air to identify their sources and temporal trends [69]. In the

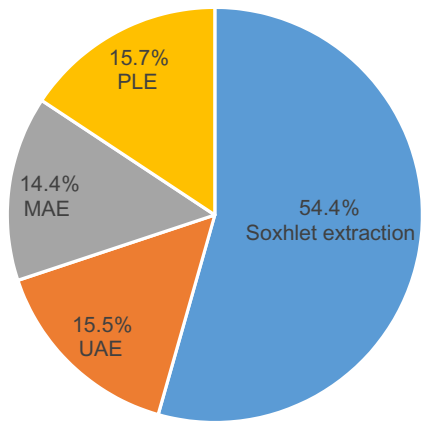

Fig. 20.3 Frequency of application of solvent extraction techniques in air samples in the period 2007–18. *(Source: Scopus. Date: October 2018. Keywords: air, environmental science, Soxhlet, sonication, MAE, and pressurized liquid extraction.)*

first step, air was collected for 24 h by a high-volume sampler (total sample volume about 820 m^3) and passed through a fiber filter and a XAD-2 resin to collect particles and the gas phase, respectively. Then, filters and resin were separately Soxhlet extracted for 24 h with acetone-hexane (1:1, v/v). This strategy revealed the presence of these compounds in both phases by using one single-step sampling, which is commonly applied in air analysis. Because the levels of OCPs were expected to be low, extracts were reduced by evaporation and fractionated by a silica gel column. The final extracts were reduced by evaporation again and analyzed by GC with an electron capture detector (ECD). This method enabled the analytes to be determined at low pg/m^3 levels and established temporal trends, which suggested that these OCPs are used exclusively not only in agriculture but also in insect control in cities. Other applications, some of which are collected in Table 20.2, used hexane or dichloromethane (DCM) to extract OCPs [70] or various proportions of hexane/diethyl ether to extract PAHs [73] and PCBs [71]. To obtain quantitative recoveries (62%–99%), an extraction time between 18 and 24 h is usually required, and the most commonly used solvent volume is about 100 mL.

As was indicated earlier, significant efforts have been made to minimize extraction time and the volume of organic solvent consumed. UAE has achieved both these goals with equipment of reasonable cost, and some studies have compared UAE with Soxhlet extraction, the reference classical technique. By way of example, one study compared the ability of Soxhlet and UAE with extract PCBs in the gas phase of outdoor air around a landfill station [71]. Air was actively sampled for 24 h on polyurethane foam (PUF) plugs that are commonly used for retaining compounds from the gas phase. Recoveries (84%–101%) were similar in both cases, but Soxhlet needed 100 mL of hexane/diethyl ether (95:5, v/v) for 5 h, while UAE only needed 40 mL of the same solvents for 15 min. In both cases, extracts were concentrated by solvent evaporation and analyzed by GC with tandem mass spectrometry (MS/MS) with the precision of both methods lower than 12% (expressed as relative standard deviation, RSD). For most applications, UAE uses less than 40 mL of organic solvents and an extraction time of 5–20 min and provides recoveries similar to those for Soxhlet extraction.

MAE is also a common option. It usually takes the same time as UAE and uses similar solvent volumes. MAE was used for the first time to extract organophosphate esters from airborne particulate matter in a suburban area [74]. Airborne particles were actively sampled by quartz fiber filters (PM_{10}) for 24 h at a flow rate of 2.3 m^3/h. Microwaves allowed a quantitative extraction (recoveries between 77% and 114%) of the analytes in only 3.5 min using a mixture of water-ethanol (1:1, v/v) at 180°C. Then, contrary to the common step of solvent evaporation, the extract was diluted with water to allow automatic analysis by immersion solid-phase microextraction (SPME) and GC-MS/MS. The final method had LODs around 0.15 ng/m^3, consistent with air levels for these analytes.

TABLE 20.2 Selected Examples of Solvent Extraction Techniques Applied in the Analysis of Air Samples

Extraction Technique	Family of Compounds	Matrix	Extraction Technique Features	Instrumental Analysis Techniques	%R	LODs	Ref
Soxhlet extraction	OCPs, PCBs	Gas-phase outdoor air	Hexane	GC-ECD	–	0.003 ng/m^3	[70]
	OCPs	Airborne particles and gas phase of outdoor air	Hexane-acetone (1:1, v/v), 24 h	GC-ECD	>80%	–	[69]
	PCBs	Outdoor air around landfill station	Hexane-diethyl ether (95:5, v/v), 100 mL, 5 h	GC-MS/MS	>85%	–	[71]
	OCPs, PCBs	Airborne particles and gas phase of urban outdoor air	Hexane-acetone (1:1, v/v), 200 mL, 8 h	GC-MS	>75%	–	[72]
	PCDDs, Fs, dl-PCBs	Gas-phase urban, rural and industrial outdoor air	Toluene-acetone (9:1, v/v), 24 h	HRGC-HRMS	>92%	0.001–0.2 ng/sample	[68]
	PAHs	Urban outdoor air	Hexane-diethyl ether (1:9, v/v), 350 mL, 18 h	LC-GC-MS	>99%	–	[73]
UAE	PCBs	Outdoor air around landfill station	Hexane-diethyl ether (95:5, v/v), 40 mL, 15 min	GC-MS/MS	>82%	0.003–0.625 ng/m^3	[71]
MAE	OCPs, PCBs	Airborne particles and gas phase of urban outdoor air	Hexane-acetone (1:1, v/v), 40 mL, 20 min, 150 W	GC-MS	>85%	–	[72]
	OPEs	Suburban airborne particles	Water-ethanol (1:1, v/v), 10 mL, 3.5 min, 180°C	SPME-GC-MS/MS	>77%	0.15 ng/m^3	[74]

Continued

TABLE 20.2 Selected Examples of Solvent Extraction Techniques Applied in the Analysis of Air Samples—cont'd

Extraction Technique	Family of Compounds	Matrix	Extraction Technique Features	Instrumental Analysis Techniques	%R	LODs	Ref
PLE	PAHs	Urban and rural outdoor air	ACN, 150°C, 1500 psi, 15 min, 3 cycles	GC-MS/MS	>43%	0.1–5 ng/sampler	[75]
	Benzothiazoles, benzotriazoles, benzensulfonamides	Urban airborne particles	Ethyl acetate, 70°C, 1500 psi, 5 min, 1 cycle	GC-MS	>60%	1.3–63.1 pg/m^3	[67]
	PAHs	Urban outdoor air	Hexane, 110°C, 500 psi, 5 min, 2 cycles	LC-GC-MS	>99%	–	[73]
	N-nitrosamines	Harbor airborne particles	Ethyl acetate, 40°C, 1500 psi, 5 min, 1 cycle	GC-MS/MS	>49%	0.1–0.2 ng/m^3	[66]
	Drugs of abuse	Urban airborne particles	MeOH and MeOH-acetone (1:1), 90°C, 1250 psi, 5 min, 2 cycles	LC-MS/MS	>23%	0.11–8.46 pg/m^3	[65]
	OCPs, PCBs	Airborne particles and gas phase of urban outdoor air	Hexane-acetone (3:1, v/v), 100°C, 1500 psi, 20 min, 2 cycles	GC-MS	>95%	0.05–0.52 pg/m^3	[72]

dl-PCB, dioxin-like polychlorinated biphenyl; *ECD*, electron capture detector; *F*, furan; *GC*, gas chromatography; *HRGC*, high-resolution gas chromatography; *HRMS*, high-resolution mass spectrometry; *LC*, liquid chromatography; *LOD*, limit of detection; *MAE*, microwave-assisted extraction; *MS/MS*, tandem mass spectrometry; *OCP*, organochlorine pesticide; *OPE*, organophosphate esters; *PAH*, polyaromatic hydrocarbon; *PCB*, polychlorinated biphenyl; *PCDD*, polychlorinated dioxin; *PLE*, pressurized liquid extraction; *%R*, %recovery; *Ref*, reference; *UAE*, ultrasound-assisted extraction.

PLE, also known as accelerated solvent extraction (ASE), is often used to overcome the main drawbacks of Soxhlet extraction. The effective extraction reached by PLE takes advantage of the increased analyte solubility at temperatures well above the boiling points of the solvents used. Although temperatures around 100°C are typical in most applications, temperatures as low as 40°C were sufficient to extract *N*-nitrosamines from PM_{10} filters [66]. On the other hand, temperatures as high as 150°C were needed to extract PAHs from a resin-based passive sampler [75]. In addition, PLE uses high pressure to keep the extraction solvent below its critical point. Typically, 1500 psi in almost all applications does not significantly affect extraction. The extraction solvent has a considerable influence on the effectiveness of the extraction, the most common being dichloromethane, hexane, ethyl acetate, acetonitrile, methanol, or their binary mixtures. Besides temperature and solvent, extraction time and number of cycles also have a great influence on the extraction. Short extraction times of around 5–10 min are usually required for contaminants in air, although typically 2–3 cycles with the same solvent are needed for quantitative extractions. Although PLE allows matrix cleanup inside the cell, which is called in-cell cleanup, this strategy is not typically required in air analysis because of the characteristics of the matrix.

Soxhlet extraction, MAE, and PLE were compared for the extraction of a group of SVOCs (OCPs and PCBs) from a high-volume sampler that collected particles with a quartz filter and gas phase with PUFs from urban outdoor air [72]. Although both phases were extracted separately, the methods followed were the same. The extraction solvent used in all the methods was hexane-acetone (3:1, v/v). However, while Soxhlet extraction was effective with 200 mL of solvent for 8 h, MAE required only 40 mL and 20 min at 150 W and PLE two cycles of 20 min at 100°C. As expected, solvent volume and extraction time were significantly lower in MAE and PLE than in Soxhlet extraction, but recoveries were higher. While Soxhlet gave recoveries up to 74%, for MAE, they were up to 85%, and for PLE, they were as high as 95%. Moreover, PLE showed good reproducibility probably because the whole extraction procedure was automated. Thus PLE was selected as the most suitable technique for carrying out the study.

Although most of the applications in air used PLE in combination with GC, PLE has also been combined with LC to determine air contaminants with low vapor pressures and high and medium polarity. One example is the determination of drugs of abuse and their metabolites [65]. Because of their physicochemical properties, only airborne particles were sampled with a $PM_{2.5}$ filter from urban air. Then, analytes were extracted by PLE using two extraction cycles with different solvents (the first with methanol and the second one with methanol-acetone (1:1, v/v)). For both cycles, temperature and extraction time were 90°C and 5 min, respectively. The final extract was concentrated by evaporation to 500 µL and analyzed by LC-MS/MS with an electrospray interface. Although recoveries were between 16% and 68%, LODs were low enough (between 0.11 and 8.46 pg/m^3) to be suitable for air concentrations.

Repeatability was also good (RSDs < 23%). Recently, a method based on PLE and GC-MS was developed for the first time to determine a group of high-production volume chemicals (benzothiazoles, benzotriazoles, and benzene sulfonamides) in airborne particles of urban and suburban outdoor air [67]. Air particles were sampled for 24 h at 30 m^3/h with a high-volume air sampler equipped with a quartz filter (PM_{10}). Then the analytes were extracted from the filter by PLE using only one cycle with ethyl acetate at 70°C and 1500 psi for 5 min. Recoveries were higher than 60%. The extract was evaporated to 1 mL and analyzed by GC-MS. LODs were between 1.3 and 63.1 pg/m^3.

20.3.2 Solid samples

The determination of organic compounds in environmental solid samples, such as soil, sediment, and sludge, frequently requires laborious multistep sample preparation procedures. This is due to the complexity of solid samples and the very low levels at which specific compounds need to be accurately determined. Traditional sample preparation procedures consist of a solvent extraction or digestion step followed by a purification of the extract. Solvent extraction is extremely efficient at transferring the compounds of interest from sample matrices that are often complex into a solution. There are numerous procedures for facilitating the transfer of analytes into the solvent: Soxhlet, a continuous extraction technique that uses a solvent at high temperatures; UAE with mechanical or ultrasound shaking; PLE, which mixes solid and solvent at high temperatures and pressures; MAE, which uses microwave heating and/or increased pressure; and supercritical fluid extraction (SFE), which uses fluids with decreased viscosity, higher permeability, and a higher diffusion rate. As in air analysis, PLE and MAE can be used instead of Soxhlet for extracting organic compounds, because they are faster than Soxhlet extraction (which takes several hours) and require much less solvent. While all of the previously mentioned are well-established approaches and have proved to be efficient for some analytes, they are less advantageous for others, as shown in a number of critical reviews [76–78].

Selected solvent extraction-based methods for the analysis of solid environmental samples are summarized in Table 20.3. Conventional solid-liquid extraction techniques such as Soxhlet are still used for some analytic procedures [79, 80]. Xiangying et al. [79] developed a simple analytic method for the simultaneous determination of multiple organic pollutants in sediment samples based on Soxhlet extraction. They used 200 mL of dichloromethane as solvent coupled with separation/cleanup on a chromatographic column packed with neutral alumina and silica gel. Five groups of pollutants were included: PAHs, OCPs, synthetic musks, UV filters, and organophosphate esters (OPEs). The method provided good recoveries with excellent reproducibility. Four sediment samples were analyzed, and most of the target compounds were found in the samples.

TABLE 20.3 Selected Examples of Solvent Extraction Techniques Applied in the Analysis of Environmental Solid Samples

Extraction Technique	Family of Compounds	Matrix	Extraction Technique Features	Cleanup	Instrumental Analysis Techniques	%R	LODs (ng/g)	Ref
Soxhlet	Multiple organic pollutants	Sediment	200-mL DCM 72 h	Alumina and silica gel column	GC-MS	63.5–126.1	0.03–0.33	[79]
SE-MASE-MIP	PAHs	Sewage sludge	30-mL hexane 16 h	–	GC-MS	17–48	0.14–12.86	[80]
UAE	OPFRs	Soil	30-mL methanol 45 min	SPE	LC-MS/MS	50–120	0.06–0.20	[81]
	Musks	Sediment	20-mL hexane/DCM (1/2; v/v) 10 min	SPE	GC-MS/MS	81–82	0.5–0.6	[82]
	Pharmaceuticals	Sewage sludge	20-mL water/methanol (1/1; v/v) 15 min	–	LC-MS/MS	50–110	< 10 for 91% of the analytes	[83]
PLE	Pharmaceuticals	Soil Sediment	Water 90°C, 500 psi, 3 cycles	SPE	LC-MS/MS	34–105	0.1–6.8	[84]
	OCPs	Sediment	DCM/hexane (4/3; v/v) 105°C, 4 cycles	Silica gel	GC-MS/MS	47–118	0.06–1.80	[85]
	Pesticides	Soil	Water/ACN (1/2; v/v) 140°C, 110 bar, 3 cycles	–	GC-MS and LC-MS/MS	12–153	6–23,000	[86]

Continued

TABLE 20.3 Selected Examples of Solvent Extraction Techniques Applied in the Analysis of Environmental Solid Samples—cont'd

Extraction Technique	Family of Compounds	Matrix	Extraction Technique Features	Cleanup	Instrumental Analysis Techniques	%R	LODs (ng/g)	Ref
PHWE	Benzothiazoles, benzotriazoles, and benzene sulfonamides	Sewage sludge	Water 80°C, 1500 psi, 1 cycle	SPE	LC-orbitrap-HRMS	>80	0.25–25	[87]
	Alkylphenols	Sediment	Water/methanol (95/5; v/v), 200°C, 2000 psi, 2 cycles	MASE	LC-MS/MS	92–103	0.024–0.600	[88]
	Pharmaceuticals	Soil Sediment	Water 100°C, 1500 psi, 3 cycles	SPE	LC-MS/MS	50–140	0.01–0.83	[89]
UMAE	Insecticides	Sediment	100-mL hexane/acetone (1/1; v/v)	SPE	GC-MS	65–141	0.27–0.70	[90]
MAME	Pharmaceuticals	Sediment	Nonionic surfactant: polyoxyethylene 10 lauryl ether (5%; v/v) (8 mL)	SPE	LC-MS/MS	>70	4–167	[91]
	Fluoroquinolone antibiotics	Marine sediment Sewage sludge	Cationic surfactant: hexadecyltrimethylammonium bromide (5%; v/v) (15 mL)	–	LC-MS/MS	> 73	0.15–0.55	[92]
MAE	PAHs	Sediment	405-μL IL (HDMImBr)	–	LC-FD	70.1–108.3	0.8–53	[93]
MAE/LDS-IT-UAEME	OPPs	Soil	MAE: water (5 mL) UAEME: toluene (20 μL)	–	GC-μECD	91–101	0.04–0.13	[94]

SFE	4-nitrotoluene and 3-nitrotoluene	Soil	CO_2 (150-μL methanol as modifier) Static time: 10 min Dynamic time: 35 min	DLLME	GC-FID	80–84	0.12 mg/Kg	[95]
	PAHs	Marine sediments	CO_2 (50-μL MeOH as modifier)	DLLME	GC-FID	67–99	0.2 mg/Kg	[96]
MSPD	PBDEs	Soil	Dispersant: bamboo charcoal	–	GC-MS	71–100	10–400 pg/g	[97]
	Antimycotic drugs	Sludge	Dispersant: C_{18}	SPE	LC-MS/MS	–	5–8	[98]
	PPCPs	Sludge	Dispersant: C_{18}	–	LC-MS/MS	50.3–107	0.117–5.55 (MQL)	[99]
QuEChERS	Aromatic organochlorines	Soil	DCM-citrate buffer (15-mL dichloromethane)	–	GC-MS	60–100	2–50	[100]
	Multiclass emerging contaminants	Soil	ACN-acetate buffer (10-mL acetonitrile)	dSPE	LC-MS/MS	60–131	0.015–3	[101]
	Steroid hormones	Soil	ACN-acetate buffer (15-mL acetonitrile)	dSPE	LC-MS/MS	75–110	0.0014–0.462	[102]

DCM, dichloromethane; *DLLME*, dispersive liquid-liquid microextraction; *dSPE*, dispersive solid-phase extraction; *FID*, flame ionization detector; *GC*, gas chromatography; *HRMS*, high-resolution mass spectrometry; *LC*, liquid chromatography; *LDS-IT-UAEME*, low-density solvent-based in-tube ultrasound-assisted emulsification microextraction; *LODs*, limits of detection; *MAME*, microwave-assisted micellar extraction; *MASE*, membrane-assisted solvent extraction; *MeOH*, methanol; *MS*, mass spectrometry; *MS/MS*, tandem mass spectrometry; *MSPD*, matrix solid-phase dispersion; *OCPs*, organochlorine pesticides; *OPFRs*, organophosphorus flame retardants; *OPPs*, organophosphorus pesticides; *PAHs*, polycyclic aromatic hydrocarbons; *PBDEs*, polybrominated diphenyl ethers; *PHWE*, pressurized hot water extraction; *PLE*, pressurized liquid extraction; *PPCPs*, pharmaceutical and personal care products; *QuEChERS*, quick, easy, cheap, effective, rugged, and safe extraction; *%R*, %recovery; *SE-MASE-MIP:* Soxhlet extraction-membrane-assisted solvent extraction-molecularly imprinted polymer; *SFE*, supercritical fluid extraction; *SPE*, solid-phase extraction; *UAE*, ultrasound-assisted extraction; *UMAE*, ultrasonic microwave-assisted extraction; *USAEME*, ultrasound-assisted emulsification microextraction; *μECD*, μelectron capture detector.

The main disadvantage of current extraction techniques for complex solid samples is that most of them still require an additional cleanup step. Sample preparation with multiple steps is tedious and susceptible to errors because analytes can be lost or contaminated. Attempts to eliminate these steps led to a single-step method known as the Soxhlet extraction-membrane-assisted solvent extraction-molecularly imprinted polymer (SE-MASE-MIP) technique for the selective extraction of PAHs as model compounds from complex solid samples [80]. This technique combines three extraction devices in a single entity to eliminate the need for a further cleanup step. Soxhlet extraction was targeted for its high efficiency and reproducibility, membrane-assisted solvent extraction for its size-selectivity, and the molecularly imprinted polymer for its specificity toward the target compounds. The extraction process takes 16 h and uses 30 mL of hexane. LODs ranged from 0.14 to 12.86 ng/g with RSD values for the 16 USEPA priority PAHs from wastewater sludge samples between 0.78% and 18%.

Novel extraction techniques were developed to reduce not only the amount of solvent but also the long extraction time associated with Soxhlet extraction, for example, UAE, MAE, SFE, and PLE [103]. Compared with the Soxhlet technique, UAE is an expensive but efficient alternative, which also extracts the analytes that may be altered in the working conditions for Soxhlet extraction. Moreover, cavitation increases the polarity of extractants and analytes, thus enhancing recovery. Other advantages of UAE over MAE and PLE are the lower cost of the apparatus, the ease operation, and the fact that it can be used with any solvent. Extraction of analytes mainly depends on the polarity of the solvent, the nature and the homogeneity of the sample, the ultrasound frequency, and the sonication time.

A cleanup step is usually performed after UAE extraction, especially when analyzing complex matrices such as sewage sludge. An SPE cleanup is typically performed, often with Oasis HLB, C_{18}, and silica gel cartridges [104, 105]. Several groups of organic compounds have been efficiently extracted from soil, sediment, and sludge samples using UAE with different organic solvents and SPE as a cleanup step [81–83]. Lorenzo et al. [81] reported UAE extraction in combination with LC-MS/MS with a cleanup step with Strata-X polymeric reverse-phase cartridge for the determination of OPFRs in soil. UAE was carried out three times with 10 mL of methanol for 15 min. Recoveries were 50%–120% and the precision less than 12% RSD. The LODs ranged from 0.06 to 0.20 ng/g dry weight (d.w.), and target compounds were detected in all soil samples analyzed from 13.8 to 89.7 ng/g (d.w.).

PLE is already a routine technique for the trace analysis of organic contaminants. It is used in many laboratories because of its ease of handling, short extraction time, low sample-amount requirements, and good performance. The small volumes of organic extracts obtained by PLE (containing analytes and soluble matrix) facilitate further concentration and cleanup, typically carried out by SPE, SPME, and gel-permeation chromatography (GPC) [84–86, 106–108]. Duodu et al. [85] evaluated

the possibility of combining the extraction and cleanup steps into a single PLE step for the extraction of OCPs from sediment samples followed by GC-MS/MS. Most of the OCPs had recoveries higher than 80% and LODs in the range 0.06–1.80 ng/g. The method is highly selective and sensitive and drastically reduces the cost and time of analysis.

Low-to-medium polarity analytes can be effectively extracted by water in pressurized hot water extraction (PHWE), a version of PLE, as the viscosity and surface tension decrease and diffusivity increases for water with an increase in temperature. PHWE is a green method that uses water at high temperatures (100–374.1°C the critical temperature of water) and high pressure so that it remains in the liquid state. PHWE was used to extract organic compounds from soil, sludge, and sediment matrices [106, 109]. After extraction, the low levels of target compounds and the coextraction of a large number of potentially interfering compounds require a cleanup and preconcentration step, with SPE being the most commonly used technique for this purpose [87–89]. A group of five benzotriazoles, four benzothiazoles, and four benzene sulfonamides were determined in sewage sludge samples by SPE-PHWE-LC. The cleanup included a tandem SPE consisting of an Oasis HLB cartridge and a Florisil cartridge. The absolute recoveries were generally over 80%, and the matrix effect was lower than 20% for most of the compounds by high-resolution mass spectrometry. The LODs ranged from 0.25 to 25 ng/g depending on the compound [87].

MAE is an effective technique for the rapid extraction of a number of trace organic pollutants from solid environmental samples, due to advantages in facilitating on-line measurements, high efficiency, and significantly lower extraction time and solvent consumption than traditional techniques [110–113]. The mass-transfer mechanism in extraction can be enhanced by ultrasonic MAE (UMAE). Li et al. [90] developed an UMAE-SPE method to isolate organophosphorus and pyrethroid insecticides from sediment. Due to a significant reduction in extraction time, the UMAE method greatly improved the extraction efficiency of thermally labile and volatile insecticides. A variety of procedures have been developed in MAE using environmentally friendly solvents (e.g., microwave-assisted micellar extraction (MAME), IL-based MAE, and microwave-assisted aqueous-solution extraction). Cueva-Mestanza et al. [91] developed a cost-effective MAME-SPE method for the simultaneous determination of eight pharmaceutical compounds in sediment samples, using a nonionic surfactant (polyoxyethylene 10 lauryl ether). A cationic surfactant (hexadecyltrimethylammonium bromide) was successfully used as an extractant with MAME and LC-MS/MS for the determination of fluoroquinolone antibiotics in coastal marine sediments and sewage sludge samples [92]. PAHs have been extracted from sediments by MAE using aqueous solutions containing aggregates of IL 1-hexadecyl-3-methylimidazolium bromide (HDMImBr) as the extracting medium followed by LC with fluorescence detection without a cleanup step to

remove the IL before injection [93]. Su et al. [94] coupled solventless MAE with low-density solvent-based in-tube ultrasound-assisted emulsification microextraction (LDS-IT-UAEME) to determine OPP pesticides in soils. The method was shown to be highly competitive in terms of sensitivity, cost, and speed of analysis.

SFE is another well-known technique used for isolating and preconcentrating organic compounds in solid environmental samples [114, 115] and can be used for a variety of solid samples. SFE requires less organic solvent, has a short extraction time, and can extract thermally labile compounds under mild conditions. Although SFE is an environmentally friendly technique, it is not in widespread use because of the relatively high cost of the equipment and the difficulty of extracting many compounds. Due to the selectivity of the SFE process, the extracts obtained by this technique have low concentrations of undesired compounds and can often be introduced directly into analytic devices without a further cleanup step. Nevertheless, a cleanup step is often required after extraction and the collecting solvent needs to be evaporated before analysis. DLLME combined with SFE is an efficient sample preparation method for solid samples. This combination prevents the solvent from vaporizing after extraction and increases the preconcentration factor for organic compounds in soils and sediments [95, 96]. It allows the determination of 4-nitrotoluene and 3-nitrotoluene in soil samples by SFE-DLLME-GC-flame ionization detector (FID) [95] and PAHs in marine sediments [96].

The matrix solid-phase dispersion (MSPD) technique and quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction are characterized by the use of small solvent volumes and facilitate a single-step extraction and cleanup. MSPD is popular because it is more straightforward (it does not require any instrumentation or specific equipment), flexible, and rugged than other sample preparation techniques. The mild extraction conditions (i.e., room temperature and atmospheric pressure) preserve analytes from degradation and denaturation [116, 117]. Nevertheless, MSPD has sometimes been used in conjunction with PLE to increase recoveries for compounds that interact strongly with the solid matrix [118]. An MSPD-GC-MS method for the analysis of eight major PBDEs in soil was developed by Yuan et al. [97] using bamboo charcoal as a dispersive sorbent. The use of charcoal results in efficient extraction, and the method is low-cost, easy to operate, and suitable for the routine monitoring of low levels of PBDEs in soil. MSPD is an attractive alternative to other sample preparation techniques for extracting pharmaceuticals and personal care products (PPCPs) with different physicochemical properties in sewage sludge samples for LC-MS/MS analysis [99]. The QuEChERS method is rapid and simple and consumes small amounts of solvent, which is a significant advantage in the era of green chemistry. It provides good recovery and sensitivity for many compounds, even in the case of multiresidue methods containing compounds of a wide polarity range [119, 120]. The QuEChERS method in its original form has only been applied in very few cases, most studies adopting procedures that have been modified

to a greater or lesser extent. UAE and/or the modification and/or the elimination of the cleanup step have been introduced to improve recoveries [102, 119]. By way of example, a multiresidue method was developed by Rouvière et al. [100] for the simultaneous analysis of 43 aromatic organochlorine compounds in soil by GC-MS. The QuEChERS method uses a water-immiscible solvent with no additional cleanup by dispersive SPE (dSPE), which reduces the cost of the analysis. This method can determine highly volatile compounds in soils, which cannot be determined by conventional methods, because they generally require water to be removed.

20.3.3 Water

Liquid-phase extraction techniques are widely used to extract organic compounds from environmental water samples. On the one hand, if these extraction techniques are being used for the first time, they are used as part of an analytic method for proof of concept. For these methods, simple, less sensitive detectors are often used, for example, UV in LC or FID or single quadrupole MS in GC. On the other hand, for determining or monitoring the presence of organic compounds, typical low concentrations require high-tech detectors based on MS. Model compounds such as PAHs, pesticides, or UV filters have often been used to evaluate emerging techniques. Table 20.4 shows selected examples of the various liquid-phase extraction techniques for the analysis of environmental water samples. The subsections later discuss the main liquid-phase extraction techniques for water analysis.

20.3.3.1 LLE Techniques

LLE is still used to analyze environmental water samples, even though it is one of the oldest sample extraction techniques and is progressively being replaced by other techniques. For instance, a group of novel brominated contaminants were extracted from 200 mL of river or sewage water samples using 100 mL of dichloromethane in triplicate. After salt addition (10% NaCl) and pH adjustment (4–5), recoveries exceeded 75% in most cases [121]. In order to avoid using large amounts of organic solvent, green or environmentally friendly solvents such as ILs, deep eutectic solvents (DESs) [155], and SUPRAS [156] have also been used. The use of surfactant-based extraction media and CPE is an alternative. For instance, a group of UV filter compounds were first extracted from river and lake water samples by CPE using a nonionic surfactant (Triton X-114) and the surfactant-rich phase further extracted by means of dSPE using magnetic nanoparticles with completely recovery (88%–97%) of the analytes [125]. These green solvents have also been used extensively with other microextraction techniques for water analysis. A common feature of LLE and CPE techniques for the extraction of water samples is that they extract more sample (usually between 100 and 1000 mL) than other LPME techniques,

TABLE 20.4 Selected Examples of the Extraction Techniques Applied in the Analysis of Environmental Water Samples

Extraction Technique	Family of Compound	Matrix (Type of Water)	Extraction Technique Features	Instrumental Analysis Technique	%R	LODs (ng/L)	Ref
LLE	Brominated flame retardants	Tap, river, sewage	Sample volume: 200 mL 3 × 100-mL DCM 10% NaCl	LC-orbitrap	59–101%	0.8–0.9	[121]
	Chloramphenicol (pesticide)	Lake, feed water from farmsteads	Sample vol: 50 mL 3-mL[C_4mim][Cl]/K_2HPO_4 (IL)	LC-UV	95–98%	100	[122]
	Benzimidazole fungicides	River, ground water	Sample volume: 20 mL 100 µL: decanoic acid/ decanoate (1:1, *w/v*) C_{10}:TBA^+ (SUPRA)	LC-FL	75–102	0.1–32	[123]
CPE	Fluoroquinolones	Lake, river, and sewage	SDS and HCl 12 M	LC-FL	83–97	7000–13,000	[124]
CPE-dSPE	UV filters	Lake, river	Sample volume: 50 mL TritonX-114 (0.5 w/v)	LC-DAD	88–97	1.5–7.5	[125]
DI-SDME	Pesticides	River	Sample volume: 4 mL 1-µL heptane	GC-MS	90–108	30–80	[126]
IL-HS-SDME	Chlorobenzenes	Tap, river, sewage	Sample volume: 10 mL 5-µL [C_4MIM][PF_6]	LC-DAD	61–100	100	[127]
Mag-IL-HS-SDME	Chlorobenzenes	Tap, pond, sewage	Sample volume: 20 mL 1-µL 1-ethyl-3-methylimidazolium tetrathiocyanatocobaltate(II)	TD-GC-MS	90–113 (Rel %R)	4–8	[128]
SFO-SDME	PBDEs	Surface	Sample volume: 40 mL 25-µL 2-dodecanol	LC-UV	46–74	10–40	[129]

HF(2)-LPME	Pesticides	Spring, rain, groundwater	Sample volume: 10 mL 3.3-μL 1-octanol	GC-MS	69–120	2–12	[130]
HF(3)-LPME	Salicylates	River, seawater	Sample volume: 10 mL 1-octanol impregnate pores 15-μL water at pH 3	LC-UV	80–114 (Rel %R)	600–1200	[131]
	Steroid hormones	Tap, sewage	Sample volume: 100 mL 10-μL di-n-hexylether	GC-MS	EF: 1500–3400	1.5–10	[132]
	Aliphatic and aromatic hydrocarbons	Strom	Sample volume: 10 mL 5-μL toluene IL impregnated	GC-MS	EF: 53–210	1–5	[133]
BT-LPME	PAHs	River	Sample volume: 15 mL 15-μL CCl_4 (5 μL analyzed)	GC-MS	92–103	2–11	[134]
EME	DBPs	Sewage	Sample volume: 23 mL Toluene (impregnated membrane) 150-μL aqueous phase 200 V	LC-UV	87–106	7–40	[135]
	Drugs	Sewage	Sample volume: 4 mL Agarose membrane 100-μL aqueous phase 25 V	LC-UV	38–74	1.5–1.8	[136]
DLLME	PAHs	Surface, river, well	Sample volume: 5 mL 8-μL C_2Cl_4 (extraction solvent) 1-mL acetone (dispersing agent)	GC-FID	71–111	7–30	[137]
	LAS, phthalates, nonylphenols	Tap, river, sewage	Sample volume: 8 mL 50-μL dichlorobenzene (extraction solvent) 1.5-mL MeOH (dispersing agent)	LC-MS/MS (QqQ)	57%–80% Except LAS C_{10} 30%–36%	9–200 (LOQs)	[138]

Continued

TABLE 20.4 Selected Examples of the Extraction Techniques Applied in the Analysis of Environmental Water Samples—cont'd

Extraction Technique	Family of Compound	Matrix (Type of Water)	Extraction Technique Features	Instrumental Analysis Technique	%R	LODs (ng/L)	Ref
ST-DLLME	Carbamate pesticides	Lake	Sample volume: 5 mL 15-μL toluene (extraction solvent) 0.5-mL ACN (dispersing agent) 0.5-mL ACN (terminating)	GC-MS	95–104	1–500	[139]
VALLME	PFAS	Seawater	Sample volume: 35 mL 10-μL octanol (extraction solvent) 5 min, 1800 rpm	LC-LTQ-Orbitrap	95–105	0.2–3 ng/l	[140]
UA-DLLME-SFO	PFAS, plasticizers, preservatives, flame retardants	Tap, surface	Sample volume: 10 mL 80-μL 1-undecanol (extraction solvent) 0.5-mL MeOH (dispersing agent) Sonication 5 min (no info kHz) Cooled ice bath 5 min	LC-QqQ	20–95	10–1400	[141]
UA-DLLME	Benzophenone UV filters	Swimming pool, river	Sample volume: 8 mL 30-mg trioctylmethylammonium chloride and decanoic acid (DES) (extraction solvent) 40 kHz (dispersing agent)	LC-UV	84–105 (Rel %R)	100–300	[142]
USAME	Musk fragrances, phthalate esters, lindane	Tap, bottle, swimming pool, river, harbor seawater	Sample volume: 10 mL 100-μL chloroform (extraction solvent) 40 kHz (dispersing agent)	GC-MS	78–114	6–133	[143]
	PCBs	Tap	Sample volume: 10 mL 200-μL isooctane (extraction solvent) 35 kHz (dispersing agent)	GC-MS	87–92	3–12	[144]

UASEME	PAHs	Tap, sewage	Sample volume: 5 mL 20-µL cyclohexane (extraction solvent) 10-µL Tween 80 (emulsifier) 40 kHz (dispersing agent)	LC-FL	EF: 90–247	0.6–62.5	[145]
UASO-HLLME	Triazole pesticides	Surface	Sample volume: 3 mL 1.3-g NaCl 650-µL ACN (extraction solvent) no info ultrasounds	GC-MS	87–119	400–14,4000	[146]
IL-UA-DLLME	Benzophenone UV filters	Tap, swimming pool, river	Sample volume: 10 mL 20-µL [HMIM][FAP](extraction solvent) 0.1-mL MeOH (dispersing agent) 35 kHz	LC-UV	71–118 (Rel %R)	200–5000	[147]
TIL-DLLME	Pyrethroid pesticides	Tap, reservoir, groundwater, river	Sample volume: 10 mL 45-µL [C_6MIM][PF_6] (extraction solvent) Heated at 70°C Cooled in ice bath	LC-UV	77–136	300–600	[148]
	UV filters	Swimming pool, tap	Sample volume: 5 mL 20-µL [C_6MIM][FAP] (extraction solvent) heated at 50°C cooled at 0°C	LC-UV	88–116 (Rel %R)	1.2–5.3	[149]
MA-DLLME	Pesticides	Tap	Sample volume: 10 mL 260-µL [N_{8881}][Tf_2N] (extraction solvent) 1-mL MeOH (dispersing agent) 200 W	LC-DAD	40–100		[150]
MSA-DLLME	Benzophenone UV filters	Lake	Sample volume: 20 mL 40-µL 1-octanol (extraction solvent) 1300 rpm (dispersing agent)	LC-UV	EF: 59–107	200–800	[151]

Continued

TABLE 20.4 Selected Examples of the Extraction Techniques Applied in the Analysis of Environmental Water Samples—cont'd

Extraction Technique	Family of Compound	Matrix (Type of Water)	Extraction Technique Features	Instrumental Analysis Technique	%R	LODs (ng/L)	Ref
Ferrofluid-LPME	PAHs	River	Sample volume: 20 mL 100-µL 1-octanol (extraction solvent) 10-mg silica-coated magnetic particles 100-µL ACN (desorbing solvent)	GC-MS	59–93	17–57	[152]
KWLPME	Benzophenone UV filters	Swimming pool	Sample volume: 20 mL 8-µL 1-octanol/ perchloroethylene (25/75, v/v) 0.8 cm of wool 30-µL ACN (desorbing solvent)	LC-UV	77–102 (Rel %R)	15,000–20,000	[153]
SBDLME	UV filters	River, seawater, swimming pool	Sample volume: 25 mL 25-µL [P6,6,6,14][Ni(hfacac)$_3^-$] (MIL) (extraction solvent)	TD-GC-MS	87–117	10–27	[154]

ACN, acetonitrile; *BT-LPME*, ballpoint tip-protected LPME; *CPE*, cloud-point extraction; *DAD*, diode array detector; *DBPs*, disinfection by-products; *DCM*, dichloromethane; *DES*, deep eutectic solvents; *DI-SDME*, direct immersion SDME; *DLLME*, dispersive liquid-liquid microextraction; *dSPE*, dispersive SPE; *EF*, enrichment factor; *EME*, electromembrane extraction; *FL*, fluorescence detector; *GC*, gas chromatography; *HF-LPME*, hollow-fiber LPME; *HS-SDME*, headspace-SDME; *IL*, ionic liquid; *KWLPME*, knitting wool LPME; *LAS*, linear alkyl sulfonates; *LC*, liquid chromatography; *LLE*, liquid-liquid extraction; *LLME*, liquid-liquid microextraction; *LOQs*, limits of quantification; *LPME*, liquid-phase microextraction; *LTQ*, linear trap quadrupole; *MA*, microwave; *MeOH*, methanol; *MIL*, magnetic IL; *MMLLE*, microporous membrane liquid-liquid extraction; *MS*, mass spectrometry; *MS/MS*, tandem mass spectrometry; *MSA*, magnetic stirring-assisted; *OPPs*, organophosphorus compounds; *PAHs*, polycyclic aromatic hydrocarbons; *PBDEs*, polybrominated diphenyl ethers; *PCBs*, polychlorinated biphenyls; *PFAS*, polyfluoroalkyl compounds; *QqQ*, triplequadrupole; *%R*, % recovery; *Rel %R*, relative %R; *SBDLME*, stir bar dispersive liquid microextraction; *SD-DLLME*, solvent-terminated DLLME; *SDME*, single-drop microextraction; *SDS*, sodium dodecyl sulfate; *SFO-SDME*, solidification floating organic drop microextraction; *SPE*, solid-phase extraction; *ST-DLLME*, solvent-based terminated DLLME; *TBA*, tetrabutylammonium; *TD*, thermal desorption; *TIL*, temperature-controlled IL; *UA*, Ultrasonication; *UASEME*, ultrasound-assisted surfactant-enhanced emulsification microextraction; *UASO-HLLME*, ultrasound-assisted salting-out homogeneous LLME; *USAME*, Ultrasound-assisted microextraction; *UV*, Ultraviolet detector; *VALLME*, vortex-assisted LLME; *[HMIM][FAP]*, 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate; *[N_{8881}][Tf_2N]*, trioctylmethylammonium bis(trifluoromethylsulfonyl)imide; *[C4MIM][PF6]*, 1-butyl-3- methylimidazolium hexafluorophosphate; *[C_6MIM][PF_6]*, 1-hexyl-3-methylimidazolium hexafluorophosphate.

which typically are applied to sample volumes between 5 and 20 mL. Nonetheless, the larger the amount of sample, the greater the volume of organic solvent required. Thus, the amount of sample has no effect on the preconcentration factor achieved.

Another way to avoid using the large solvent volumes of required by LLE and to reduce the number of manipulation steps is to use LPME techniques. For water samples, DLLME, with all its variations, is one of the main techniques used. Nonetheless, other microextraction techniques are also often used. Fig. 20.4 shows the distribution of various LPME techniques for water analysis.

20.3.3.2 Single-Drop Microextraction

SDME was the first solvent-based microextraction approach, and it is now gradually being replaced by other LPME techniques to avoid the instability of the droplet giving a lack of robustness. Despite this, SDME has been used to determine different compounds from water samples, in different modes, for example, DI-SDME [126], HS-SDME [128, 157], continuous-flow microextraction (CFME) [158], and solidification floating organic drop microextraction (SFOD-SDME) [159]. The solvent used in SDME for environmental aqueous samples is similar to the solvents used in other applications, although it must have a low vapor pressure, low water solubility, and a lower density than water [160]. The choice of solvent also depends on its extraction capacity and selectivity. Some examples of typical solvents are n-hexane, toluene, 1-octanol, 1-dodecanol, and undecane. 1-Undecanol, 2-dodecanol, and n-hexadecane were compared in the SDME extraction of a group of trihalomethanes from drinking water. A 7-μL droplet of 1-undecanol exposed for 15 min floated on the surface of 10 mL of drinking water at 60°C containing 3-M NaCl and stirred at 750 rpm provided the best extraction efficiency for the selected compounds [161]. ILs have also been used as alternative extractants to improve the stability of the droplet. For instance, Vidal et al. [127] extracted a group of chlorobenzenes from 10 mL of water using HS-SDME and a 5-μL microdrop of the IL 1-hexyl-3-methyl hexafluorophosphate. Later, it was shown that 1 μL of a magnetic ionic liquid in HS-SDME was sufficient to extract the same group of compounds from 20-mL aqueous samples [128]. The magnetic features of the ionic liquid improved the extraction, which, despite the low volume of ionic liquid, was robust enough to achieve better figures of merit [127].

20.3.3.3 Membrane-Assisted Microextraction Approaches

Although some authors assert that the droplet is quite robust and reproducible during the extraction, protecting this droplet with membranes has improved its stability and reproducibility. Thus HF protected LPME or HF-microporous membrane liquid-liquid extraction (HF-MMLLE) has gained in popularity since it was first used. This technique was used to extract such organic compounds as haloacetic acids, BTEX,

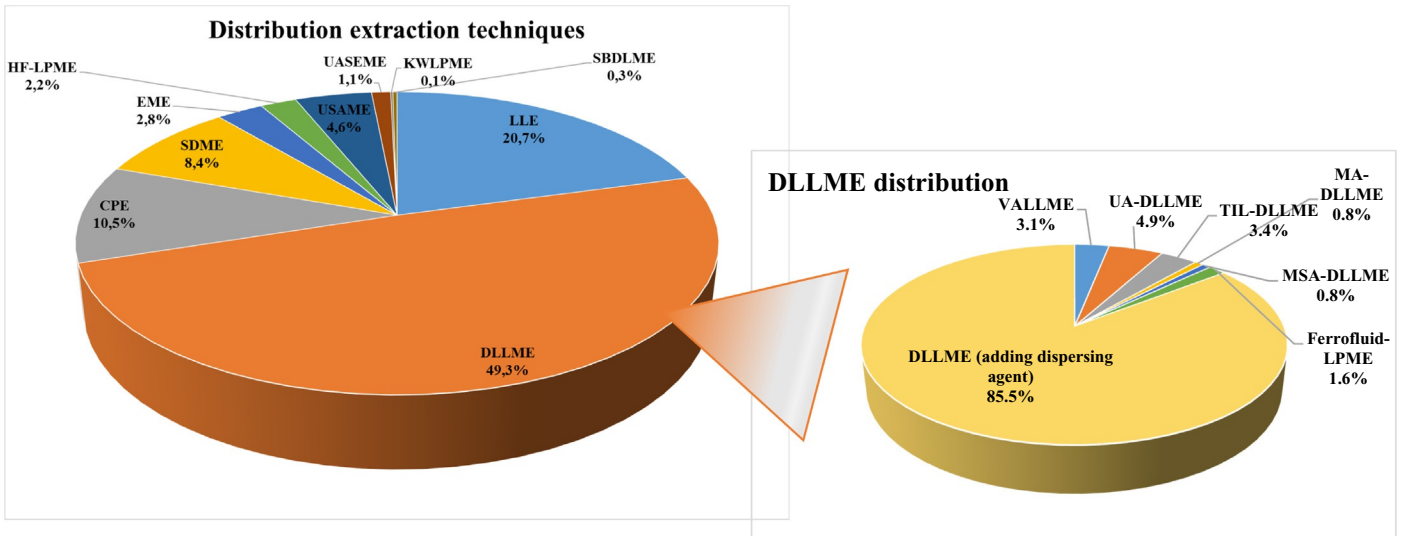

Fig. 20.4 Frequency of application of solvent extraction techniques in water samples in the period 2007–18. *(Source: Scopus. Date: October 2018. Keywords: water, environment, and the name of the extraction techniques defined in Section 3.3.)*

haloethers, sulfonamides, phenols, drugs, estrogens, and pesticides from environmental water samples [34]. In the two-phase approach, typically, 1-octanol and toluene are used as the extractant. However, the latter is largely avoided because, in spite of being membrane protected, it often partially or totally evaporates after the extraction process. This is because extraction by this technique takes longer (from 20 to 400 min) than SDME, which generally takes only 5–20 min. The determination of pyrethroid insecticides with HF-LPME in the two-phase mode using 1-octanol followed by GC-MS was performed after optimizing the variables for the extraction. It was found that extraction efficiency increased with extraction time up to 6 h, when equilibrium was attained [130]. It should be mentioned that the volume of organic solvent depends on the length of the HF and can be greater, than in SDME. However, only part of the extract can be analyzed, so the LODs of the method cannot be improved. Toluene, dihexyl ether, hexane, or other low-boiling-point solvents are more widely used in the three-phase approach. Some authors have also resorted to ILs to impregnate the membrane. Basheer et al. [133] evaluated the combination of toluene with 1-butyl-3-methylimidazolium ionic liquids with different anions as solvent in a three-phase HF-LPME followed by GC-MS to determine a group of aliphatic and aromatic hydrocarbons from storm water. In this three-phase approach, IL impregnated the membrane, and toluene was the acceptor phase. The HF-LPME approach was better than SPME and gave higher relative recoveries and selectivity. The enhanced selectivity is attributed to the fact that the pores of the HF act as a barrier preventing the introduction of larger particles. Ji et al. proposed ballpoint tip-protected liquid-phase microextraction (BT-LPME) in which the droplet of organic solvent is confined in the hollow cavity of a bullet-shaped ballpoint tip [134]. BT-LPME was used to extract PAHs from river water. As the droplet is protected, the spinning speed can be maximized. In this particular study, it was set at 1000 rpm, but it was able to stand up to 1500 rpm. Taking advantage of this, the authors compared the stability of the 2-μL carbon tetrachloride SDME droplet with the 15-μL droplet of the same solvent in BT-LPME. For an extraction time of 20 min, a stirring speed of 250 rpm in SDME dissolved the drop, whereas in BT-LPME the speed could be up to 1250 rpm. This faster speed led to larger enrichment factors for the same compounds. Other membrane-assisted LLE techniques have been proposed to the extent that in recent years, the original supported liquid membrane (SLM) extraction technique has largely been replaced for the extraction of organic compounds from water samples. On the contrary, EME in which an electric potential applied across the membrane promotes the transport of the compounds by electromigration has been utilized in some studies. Two families of disinfection by-products (DBPs) were extracted from sewage water with EME under the following conditions: toluene as organic solvent and alkaline aqueous solution as acceptor and with a positive electrode in the acceptor solution and a negative electrode in the sample. Several parameters including sample pH; extraction time; ionic strength;

and, in particular, the voltage applied (200 V in this study) were optimized [135]. Under these conditions, recoveries up to 87% and LODs between 7 and 40 ng/L were achieved. Recent EME applications have focused on improving analyte transfer to the acceptor phase by designing novel supporting materials that reduce or avoid the use of organic solvents. For example, agarose gel was used as a membrane in EME to extract drugs from sewage water samples [136] or chlorophenol compounds from river and lake water samples [162]. Another strategy, which has been successfully tested for the extraction of herbicides in river water, is to add surfactants (e.g., Triton X-114) to the acceptor phase [163].

20.3.3.4 Dispersive Liquid-Liquid Microextraction

DLLME is the microextraction technique most widely used for the analysis of water samples. DLLME was presented in 2006 for the extraction of PAHs from environmental water samples. More specifically, a mixture containing 1 mL of acetone (dispersing agent) with 8 μL of tetrachloroethylene (extraction solvent) was quickly injected into 5 mL of aqueous sample and the mixture shaken. The cloudy solution was centrifuged, and the sedimented phase (2 μL) was analyzed by GC [137]. Since then, the number of applications has increased considerably, and they cover several types of organic compounds (PAHs, pesticides, phthalates, linear alkyl sulfonates [LASs], UV filters, personal care products, BTEXs, musks, phenols, PBDEs, hormones, etc.) extracted from different types of water samples (drinking, tap, sewage, river, spring, well, lake, swimming pool, fish farm, etc.). Proof of this is that some reviews have been published in recent years that are exclusively on DLLME [164–167].

The typical volume of aqueous samples is 5 or 10 mL, for which the pH and ionic strength are suitably adjusted. The most usual dispersing agents are methanol, acetone, and acetonitrile with volumes between 0.5 and 2 mL. Typical extraction solvents in volumes on the microliter scale (normally ranging from 10 to 100 μL) are halogenated solvents such as carbon tetrachloride, chloroform, chlorobenzene, tetrachloroethylene, and alcohols such as 1-octanol and dodecanol and other low-density solvents such as toluene, n-hexane, or cyclohexane [164, 166]. When these latter solvents are used, the technique is known as low-density extraction solvent-based solvent-terminated DLLME (ST-DLLME or SD-DLLME). Moreover, in ST-DLLME, the phases are separated by adding a disperser solvent and not by centrifugation, which reduces the total extraction time. For example, for the extraction of carbamate pesticides from a lake water sample, a method based on ST-DLLME followed by GC-MS was developed. A mixture containing 0.5 mL of acetonitrile (dispersing agent) with 15 μL of toluene (extraction solvent) was rapidly injected into 5 mL of aqueous sample, and an emulsion was formed. After 2.5 min, 0.5 mL of acetonitrile (demulsifier solvent or terminated solvent) was injected to break the emulsion, and two phases were formed with no need for centrifugation.

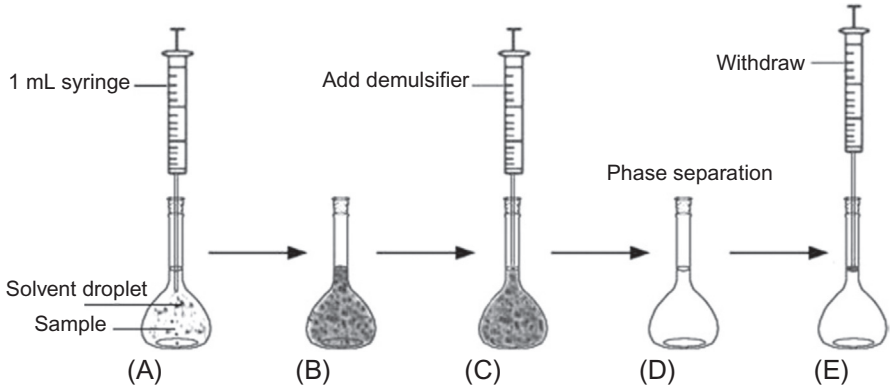

Fig. 20.5 Steps involved in ST-DLLME in the example of the extraction of a group of carbamate pesticides from lake water samples. (A) Injection of extraction solvent and disperser solvent into aqueous sample, (B) formation of emulsion for extraction, (C) addition of terminating solvent to break up the emulsion, (D) phase separation, and (E) collection of low-density extraction solvent in the upper layer. *(From Chen H, Chen R, Li S. Low-density extraction solvent-based solvent terminated dispersive liquid–liquid microextraction combined with gas chromatography-tandem mass spectrometry for the determination of carbamate pesticides in water samples. J Chromatogr A 2010;1217:1244–8 with permission of Elsevier.)*

A total of 1 μL of the organic phase was directly injected into the GC-MS [139]. Fig. 20.5 illustrates the steps involved for the extraction of carbamate pesticides by ST-DLLME from a lake water sample.

The main drawback of this technique is that it requires a dispersing solvent that often decreases the partition constant of the analytes into the extraction solvent, and strategies that favor the microemulsion of the extraction solvent have been proposed as a solution. For the extraction of *per*- and polyfluoroalkyl compounds (PFAS) from seawater, vortex-assisted LLME (VALLME) was selected because it does not require the samples to be filtered, which may retain PFAS. In this case, instead of adding a dispersing solvent, a microemulsion was attained by agitating 100 μL of octanol and 35 mL of seawater in a vortex. Complete recoveries were obtained (95%–105%), which were higher than for a similar group of compounds extracted from larger volumes (i.e., 100–1000 mL) of environmental water samples with SPE [140]. A similar group of PFAS was included in the multiresidue extraction (including hormones, plasticizers, preservatives, and flame retardants) using DLLME. In this case the microemulsion and phase separation were assisted by UAE for 5 min, and the resulting organic droplets were cooled in an ice bath for 5 min, resulting in solidified droplets (i.e., SFOD). The technique used was UAE-DLLME-SFOD. Among compounds studied, PFAS were the group most influenced by the length of sonication (the longer it was, the higher the recovery) [141]. García-Jares was the first to use

ultrasounds to promote emulsification in UAEME [143]. UAEME was used to extract musk fragrances, phthalate esters, and lindane from different water samples (including bottled mineral water and seawater), using chloroform as extraction solvent. For some compounds, the addition of surfactant in combination with ultrasounds helped dispersion and reduced the extraction time. This technique is known as ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME), which was used, for example, to extract PAHs from tap and sewage water [145]. Another modification, known as ultrasound-assisted salting-out homogeneous liquid-liquid microextraction (UASO-HLLME), was used for the extraction of triazoles from surface water in which the addition of the salt (NaCl) avoids the need for a second solvent, [146]. In a few cases, ionic liquids and decanoic acid were used as extraction solvents for UV filters in water samples by UAE-DLLME in which the conventional organic solvent was replaced by octylmethylammonium chloride and decanoic acid [142] or the IL 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate [147], both of which were selected after testing different DESs and ILs, respectively. The variant temperature-controlled ionic liquid DLLME (TIL-DLLME) in which temperature variation is used to promote solubility of the ionic liquid and later the phase separation was used to extract organic compounds from water samples. Zhang et al. [149] used this approach to extract UV filters from swimming pool and tap water samples. In detail, the IL (20 μL of 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate) was dissolved in the sample (5 mL) by heating to 50°C, which also promoted the migration of the analytes into the IL phase. Later, the solution was cooled to 0°C and centrifuged to separate the phases. The authors also compared the features of TIL-DLLME with other solvent-based microextraction techniques (i.e., SDME, HF-LPME, and IL-DLLME) and found that apart from the shorter extraction time (the increase in temperature means that equilibrium is reached quickly), another significant advantage is that TIL-DLLME does not require a dispersant agent, which simplified the process and led to higher extraction efficiency. Another approach, MAE-DLLME, was proposed for the extraction of pesticides from tap water and magnetic stirring-assisted DLLME (MSA-DLLEE) to extract UV filters from lake water samples [151]. As indicated in Fig. 20.4, the use of these techniques is less common than for other microextraction techniques. They are often applied merely for the purpose of evaluation.

20.3.3.5 Other Microextraction Techniques

Lee's research group [152] presented one of the more unusual LPME techniques—ferrofluid-based LPME followed by GC-MS—to determine PAHs in river water. In this extraction, 1-octanol (extraction solvent) was confined within the pores and interstices of the silica-coated magnetic particles that accelerated the extraction process. The method showed high extraction efficiency with low LODs 17–57 ng/L, and the recoveries for the target analytes were between 59% and 93%. The same research

group also proposed using knitting wool (knitting wool LPME-KWLPME-) as the extractant phase holder for a group of benzophenone UV filters when analyzing swimming pool water [153]. Stir bar dispersive liquid microextraction (SBDLME) was used for the extraction of UV filters from bathing water (river, sea, and swimming pool) [154] and for PAHs from river, tap, and rain water [168]. The inclusion of a magnetic ionic liquid in SBDLME allows the rotation of the coating. Moreover, the recoveries were in the range 87%–117%, and the LODs attained when SBDLME was coupled to TD-GC-MS were at low nanogram per liter concentration levels.

20.3.3.6 Combination of LPME Techniques With Other (Micro) extraction Techniques

DLLME was combined with other extraction methods, mainly dSPE, to achieve high analyte preconcentration and for further sample cleanup for the extraction of PAHs, chlorophenols, 4-nonylphenol, dodecyl alcohol ethoxylates, and pesticides in surface water samples. As an example, for the extraction of PAHs, 20 μL of n-octanol was rapidly injected into 20 mL of river water and the mixture vortexed for DLLME. Subsequently, as part of the dSPE protocol, 10 mg of magnetic nanoparticles was added and stirred for 1 min to retrieve the n-octanol. Next, the nanoparticles were separated with a magnet and eluted with 100 μL of acetonitrile, a fraction of which was analyzed by GC-MS [169]. Thus, dispersive SPE addressed some of the limitations of DLLME such as the need for a third solvent or the collection of the extraction solvent. EME was also combined with DLLME (EME-DLLME) to extract chlorophenols from drain water and with SPE (SPE-EME) to extract chlorophenoxy acid herbicides from river and sea water [164, 167, 170, 171]. However, it is expected that more combinations will be made in the future to enhance their potential. In general, these combinations enable a higher volume of sample to be extracted, which clearly lowers method detection limits.

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Chapter 21

Application in Food Analysis

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Chapter Outline

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Abbreviations

AALLME	air-assisted liquid-liquid microextraction
BPA	bisphenol A
BFRs	brominated flame retardants
DLLME	dispersive liquid-liquid microextraction
DES	deep eutectic solvents
QuEChERS	quick, easy, cheap, effective, rugged, and safe extraction
GC-ECD	gas chromatography-electron capture detector
GC-FID	gas chromatography-flame ionization detector
GC-MS	gas chromatography-mass spectrometry
GC-MS/MS	gas chromatography-tandem mass spectrometry
HHPP	high hydrostatic pressure processing
HF-LPME	hollow-fiber liquid-phase microextraction
HPLC-DAD	high-performance liquid chromatography with diode array
HPLC-UV	high-performance liquid chromatography with ultraviolet
LPME	liquid-phase microextraction
MAE	microwave-assisted extraction
PAHs	polycyclic aromatic hydrocarbons
SDE	single-drop extraction
SE	Soxhlet extraction
SPE	solid-phase extractions
UAE	ultrasound-assisted extraction
UHPLC-MS/MS	ultrahigh-performance liquid chromatography with tandem mass spectrometry
UPLC-HRMS	ultra-performance liquid chromatography/high-resolution mass spectrometry
SULLE	sugaring-out liquid-liquid extraction
CCSHLLE	countercurrent salting-out homogenous liquid-liquid extraction
GPC	gel permeation chromatography
dSPE	dispersive solid-phase extraction

21.1 Introduction

Food includes a diverse and complex number of substances such as proteins, carbohydrates, lipids, vitamins, and minerals that are essential to human health and well-being, together with a plethora of microconstituents, including many beneficial phytochemicals. However, food production and processing (homemade or industrial) can affect their composition, quality, and safety. Therefore a comprehensive elucidation of food composition and evaluation of food safety require the use of the best analytic methods. In all food analytic methods, sample preparation is a crucial step to assure a good extractive yield of the analytes of interest and simultaneously remove as much as possible interfering matrix components.

Liquid-phase extraction is the prevailing sample preparation technique used in food analysis. In the past decade, particular attention has been given to possible improvements that make it possible to meet the challenge of developing faster and environment-friendly methods. Therefore novel liquid-phase extraction techniques such as microwave- (MAE) and ultrasound-assisted extraction (UAE);

pressurized liquid extraction (PLE); quick, easy, cheap, effective, rugged, and safe extraction (QuEChERS); single-drop extraction (SDE); dispersive liquid-liquid microextraction (DLLME); and extraction with ionic liquids or deep eutectic solvents (DES) have been applied with success in food analysis.

The following review will focus on the latest developments in novel liquid-phase extraction techniques applied in the analysis of major (proteins, carbohydrates, and lipids) and micronutrients (vitamins) and in the detection and quantification of hazardous contaminants from different sources, namely, pesticide residues, mycotoxins, brominated flame retardants (BFRs), bisphenol A (BPA), and polycyclic aromatic hydrocarbons (PAHs).

21.2 Nutrients

21.2.1 Macronutrients

Proteins along with peptides and amino acids are important constituents of food. They contribute to the structure and flavor of food and are essential to human life, responsible for multiple functions in our body, including building tissue, cells, and muscle, and the production of hormones and antibodies. Quantification of total protein in food can be achieved by direct or indirect methods. Direct methods are dependent on amino acid composition (e.g., biuret, Lowry, UV/Vis spectrophotometry, and IR spectrophotometry), while in indirect methods the total protein content is based on the determination of the total organic nitrogen (Kjeldahl) or after chemical reactions with functional groups within the protein (Dumas) [1]. Despite minor progress in this field, the advent of novel extraction techniques enabled significant advances to be achieved in the determination of specific proteins, peptides, or amino acids. Ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and high hydrostatic pressure techniques were successfully applied for either the isolation or extraction of proteins in food and in their digestion with a view to the determination of peptides and amino acids [1].

Ly et al. [2] proposed a UAE technique for the extraction of proteins from defatted rice bran meal. The initial extraction rate and extraction constant in the proposed technique were 3.48 and 2.20 times, respectively, higher than those for a conventional extraction while providing an equivalent or better protein yield. Altuner et al. [3] proposed the extraction of the total amounts of proteins from *Cedrus atlantica* pollens through high hydrostatic pressure processing (HHPP) using a pressure of 220 MPa for 10 min. This extraction allowed a maximum protein concentration of about 18 μg/mL compared with about 1.9 μg/mL in 24 h by a conventional extraction. Lin et al. [4] proposed a novel MAE protein digestion method using trypsin-immobilized magnetic nanoparticles. The magnetic nanoparticles acted as

a substrate for enzyme immobilization absorber for microwave irradiation, thus improving the efficiency of microwave-assisted digestion. The process provides equivalent or better digestion efficiency when compared with the conventional in-solution digestion, with the advantage of facilitating the top-down approach for protein isolation followed by mass spectrometric identification.

Carbohydrates present in foods are one of the major energy sources for humans. Moreover, they contribute to sweetness, appearance, and textural characteristics of many foods and are also precursors of aroma and color substances formed during food processing. Carbohydrates are usually classified as monosaccharides, oligosaccharides, and polysaccharides. Monosaccharides are polyhydroxy aldehydes or ketones, with 3–8 carbon atoms. Oligosaccharides are polymers containing a small number (3 to approximately 10) of monosaccharide residues connected by glycosidic linkages, while polysaccharides are the designation commonly used for larger polymers. While monosaccharides and oligosaccharides are water soluble, polysaccharides are usually water insoluble. A huge number of analytic techniques have been developed to measure the total or specific concentration of food carbohydrates. Whatever the case a previous separation of carbohydrates from other major food components, such as lipids and proteins, is crucial.

The classic liquid extraction based on mixtures of water with alcohols of low molecular weight such as ethanol or methanol have been replaced by ionic liquids or DES for dissolution and extraction of carbohydrates [5]. These novel extraction solvents are green solvents characterized by high thermal stability. Liu et al. [6] propose a MAE extraction using choline chloride and oxalic acid dihydrate as DES extractant for the separation of lignocellulose from wood biomass. The best results were obtained using a mass-to-solvent ratio of 1:20 at 80°C, 800-W microwave energy for 3 min. Alternative techniques such as UAE and MAE have been used for the extraction of polysaccharides from vegetable foods [7]. Compared with conventional extraction reference techniques, UAE and MAE provided higher extraction efficiency [7]. Wang et al. [8] proposed an UAE method using water as extractant, ultrasonic power of 146 W, extraction time of 14.5 min, and extraction temperature of 60°C, for the extraction of two polysaccharides (APS1 and APS2) from *Artemisia selengensis* Turcz. Bagherian et al. [9] investigated the effects of microwave power and heating time for the extraction of pectins from grapefruit with acidified water (pH 1.5). The highest total amount of pectin was 27.81% (w/w) obtained after 6 min at 900 W by MAE.

PLE has been used for the fractionation of carbohydrates. The group of Ruiz-Matute used PLE for the isolation of lactulose from lactose using 70:30 (v/v) ethanol/water at 40°C and 1500 psi [10]. Latter the same group proposed a new procedure that combines the use of activated charcoal and PLE to obtain enriched fractions of di- and trisaccharides from honey [11]. Samples were adsorbed onto activated charcoal and packed into a PLE extraction cell and extracted with

ethanol/water mixtures. Optimum results were obtained at 10 MPa and 40°C using two consecutive PLE cycles: firstly, 1:99 (v/v) ethanol/water for 5 min followed by 50:50 (v/v) ethanol/water for 10 min. Compared with other methodologies for the fractionation of honey carbohydrates, PLE was faster, required less solvent, and minimized sample handling.

Lipids have a central role in human life as the major source of energy. In food, lipids contribute to both flavor and texture. The major lipid groups include triacylglycerols, phospholipids, steroids, and waxes; in common, they present both high hydrophobicity and high solubility in apolar organic solvents. Owing to lipid complexity, most of the methods used for both total lipid estimation or for further analysis of specific lipid compounds require a previous lipid extraction [12]. Neutral lipids (triacylglycerols) are usually extracted with nonpolar solvents such as petroleum ether, hexane, or supercritical carbon dioxide. In samples containing phospho- or glycolipids, polar solvents such as methanol are used for lipid isolation. Standard techniques such as Soxhlet extraction (SE) based on solvent extraction or Weibull-Berntrop, Röse-Gottlieb, Mojonnier, Folch, Werner-Schmid, and Bligh-Dyer based on acid, alkaline, or enzymatic hydrolysis before solvent extraction can be used to evaluate lipid content [13]. These techniques require a long extraction time and a large solvent volume, and some methods require high temperatures that can promote changes in the extract composition. In the last decade, alternative extraction techniques to overcome these shortcomings have been considered. For example, some of the newer proposals are based on UAE, PLE, or MAE.

Péres et al. [14] compared the classical extraction technique for plants (SE) with UAE and PLE for the determination of terpenes (terpenic alcohols and phytosterols), fatty acids, and vitamin E from leaves of *Piper gaudichaudianum* Kunth. PLE was simpler and more effective than SE or ultrasound extraction techniques for isolating these compounds from plants. Pieber et al. [15] tested an automated PLE technique (ASE) to extract polyunsaturated fatty acids from marine microalga *Nannochloropsis oculata*. The highest extraction yield was obtained with ethanol (36 ± 4 mass%) compared with lower yields for n-hexane (6.1 ± 0.3 mass%). Afolabi et al. [16] evaluated MAE to obtain a lipid extract from eel fish with ethanol as the extraction solvent. This procedure produced a good yield of free fatty acids and acid value with a shorter extraction time and higher reproducibility. The maximum extraction yield (16.13% w/w) was obtained at a microwave power of 800 W; free fatty acids and acid value of 1.35 and 2.69 mg KOH/g, respectively, confirmed the high quality of the extract obtained. Gutte et al. [17] proposed an extraction of flaxseed oil by ultrasonic treatment and yield of omega-3 fatty acids. Ultrasonic treatment at 40 kHz, temperature 30°C, extraction time 40 min, and sample-to-solvent (n-hexane) ratio of 1:10 gave the best results. UAE improved the extraction yield by 11.5% with a similar amount of solvent.

21.2.2 Micronutrients

Vitamins are minor but essential components of food, with an important role in the normal growth, maintenance, and functioning of the human body. In general, vitamins can be divided according to their polarity into two groups: fat-soluble vitamins such as A, D, E, and K1 and water-soluble vitamins, which includes all vitamins of the complex B and vitamin C. Since vitamins are present at low concentrations in foods that contain a large amount of potentially interfering substances, analytic methods need to be both sensitive and specific. Additionally, vitamins are often present in multiple forms, including coenzymes and other functional derivatives, some of which are chemically unstable [18]. Therefore an accurate extraction and purification of the sample is crucial to improve the whole analytic performance (e.g., selectivity, sensitivity, and accuracy). The older SE and heating under reflux techniques have been replaced in the last decade by eco-friendly and effective sample preparation techniques including UAE and DLLME [18]. Chen et al. [19] optimized an UAE using water for the extraction of vitamin B from rice bran. Under the optimum conditions a good extraction efficiency of vitamin B (purification factor obtained from the ratio extraction efficiency of vitamin B from total yield was 4.55) and recovery (recovery rate was 93%) was obtained at 323 K with a solvent-to-solid ratio of 10 for 1.5 h in dried, defatted rice bran. A DLLME procedure was proposed by Viñas et al. [20] for the extraction of vitamins D and K in foods. Briefly the sample was mixed with acetonitrile (3 mL), which functioned as the dispersion solvent, with carbon tetrachloride (150 μL, extraction solvent) and injected into water (6 mL); after vortexing and centrifugation the sedimented phase was collected and evaporated to dryness. The residue was reconstituted in acetonitrile and was analyzed by LC-UV and liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS). This method eliminates matrix interference and is sensitive with an improved limit of detection compared with other methods.

21.3 Chemical Hazards

Monitoring chemical hazards in foodstuffs is a complex issue. Therefore a huge investment in time and effort is placed in these activities by regulatory and research laboratories to develop greener, faster, more precise, and more accurate methods, able to ensure the safety of food commodities. A range of novel sample preparation techniques based on liquid extraction allied to improved instrumental techniques were utilized in recent years for the determination of agrochemical contaminants (pesticide residues), natural toxins (mycotoxins), environmental contaminants (BFRs), packing contaminants (BPA), and processing contaminants (PAHs) in foodstuffs.

21.3.1 Pesticides

Pesticides have been used for decades to protect food sources from rodents, weeds, insects, and fungi. Despite their economic benefits, they are by nature toxic compounds, representing a risk to humans and wildlife. Therefore pesticide application and pesticide residue levels in food and water supplies are regulated by national and international regulatory agencies. To ensure compliance with the rules, scientists seek better approaches for the evaluation of pesticide residue levels in all kinds of foods. Due to the large number of pesticide classes that are used in agriculture, such as fungicides, insecticides, herbicides, acaricides, and nematicides, belonging to different chemical families, namely, organophosphate, organochlorine, carbamate, ureas, and pyrethroids, multipesticide residue analyses are required.

Traditional approaches to extract pesticide residues from food involve the use of water-miscible solvents (e.g., methanol and acetonitrile) followed by liquid-liquid partitioning with an organic solvent, usually *n*-hexane saturated with acetonitrile [21] or a mixture of ethyl acetate/cyclohexane [22]. These extracts are typically subjected to further cleanup, such as solid-phase extraction (SPE), gel permeation chromatography (GPC), or dispersive solid-phase extraction (dSPE) [23] before analysis. Li et al. [24] extracted herbicides from peanuts by ultrasound extraction using ethyl acetate. The isolation of the analytes from coextracted fat was achieved by dSPE using MIL-101(Cr) as sorbent. In this step the analytes are adsorbed on MIL-101 (Cr) with the fat remaining in solution. The herbicides were isolated and determined by LC-UV. Despite the quality of the extracts obtained, traditional liquid extraction procedures are time-consuming and laborious and require large amounts of potentially hazardous solvents. Thus some improvement of LLE procedures based on different microextraction procedures, DLLME, single-drop microextraction (SDME), hollow-fiber liquid-phase microextraction (LPME), air-assisted liquid-liquid microextraction, sugaring-out liquid-liquid extraction (SULLE), and countercurrent salting-out homogenous liquid-liquid extraction (CCSHLLE), has been described.

DLLME was used for the extraction of pesticides in juices [25], tomato [26], orange and orange juice [27], tea [28], and baby foods [29], among other matrixes [30]. In these studies the pesticide residues were extracted with minute amounts of halogenated solvent such as tetrachloroethane, chlorobenzene, tetrachloroethene, and carbon tetrachloride [25–27, 30] with a higher density than aqueous solutions, in the presence of a dispersive solvent such as acetonitrile or acetone, facilitating the enrichment of the pesticides in the sedimented (bottom) phase. However, other solvents such as 1-dodecanol, 2-dodecanol, n-hexadecane, and n-hexane, with a lower density than water, were applied as extraction solvents in DLLME for multipesticide residue analysis [31, 32]. Moinfar and Hosseini [32] used n-hexane for the determination of 10 organophosphates in tea. Ionic liquids like 1-hexyl-3-methylimidazolium hexafluorophosphate [33], 1,3-dibutylimidazolium hexafluorophosphate [34], and 1-octyl-3-methylimidazolium hexafluorophosphate [35] have

been used for the extraction of pesticides by DLLME. Wang et al. [36] developed an IL-DLLME with analysis by LC-UV for the determination of triazines in honey. A mixture of 1-hexyl-3-methylimidazolium hexafluorophosphate (extraction solvent) and Triton X 114 (dispersion solvent) injected in an aqueous solution of honey (2 g of honey with 20 mL of water) was used. The detection limits for chlortoluron, prometon, propazine, linuron, and prebane were between 5 and 10 μg/kg. Although ionic liquids are claimed to be environmentally friendly and a suitable alternative to traditional organic solvents, they are expensive, and their impact on the environment is under discussion [37]. Because of doubts about the greenness of ionic liquids, a new generation of "sustainable" solvents has been developed, the so-called deep eutectic solvents and natural deep eutectic solvents [38]. These solvents are composed of two nontoxic components, one of which is a hydrogen-bond acceptor (tetraalkylammonium or phosphonium salts), while the other (acids, alcohols, amines, or carbohydrates) is a hydrogen-bond donor [39]. One of the most common components is choline chloride (ChCl), which is an inexpensive, biodegradable, and nontoxic salt. A mixture of ChCl and 4-chlorophenol (1,2 M ratio) was used in DLLME for the extraction of nine pesticides in fruits and vegetables prior to gas chromatography with flame ionization detection (GC-FID) [40]. The limits of detection (LODs) and quantification (LOQs) for the selected pesticides were in the ranges of 0.24–1.4 and 0.71–4.2 μg/L, respectively.

SDME using a microdrop of organic solvent at the tip of a syringe needle immersed in an aqueous solution or exposed to the headspace above a sample was used for the extraction of pesticide residues from several food matrices [30] such as juices [41], honey [42], vegetables [43], and tea [44]. In most cases, toluene was used as the extraction solvent since it is stable, is less toxic than similar solvents, possesses higher extraction efficiency, and is compatible with GC analysis. Despite good performance, SDME is not viewed as suitable for online preconcentration and has been utilized to only a limited extent.

In HF-LPME the acceptor solvent is immobilized in the cavity of the hollow fiber (polypropylene membrane), the pores of which act as a filter preventing interference from high-molecular-weight molecules present in the sample matrix. Although not extensively used for pesticide analysis in food samples, HF-LPME has been used for the extraction of pesticide from alcoholic beverages (wine and beer), using 1-octanol as solvent [45], and from vegetables with dihexyl ether as extraction solvent [46] and also for the extraction of fungicides from orange juice, using 2-octanone as extraction solvent [47]; organochlorine pesticides from tomato and strawberry, using a mixture of toluene; and hexane as extraction solvent [48] and pesticides from grapes with pressurized hot water as solvent [49]. HP-LPME affords a suitable sensitivity for use in pesticide residue analysis. However, the extraction procedure requires the presence of the analytes in a solution, restricting applications to liquid samples [30]. Timofeeva et al. [50] used sugaring-out liquid-liquid extraction (SULLE) with liquid

chromatography-tandem mass spectrometry (LC-MS/MS) to determine malathion, diazinon, imidacloprid, and triadimefon. The pesticides were extracted into a water-miscible organic solvent—acetonitrile—followed by the separation of the organic phase using glucose as the sugaring-out reagent. LODs obtained ranged from 0.003 to 0.03 mg/L. Farajzadeh et al. [51] used countercurrent salting-out homogenous liquid-liquid extraction (CCSHLLE) followed by DLLME to isolate 11 pesticides from aqueous samples (fruit juices, well water, and river water) prior to analysis by GC-FID. An aqueous solution of the analytes containing acetonitrile and 1,2-dibromoethane was transferred into a narrow bore tube partially filled with sodium chloride forming fine droplets of organic phase that separated out as a layer above the aqueous solution. The organic layer was removed and injected into deionized water for analyte enrichment. The method provided low LODs from 0.34 to 5 μg/L with a relative standard deviation (RSD) $<7\%$.

Currently, QuEChERS is extensively used for multipesticide residues analysis in food. Initially introduced by Anastassiades et al. in 2003 [52] for the multiclass, multiresidue analysis of pesticides in fruits and vegetables, this technique involves an acetonitrile salting-out extraction followed by a dispersive solid-phase extraction (dSPE) cleanup. Buffering the sample at pH 5 during the extraction was shown to provide an optimum balance for high recoveries ($>70\%$) for some pH-dependent pesticides (e.g., pymetrozine, imazalil, and thiabendazole) independent of the fruit/vegetable matrix [53]. Therefore two different procedures, one based on strong acetate-buffering conditions (JAOAC) and the other on weaker citrate-buffering conditions (Wiley), were evaluated through extensive interlaboratory trials for dozens of pesticides in different matrices by GC-MS and LC-MS/MS. Both techniques successfully met statistical criteria for acceptability from independent scientific standard organizations, with the acetate-buffering version becoming an AOAC Official Method 2007.01 [54] and the citrate-buffering version being named European Committee for Standardization (CEN) Standard Method EN 15662. Costa et al. [55] compared the efficiency of the original citrate and acetate QuEChERS techniques for extraction of 10 pesticide residues in canned and fresh peach. The original QuEChERS technique allowed more compounds within an extraction efficiency between 80% and 120% to be extracted. The citrate-buffered QuEChERS was used for the extraction of 52 pesticides in honey and honeybees followed by LC-MS/MS analysis [56]. LOQs ranged from 0.2 to 10 ng/g and from 0.03 to 10 ng/g for honey and honeybee matrices, respectively. Acetate-buffered QuEChERS and DLLME were compared for the extraction of 24 pesticide residues in baby food followed by GC-MS analysis [29]. Both sample preparation techniques achieved suitable performance criteria, including selectivity, linearity, recovery, and precision. A higher enrichment factor was observed for DLLME. Nevertheless, dSPE provided a more effective removal of matrix coextractives than DLLME, which contributed to lower matrix effects.

Recent applications of QuEChERS have focused on the optimization of cleanup procedures. In general the sorbents used in the dSPE step are "primary secondary amine" (PSA), which is effective for the removal of fatty acids, C18; for the removal of nonpolar matrix components and graphitized carbon black (GCB); and for the removal of chlorophylls, in combination with anhydrous magnesium sulfate ($MgSO_4$). Machado et al. [57] used a QuEChERS procedure with addition of calcium chloride in the cleanup step. Ninety-eight pesticide residues in globe artichoke were detected by GC-MS and LC-MS/MS, with LODs from 0.005 to 0.025 mg/kg and from 0.003 to 0.015 mg/kg, respectively. Han et al. [58] optimized a LC-MS/MS method for the determination of 70 pesticide residues in leek, leaf lettuce, and garland chrysanthemum based on a modified QuEChERS procedure using multiwalled carbon nanotubes (MWCNTs) as a sorbent, to remove interferences from pigments. The performance of MWCNTs for the cleanup of extracts was superior to GCB and PSA. The method afforded good analytic performance with LOQs from 0.3 to 7.9 g/kg.

21.3.2 Mycotoxins

Mycotoxins are a relatively large and chemically diverse group of toxic secondary metabolites of filamentous fungi, especially those belonging to the genus *Aspergillus, Penicillium, Fusarium*, and also *Claviceps* and *Stachybotrys*, affecting mainly cereal crops and cereal foodstuffs [59, 60]. Approximately 300–400 mycotoxins have been identified so far [60–62]. However, only a few of these are considered of safety and economic concern, namely, aflatoxins (AFs), fumonisins (FMs), ochratoxins (OTs), trichothecenes (TRCs), zearalenone (ZEN), patulin (PAT), and ergot alkaloids [61, 63]. The determination of mycotoxins is quite complex since they represent structurally diverse chemical compounds at low concentrations in a vast range of matrices and sometimes in various combinations [59, 64]. Of utmost importance to obtain a representative laboratory sample are sampling and grinding. Criteria regarding sampling for mycotoxins are given in EC regulation EC/401/2006 (European Union 2006). Although this regulation does not set requirements for the preparation or size of laboratorial samples, typical sample sizes are 10–50 g. Generally the extraction of mycotoxins from liquid foods (wine, juice, and milk) has been achieved by liquid-liquid extraction using mixtures of organic solvents and water, such as ethyl acetate/hexane for extraction of PAT in juice [65] and acetonitrile/water (80:20) for extraction AFs in milk [66] and ochratoxin A (OTA) in wine [67]. For extraction from solid matrices (cereals and dried fruits), acetonitrile and methanol are commonly chosen [68]. The majority of mycotoxins are soluble in these solvents, and both are volatile and compatible with LC-MS/MS analysis. However, sometimes, it may not be possible to extract all mycotoxins of interest with adequate yields. For example, the extraction of acidic fumonisins is only achieved with a

solvent mixture containing water at a low pH. The adjustment of pH with sulfuric acid or an organic acid, such as formic or acetic acid, is a common step in this case. Liquid extraction is generally performed by conventional methods like shaking with a horizontal shaker [69] or simultaneously with Ultra Turrax Homogenizer [68]. Sulyok and coworkers employed the dilute-and-shoot technique for the determination of many mycotoxins, including glucoside adducts such as deoxynivalenol-3-glucoside, in wheat and maize [70] and other matrices [71]. The extraction is usually performed with acetonitrile/water/acetic acid 79:20:1 (v/v/v) on a rotary shaker. Subsequently, after centrifugation, the extract was diluted with an equal volume of dilution solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). This technique allows the simultaneous determination of hundreds of compounds, although a mass spectrometer with high sensitivity is required. In some applications, different energy sources have been used for the extraction, like MAE and UAE. A pretreatment technique using MAE followed by SPE was developed by Chen and Zhang [72] for the extraction of AFs from grains and grain products. To perform MAE, 12 mL of acetonitrile was added to 3 g of sample and heated at 80°C for 15 min at 350 psi. The extract was cleaned up on a Sep-Pak silica cartridge followed by derivatization with trifluoroacetic acid prior to liquid chromatography with fluorescence detection (LC-FLD). The method met EU requirements of 2 and 4 μg/kg for AFB1 and total aflatoxin content. Wang et al. [73] used an ultrasonic bath for the extraction of AFs, FBs, DON, and ZEN from corn with acetonitrile/water (84:16 v/v, containing 1% acetic acid) for 20 min at room temperature prior to analysis by LC-MS/MS.

The extraction of multimycotoxins has been performed mostly in a single step followed by a cleanup procedure to purify the sample extract from coextracted interferences. The sample cleanup procedures commonly used include SPE with reversed-phase sorbents (e.g., C18), strong cation or anion exchangers (SCX and SAX), or polymeric materials. Some cleanup procedures use specific SPE sorbents such as immunoaffinity or molecularly imprinted polymers [59].

Nowadays, QuEChERS is an established extraction procedure for mycotoxin analysis on a wide range of samples. This approach involves an acetonitrile extraction followed by salting-out the mycotoxins into the acetonitrile phase with sample cleanup by dispersive SPE. Magnesium sulfate and sodium chloride are used in the extraction step to reduce sample water, while in the cleanup step, sorbents like PSA, C18, or alumina are used to retain coextracted compounds. For an efficient extraction of some mycotoxins; changes in the salts used, in their quantity, or in the amount of C18; or addition of formic acid, water or methanol to the extraction solvent may be used. For dried matrices a swelling step with water is recommended to make samples more accessible to the extraction solvent [59]. Bryła et al. [74] developed a modified QuEChERS procedure for the determination of mycotoxins in wheat by liquid chromatography with high-resolution mass spectrometric detection (LC-HRMS). The extraction solvent consisted of a mixture of water and 10% formic acid in

acetonitrile to which magnesium sulfate, sodium chloride, sodium citrate dihydrate, and sodium citrate dibasic sesquihydrate were added. Extraction with n-hexane was used to eliminate the lipid faction. Finally, magnesium sulfate, C18, neutral alumina, and PSA were added to the extract to remove coextracted matrix compounds. This method provided LOQs between 1 and 65 μg/kg. Cunha et al. [75] reported a modified QuEChERS procedure using a combination of C18 with *Z*-Sep as dispersive SPE cleanup for the determination of 16 mycotoxins in nuts by LC-MS/MS. Recoveries of 70%–93% and repeatability (RSD $\leq$ 13%) were obtained for most mycotoxins with LOQ values from 1.25 to 5 μg/kg.

Amelin et al. [76] developed a QuEChERS procedure combined with dispersive liquid-liquid microextraction (DLLME) and derivatization with trifluoroacetic anhydride for the determination of trichothecenes in grain and mixed feed by gas chromatography with electron capture detection (GC-ECD). DLLME was used for the extraction of mycotoxins in liquid samples, such as PAT in apple juices [77], zearalenone in beer samples [78], OTA in wine [79], and AFs in milk [66]. In all these procedures, chloroform was used as the extraction solvent together with acetonitrile [66, 78, 80] or propanol [77] as dispersion solvent. A novel approach combining immunoaffinity column (IAC) and DLLME was proposed for the preconcentration of AFs in edible oils for LC-FLD analysis [81]. Samples were extracted by IAC and the acetonitrile eluent used as the dispersion solvent for DLLME with chloroform as extraction solvent. A rapid two-step microextraction technique using ionic liquid-based DLLME (IL-DLLME) and magnetic nanoparticle SPE was developed by Zhao et al. [82] for the preconcentration of AFs in animal feedstuffs for LC-FLD analysis. 1-Octyl-3-methylimidazolium hexafluorophosphate was used as the extraction solvent for DLLME to extract AFs. The hydrophobic pelargonic acid-modified ferrite magnetic nanoparticles were used to isolate the AF-containing ionic liquid from the DLLME step. No statistically significant difference at the 95% confidence level was observed between the ionic liquid-DLLME method and other LC-FLD methods using IAC cleanup.

21.3.3 Brominated flame retardants

Brominated flame retardants (BFRs) are substances added to a wide variety of industrial and household articles to reduce their flammability. They are typically mechanically blended with polymeric materials and are released into food and the environment from these materials. BFRs are typically technical mixtures of variable composition (e.g., DE-71, DE-79, or Saytex 102E) consisting of polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDDs), tetrabromobisphenol A (TBBPA) and other phenols, and polybrominated biphenyls (PBBs). These relatively nonpolar compounds undergo bioaccumulation (the increase in

concentration of a substance in an organism) and biomagnification (the increase in concentration of a substance in organisms at successively higher trophic levels). Also, their toxicity in human and wildlife associated mostly with endocrine disruption has raised concern about their increasing presence as environmental contaminants.

The assessment of BFR levels in foods generally requires the extraction of fat. For this purpose, Soxhlet and pressurized liquid extraction with n-hexane, hexane/dichloromethane, hexane/acetone, or toluene/acetone mixtures are generally used. Lipids are typically hydrolyzed with sulfuric acid added to the extract or mixed with silica gel. For cleanup SPE on neutral alumina [38, 83, 84], silica gel [85], Florisil [86], or other adsorbents [87] are typically used. Gel permeation chromatography is also reported [88]. For food products the concentration of BFRs should be based on fat content in addition to sample amount. Bichon et al. [89] developed a PLE extraction procedure for the extraction of PBDEs, HBCDDs, and TBBPA in food and feed. A toluene/acetone mixture (70:30, v/v) was used as extraction solvent with three extraction cycles of 5 min at 120°C and 100 bars pressure with a purge delay of 150 s for a 34-mL cell. The extract was purified sequentially on acidified silica, Florisil, and carbon columns into four fractions that were analyzed by GC-MS/MS or LC-MS/MS. Garcia-Bermejo et al. [90] reported the extraction of PBDEs, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), and decabromodiphenylethane (DBDPE) from functional food rich in omega-3 polyunsaturated fatty acids. Liquid samples (i.e., cow's milk, chicken eggs, and soy-based products) were lyophilized, and solid samples (biscuits and soy lecithin) were ground before extraction. The amount of sample equivalent to 4 g of fat was homogenized with anhydrous sodium sulfate and activated silica gel and packed into glass columns between two layers of anhydrous sodium sulfate and the BFRs eluted with n-hexane. The extracts were additionally fractionated on carbon SPE columns for congener-specific analysis by GC-MS/MS. Berton et al. [91] determined selected PBDEs (BDE-47, BDE-100, BDE-99, and BDE-153) in milk samples (powdered milk and freeze-dried human milk) by ultrasound-assisted extraction (UAE), cloud-point extraction (CPE), and ultrasound-assisted back extraction (UABE). UAE was used as a leaching technique and CPE and UABE to preconcentrate the analytes. Briefly, dried milk samples were extracted using UAE with hexane/acetone (70:30 v/v), centrifuged, and the supernatant collected. The procedure was repeated twice, and the extracts were combined and evaporated to dryness. The residue was dissolved in 5 mL of deionized water with the addition of surfactant (0.5 mL of 1% w/v Triton X-114) and 0.56 mL of 4% w/v sodium chloride solution. Above 55°C a surfactant-rich phase enriched in lipophilic proteins separates out leaving the hydrophilic proteins behind in the aqueous phase. The surfactant-rich phase was subjected to ultrasound-assisted back extraction by addition of 90 μL of isooctane and

analyzed by GC-ECD. LODs in the range 0.05–0.50 ng/g were obtained. The recovery of PBDEs at two different concentrations (1 and 25 ng/g) was in the range 68%–70%.

Cruz et al. [84] presented a cost-effective and fast method for the analysis of seven PBDEs and eight methoxylated PBDEs (MeO-PBDEs) in seafood matrices (muscle, the liver, and plasma) and feed using a QuEChERS extraction for solid samples and DLLME for plasma. Solid samples were lyophilized and mixed with acetonitrile/toluene (4:1, v/v) and inorganic salts ($MgSO_4$ and NaCL). Extracts were further treated with EMR-Lipid (200 mg) followed by magnesium sulfate plus *Z*-Sep +. Liquid samples were mixed with acetonitrile and concentrated by DLLME using trichloroethylene as the extraction solvent. LODs lower than 65 pg g^{-1} wet weight (WW) for muscle, 5.35 ng g^{-1} WW for the liver, 4.50 ng g^{-1} WW for feed, and 0.60 ng mL^{-1} for plasma were obtained. Compared with traditional extraction methods, solvent consumption and time were significantly reduced.

21.3.4 Bisphenol A

Bisphenol A is a synthetic chemical used in the manufacture of certain plastics and resins since the 1960s. BPA can be found in polycarbonate plastics; used in the manufacture of food storage and beverage containers and in other consumer goods and in epoxy resins; and used to coat the inside of metal products, such as food cans, bottle tops, and water supply lines. Human exposure to BPA occurs mainly via diet. A major source is packaged food and beverages contaminated with BPA migrating from the packaging material [92]. Since BPA is typically found at low levels, a wide range of extraction and cleanup techniques have been developed for its analysis in food products.

Solid-liquid extraction is a common procedure for the extraction of BPA from food, with acetonitrile the most common solvent, although others like acetone [93], methanol [94], ethanol [95], and dichloromethane [96] have also been used. The choice of solvent depends on the food matrix. This procedure is robust and simple but leads to high solvent consumption (40–300 mL) and long extraction times (20–120 min) [97]. To overcome these limitations, MAE and PLE have been used for the extraction of BPA from food such as fish [98] and meat [99]. Pedersen et al. [98] used a MAE and LC-MS for the determination of BPA, p-octylphenol, and 4-tert-octylphenol in fish tissue. Thawed fish (1 g) was extracted by MAE with a mixture of 20 mL of dichloromethane/methanol (2:1, v/v) followed by SPE cleanup. Shao et al. [99] used PLE and LC-MS/MS for the determination of BPA, octylphenol (OP), and nonylphenol (NP) in meat. A 10-g portion of meat (pork, rabbit, duck, or chicken) was mixed with celite and activated neutral alumina and packed into the base of a PLE cell for extraction with dichloromethane. The extract was cleaned up by solid-phase extraction on an aminopropyl column eluting the

analytes with 10 mL of methanol/acetone (50:50, v/v). Recoveries ranged from 91% to 100% with LOQs of 1.00, 0.20, and 0.40 μg/kg for BPA, NP, and OP, respectively.

QuEChERS provides an alternative approach for the extraction of BPA from food matrices. It can be applied to solid or liquid matrices employing different cleanup steps depending on the food matrix. Jakimska et al. [100] used QuEChERS and LC-MS/MS for the determination of 19 endocrine disruptors (EDCs) including BPA in various fish species. The extraction was performed with acetonitrile combined with sodium acetate and magnesium sulfate followed by dSPE cleanup with $MgSO_4$, PSA, and C18. Recoveries of 40%–103% were obtained for most compounds with low LODs of 0.002–3.09 ng/g for fish homogenates and acceptable precision (RSD $< 20\%$). The QuEChERS method was combined with DLLME by Cunha et al. for the extraction of BPA and BPB from canned fish [101] and canned vegetables and fruits [102] for analysis by GC-MS. After extraction of the analytes with acetonitrile and a mixture of magnesium sulfate and sodium chloride, an aliquot of the extract was further cleaned up by DLLME using tetrachloroethylene as the extraction solvent and acetic anhydride as derivatizing reagent. The latter facilitated the simultaneous acetylation of BPA and its transfer to the tetrachloroethylene phase. Recovery of BPA and BPB exceeded 68% with LODs of 0.2 μg/kg for BPA and 0.4 μg/kg for BPB.

21.3.5 PAHs

Polycyclic aromatic hydrocarbons (PAHs) are toxic substances present in the human environment at low concentrations. EU regulations list 15 PAHs, benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*ghi*]perylene (BghiP), benzo[*a*]pyrene (BaP), chrysene (CHR), dibenzo[*a*,*h*]anthracene (DBahA), indeno[1,2,3-*cd*]pyrene (IP), benzo[*a*]fluoranthene (BaF), cyclopenta[*cd*]pyrene (CPcdP), dibenzo[*a*,*e*]pyrene (DBaeP), dibenzo[*a*,*h*]pyrene (DBahP), dibenzo[*a*,*i*]pyrene (DBaiP), dibenzo[*a*,*l*]pyrene (DBalP), and 5-methylchrysene (MCH), as priority contaminants that should be monitored in the different environmental compartments and also in various food products [103]. Maximum levels have been set for PAHs in key foodstuffs, for example, smoked meat and smoked meat products, smoked fish and smoked fish products, and oils and fats, via Commission Regulation (EC) No 1881/2006 [104], in the framework of EU legislation, which sets maximum levels for chemical contaminants in foodstuffs. The techniques used for the extraction of PAHs depend on the nature of the food matrix. The extraction of PAHs from fatty foods usually consists of saponification of lipids with methanolic or ethanolic potassium or sodium hydroxide solution and isolation of the PAHs with cyclohexane [105] or n-hexane [106] from the unsaponifiable fraction. For nonfatty foods such as vegetables, fish, and meat, PAHs can be extracted by solid-liquid extraction using toluene [107], *n*-hexane [108], or a mixture of cyclohexane and ethyl

acetate [109], respectively. Soxhlet extraction using dichloromethane [110] alone or mixed with *n*-hexane [111] is also popular. After the initial extraction step, a cleanup procedure by SPE with C18, Florisil, alumina, silica gel, or another sorbent is necessary to reduce the amount of interfering coextracted compounds. To reduce extraction time and solvent consumption, new extraction techniques such as PLE and MAE have been developed, in many cases allowing an appreciable level of automation [112]. The solvents used in PLE and MAE are those utilized in the classical extraction techniques. Purcaro et al. [113] utilized MAE with n-hexane to extract PAHs from 2 g of lyophilized smoked meat samples at 115°C for 15 min for analysis by LC-FLD. The procedure provided better extraction efficiencies when compared with classical solvent extraction and LOQs lower than 0.2 μg/kg wet weight for all PAHs, except for fluoranthene (0.3 μg/kg), pyrene (0.6 μg/kg), and indeno[1,2,3-*cd*]pyrene (0.4 μg/kg). PLE and GC-MS were used for the determination of PAHs in smoked cheese [111]. For the extraction a 100-mL extraction cell was filled with 18-g activated silica gel. Dichloromethane/hexane (15:85, v/v) was used as the extraction solvent. The proposed method meets the required EU method criteria for analyzing benzo[*a*]pyrene in foods. QuEChERS extraction was used to extract PAHs from food matrices (fish, meat, teas, and vegetables) [112]. Three different sorbents, C18 + PSA, *Z*-Sep, and Z-Sep Plus, all combined with magnesium sulfate, were evaluated for the cleanup of PAHs and other persistent organic pollutants from fish [114]. The best results were obtained using Z-SEP as cleanup prior to low-pressure GC-MS/MS analysis. The method provided good detection limits 0.1–0.5 μg/kg for PCBs, 0.5–10 μg/kg for PBDEs, 0.5–5 μg/kg for select pesticides and PAHs, and 1–10 μg/kg for flame retardants.

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Chapter 22

Extraction of Plant Materials

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Chapter Outline

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22.1 Introduction

The diversity of flora has always been a precious resource for exploitation in human society. Not only do plants play a significant role in maintaining the production of oxygen through photosynthesis for living organisms to survive, but they also act as the fundamental source of food and provide medicinal values for boosting the well-being of humans. In particular, lots of bioactive compounds present in plants, such as lipids, pigments, oils, flavors, and fragrances, are widely used in a range of different industries, including the food, pharmaceutical, and cosmetics sectors. In recent years, there has been increasing interest in the utilization of different plant components or plant species as biomonitors to indicate pollution levels in a specific region. Due to the broad coverage and cost-effectiveness of vegetation, plant materials, especially plant leaves, are useful alternative adsorbents to replace the expensive and large sampling tools for evaluating pollution status.

22.1.1 Importance of plant analysis

22.1.1.1 Medicinal Value

The analysis of plant materials is crucial because the natural products extracted from plants offer precious materials for the prevention and treatment of diseases. A recent publication highlighted the utilization of natural products that combined with dendrimers act as anticancer agents [1]. Other research has successfully translated a natural product, withaferin A, into therapeutics by developing chemoproteomics-enabled covalent ligand discovery platforms for targeting treatable hotspots, which can impair breast cancer pathogenicity [2]. George et al. summarized the roles played by different subclasses of plant flavonoids, including flavones, flavanones, and anthocyanidins in cancer chemoprevention to protect DNA from various carcinogens [3]. A comprehensive review regarding the research trends, applications, and mode of actions of different plant products against Alzheimer's disease was presented by Dey et al. [4]. This study drew specific attention to a series of traditional herbal medicines that originate from India and China, which demonstrate promising potential in treating Alzheimer's disease. Different chemical classes of natural products in plants comprising alkaloids, phenolics, phenylpropanoids, and terpenoids have shown therapeutic effects of inhibiting the proliferation of vascular smooth muscle cells, hence lowering the risk of atherosclerosis, pulmonary hypertension, and stenosis [5].

Concurrent with the escalating amount of chemicals at present, bioactive compounds isolated from plant materials exhibit excellent hepatoprotective effects to prevent and treat chemical-induced liver injuries through lowering inflammation, impeding apoptosis, and ameliorating necrosis [6].

22.1.1.2 Nutritional Value

Bioactive substances extracted from plant materials also act as natural replacements for synthetic antioxidants or food additives, which supplement nutritional values and health benefits of foods. Phenolic compounds in blackcurrant, blackberry, bearberry, blueberry, cranberry, cloudberry, strawberry, and grape berries were proven to be natural antioxidants that inhibit lipid and protein oxidation in meat products [7]. A study by Sganzela and coworkers evaluated the nutritional properties of Uvaria pulp (*Eugenia pyriformis*) and indicated that higher concentrations of two bioactive compounds, namely flavonoids and phenolics, were proportionate to higher antioxidant activity [8]. Characterization of the nutritional value and content of bioactive compounds was assessed in cardoon plants (*Cynara cardunculus*), illustrating the abundance of saturated fatty acids, total phenolic compounds, and carbohydrates in different edible parts of the plant [9]. In terms of antimicrobial properties, three chosen phytochemicals, *trans*-cinnamaldehyde, (−)-epi-gallocatechin gallate, and [10]-gingerol, studied by Cetin-Karaca and Newman showed varying degrees of inhibitory activity against both *Bacillus cereus* and its spores [10]. Pires et al. carried out an intensive investigation of the beneficial properties of fruit and stems for goji (*Lycium barbarum* L.) [11]. Apart from exhibiting rich sources of sugars, organic acids, fatty acids, tocopherols, and flavonols, this study also verified that the stems of goji showed higher antibacterial activity against Gram-negative bacteria, while fruits presented higher activity against Gram-positive bacteria.

22.1.1.3 Economic Value

Phytochemicals are widely used in commercial products due to significant advantages such as biodegradability and eco-friendliness, hence demonstrating the commercial value of natural plant materials. According to Prakash et al., a wide range of essential oils categorized as generally recognized as safe by the United States Food and Drug Administration are effective against storage fungi, mycotoxin secretion, and lipid peroxidation [12]. This indicates the prospects of utilizing plant essential oils as natural food preservatives, flavors, or additives to replace synthetic compounds used at present. A plethora of plant secondary metabolites that range from terpenes, limonene, salicylic acid, and strigolactones can be categorized into three levels of plant chemodiversity based on the influences of these compounds on ecological processes, and some of the roles include toxin, repellent, and detractant [13]. The primary phytochemicals that were detected in the essential oils of genus

Mentha exhibit effective antimicrobial and insecticidal activity, therefore Mentha oils are produced commercially as an insect repellent [14]. Despite the high effectiveness and low toxicity toward nontarget vertebrates and the multiple mechanisms of action of essential oils as biopesticides, the underpinning questions regarding the development of an efficient stabilization process and the toxicity against nontarget species remain unresolved [15]. Bel-Rhlid et al. reviewed the biotechnology techniques to produce natural flavors as food odorants by extracting flavor volatiles like carotenoid and glycosides from plant sources [16]. The renowned antiaging, antioxidant, and antiinflammatory properties of secondary metabolites in plants such as polyphenol compounds, terpenoids, and organic acids have been widely integrated into cosmetic products to replace the synthetic chemicals with numerous side effects [17].

22.1.1.4 Environmental Value

From an environmental protection perspective, analysis of plant materials provides useful information on the degradation and remediation of contaminants released into the environment. An extensive review regarding the potential of terrestrial and aquatic plants, macro- and microalgae, and cyanobacteria for remediation of radioactively contaminated water was recently published, which discussed in-depth environmental factors that influence the remediation performance of plants toward nearly 20 radionuclides [18]. In light of depleting natural resources, a technology called plant microbial fuel cell (PMFC) was invented to generate renewable energy. Concurrent with evaluating the efficiency of PMFC for electricity production, Guan et al. used a PMFC system to remove 99% of the carcinogenic hexavalent chromium (Cr (VI)) from metal-polluted soil [19]. Apart from radioisotopes and toxic heavy metals, different species of plants, individually or in partnership, play a significant role in the bio- and phytoremediation of organic xenobiotics in the environment [20].

22.1.2 Types of analytes

22.1.2.1 Phytochemicals

Different classes of phytochemicals, terpenoids, phenolic compounds, glucosinolates, betalains, organic acids, and carbohydrates, are the cornerstone of plant analysis due to a multitude of benefits. Apart from the typical plant-derived volatile organic compounds in the terpenoid family, such as mono- and sesquiterpenoids, Yazaki et al. elaborated the ecological interactions of other "atypical" terpenoids, including glycosylated terpenes and composite-type terpenoids [21]. Carotenoids, anthocyanins, and betalains are the major natural pigments that can be used to produce food colorants or functional ingredients with proven health effects [22]. Despite lacking a comprehensive understanding of the mechanisms of cancer-chemopreventive

phytochemicals in medicinal plants, the significance of these kinds of phytochemicals, namely isothiocyanates, withaferin A, and honokiol, need more evidence to be popularized for cancer prevention [23]. A variety of phenolic compounds, which can be classified into monophenols, polyphenols, aromatic acids, and phenylethanoids, are also the emphasis of phytochemical analysis because of their therapeutic properties and nutraceutical values [24].

22.1.2.2 Environmental Pollutants

In contrast to the utilization of conventional active or passive sampling methods for the evaluation of pollution levels, different parts of plants are widely used as alternative passive samplers for biomonitoring purposes. Two aquatic plants, namely *Apium nodiflorum* and *Potamogeton pectinatus*, were employed for the examination of river quality by measuring both macro- and micronutrients, as well as toxic elements [25]. Zhao et al. correlated the concentration of polycyclic aromatic hydrocarbons (PAHs) in *Salix matsudana* leaves with their concentration in air, which showed good reliability as a biomonitor that is not significantly affected by meteorological parameters or leaf characteristics [26]. In another study that used *Tradescantia pallida* as a biomonitor, the clastogenicity and mutagenicity of diesel and biodiesel in exhaust emission were evaluated [27]. Apart from passive biomonitoring by using resident species, García-Seoane made an extensive study of the active biomonitoring approach by the use of transplantation techniques to measure pollutants in the coastal environment [28]. As many as 40 microalgae species across 24 genera are employed in the oceans to monitor levels of inorganic contaminants. While mosses are effective for biomonitoring trace elements, extending the exploitation of terrestrial mosses to worldwide application needs further standardization and verification [29].

22.1.3 Varieties of liquid-phase extraction methods

The qualitative and quantitative analysis of phytochemicals in stems, leaves, and roots of plants can be realized by selecting the most effective extraction methods, in which liquid-phase extraction (LPE) techniques represent some of the commonly used methods. The technical terms for the extraction methods were defined and abbreviated according to IUPAC recommendations [30]. The extraction process is necessary to obtain an adequate amount of high-quality bioactives from plants, and comprehensive overviews on the pros and cons, applications, mechanisms, and precautions of each extraction technique have been published elsewhere [31,32]. Patra and coworkers illustrated the three main categories of extracting bioactives from plants, which ranged from the basic extraction methods (maceration, decoction, digestion, infusion, boiling under reflux, and percolation), advanced extraction methods (aqueous alcoholic extraction, countercurrent extraction, and

Soxhlet extraction) to the advanced extraction techniques (pressurized liquid extraction (PLE), pressurized hot water extraction (PHWE), supercritical fluid extraction (SFE), enzyme-assisted extraction (EAE), and subcritical water extraction) [33]. Due to the drawbacks of these conventional LPE methods, the integration of more than one extraction technique such as coupling ultrasound-assisted extraction (UAE) with microwave-assisted extraction (MAE) and combining UAE with EAE is intended to improve the extraction efficiency [34]. Similarly, the introduction of greener organic solvents in the extraction process, including ionic liquids (ILs) [35] and natural deep eutectic solvent (NADES) [36], are intended to improve the extraction and separation efficiency, and at the same time fulfill green chemistry criteria [37]. In accordance with the principle of green analytical chemistry, miniaturized and eco-friendly liquid-phase microextraction (LPME) methods, including liquid-liquid microextraction, dispersive liquid-liquid mixroextraction (DLLME), and single-drop microextraction, gradually gained attention in the field of plant analysis [38].

22.1.4 Challenges and opportunities of phytochemical analysis

A review by Wolfender et al. [38] pointed out that the separation, detection, and identification of natural products from distinctive plant matrices, which are essential analytical processes for metabolite fingerprinting, metabolite profiling, and metabolite target analysis, are hindered by the chemodiversity and intrinsic physicochemical properties of these phytochemicals [39]. Furthermore, the wealth of isomeric forms, polarity, and volatility, as well as the low abundance of bioactive substances in plants, pose a great challenge for the identification of compounds with similar mass spectra [40]. The trace amount and different types of phytohormones (acidic, alkaline, and neutral) in plant matrices make the simultaneous determination of multiclass phytohormones difficult [41]. To overcome the challenges of determining phytochemicals in plant matrices, sample pretreatment methods for the isolation, separation, and preconcentration of these compounds are indispensable to obtain purified natural products [42]. Along with technological advances, modern detection techniques like mass spectrometry with various ionization methods play a crucial role in realizing the accurate determination of phytochemicals [43].

22.2 LPE Methods for the Analysis of Pollutants

The aforementioned advanced LPE techniques are widely used for the determination of varying pollutants, including emerging contaminants from plant materials, with the utilization of polar organic solvents to extract compounds of a polar or ionic nature and nonpolar/nonwater-miscible solvents for the isolation of nonpolar compounds [44]. In a study to evaluate the role of two mangrove species, namely

Excoecaria agallocha L. and *Kandelia obovata* on iron plague formation, immobilization, and uptake of PAHs and polybrominated diphenyl ethers, liquid-liquid extraction (LLE) was used to extract these contaminants, while the freeze-dried tissues of leaves, stem, and root were extracted by PLE [45]. Microwave-assisted digestion in a mixture of hydrochloric acid, nitric acid, and perchloric acid (3:1:1) was employed by Zhang et al. to predict the bioaccumulation of antimony and arsenic in maize (*Zea mays* L.) [46]. Soxhtlet extraction was used to extract 12 toxaphene congeners and 8 organochlorine pesticides from amaranth to evaluate its bioaccumulation characteristic, which indicated that this plant species is a potential candidate for phytoremediation [47]. Wu and Zhu used a sequential ultrasonic extraction procedure to isolate the bioavailable fraction of organic contaminants [48]. Three types of extraction solvent were used to determine the uptake of multiclass organic contaminants by eight plant species. In contrast to these LPE methods, LPME techniques are rarely used for the analysis of pollutants in plant materials.

22.3 LPE Methods for Extracting Phytochemicals

22.3.1 Pressurized liquid extraction

The extraction conditions for PLE at elevated temperature and pressure have the advantage of reducing consumption of organic solvent and faster extraction times. The selection of green extraction solvents like water or ethanol adds an eco-friendly benefit to this technique. In a recent study that compared the extraction yield and recovery of phytochemicals in *Morus nigra* L. leaves by three extraction methods, namely PLE, SFE, and maceration, PLE afforded higher yields and more polarity-diverse compounds within a shorter extraction time [49]. Figueroa et al. analyzed the phytochemicals in avocado peel by PLE and determined a total of 61 bioactive compounds belonging to 11 families, dominated by procyanidins, flavonols, and hydroxybenzoic and hydroxycinnamic acids [50]. Even though PLE techniques are generally more efficient than conventional extraction methods, several parameters, such as extraction temperature and solvent type, need to be optimized to maximize extraction yield and to avoid the degradation of phytochemicals [51].

22.3.2 Pressurized hot water extraction

Since the introduction of PHWE (or subcritical water extraction) in the 1990s for analysis of environmental samples, this method has gained popularity for the extraction of bioactives from plant and biological matrices in both laboratory analysis and industrial processes [52]. Phytochemicals in plants can be effectively extracted by using subcritical water as the extraction solvent under elevated pressure and

temperature. Detailed information regarding the setup, properties, mechanisms, benefits, and the application of PHWE for the extraction of bioactive compounds for pharmaceutical and nutritional use was described by Zakaria and Kamal [53]. In addition to the simple procedure (without the necessity for further cleanup), low operational cost, and "green extraction" properties, most importantly the phytochemicals extracted can be safely consumed and directly added into consumer products [54]. The highest recovery of phenolic compounds and sugars from different edible plants for use as ingredients for functional foods was obtained through optimizing the extraction parameters such as extraction temperature, solvent selection, and the addition of acid or enzyme [55]. Enzymatic hydrolysis achieved the highest extraction yield with comparatively larger amounts of phenolic compounds detected in rose (*Rosa hybrida*) than in camellia (*Camellia japonica*) and roselle (*Hibiscus sabdariffa*) flowers. Deans et al. utilized PHWE in an extensive taxonomic investigation of the phytochemical constituents in *Bellendena montana*, *Cenarrhenes nitida*, and nine plant species from the *Persoonia* genera, all endemic to Tasmania [56]. Of the 11 different glycosides isolated from these plants, 6 were newly identified compounds, including rare examples of arbutin derivatives featuring tiglic acid ester moieties. The extraction conditions of PHWE can also induce the formation of antioxidants that were initially nonexistent in plants [57]. Three main mechanisms of antioxidant formation during PHWE extraction were identified: hydrolysis at high temperature causing production of new compounds not originally present in the sample; simultaneous extraction and degradation of thermally labile compounds; and the promotion of Maillard and caramelization reactions yielding Amadori compounds and other reducing sugars by application of high temperatures in the presence of water.

22.3.3 Supercritical fluid extraction

The favorable physicochemical properties of SFE solvents include high diffusivity, low viscosity, and adequate density facilitating higher mass transfer of analytes from the solid phase into the extraction solvent together with the good solvation properties of fluid phases of liquid-like densities. General information for the selection of extraction conditions for phytochemicals is given elsewhere [58]. In recent years, an increasing effort has been diverted to study the bioactive components in waste materials to fulfill the "zero waste" concept for minimizing waste generation. Alvarez et al. reported the use of supercritical fluid extraction to valorize soybean expeller, which is a common waste from the production of soybean oil [59]. The highest total polyphenol content (represented by gallic acid) and flavonoid content (represented by quercetin) were obtained at lower temperatures with addition of ethanol to supercritical fluid carbon dioxide as cosolvent. Both polar and nonpolar bioactive substances were simultaneously extracted under the foregoing conditions. Similarly,

SFE was employed for the profiling of phenolic compounds in discarded "horchata" by-products from tiger nuts (*Cyperus esculentus*) [60]. Supercritical carbon dioxide pressure was the primary optimizing parameter that determined the differences in phenolic profiles. Higher pressure increased the yield of lipophilic phenolic compounds compared with lower-pressure extracts or conventional solvent extraction.

22.3.4 Ultrasound-assisted extraction

Ultrasonication is one of the nonthermal processing techniques commonly applied in phytochemical analysis, which can improve quality while maintaining microbiological safety for preservation purposes [61]. A comprehensive identification of hydroxycinnamic acid amides (HCAAs) in different parts of wheat, maize, and rice was achieved by UAE coupled with liquid chromatography-high resolution mass spectrometry analysis [62]. The authors constructed a mass spectral database for 846 HCCAs, and successfully identified 74 HCCAs in these plant species, 42 of them for the first time. UAE was also used to extract polyphenols, glucosinolates, carotenoids, chlorophylls, and ascorbic acid from Brassicaceae sprouts [63]. This work drew attention to the relationship between phytochemical types and enzyme activity, suitable for use as an indicator of food quality. A sequential approach was devised by Pham and coworkers using UAE to produce crude extracts from *Catharanthus roseus* with further fractionation by LLE [64]. Phytochemicals were isolated according to polarity in different solvents and the solvent extracts used in screens for antioxidant, antimicrobial, and cytotoxic activity.

22.3.5 Enzyme-assisted extraction

The mechanism of enzymatic action is primarily dependent on the utilization of specific enzymes to hydrolyze the cell wall, thereby promoting the release of active constituents into the bulk solution for effective extraction [65]. Influential factors that determine the extraction efficiency of EAE include solvent pH, reaction temperature, enzyme concentration, and enzyme type. According to Nadar et al., most bioactive compounds are entrapped within the cell wall in plant matrices, hence using enzymes to disrupt or solubilize the cell wall structure leads to effective extraction of intracellular biomolecules [66]. As much as a twofold increase in extracted plant material and a 94% increase in glucose content was extracted using a mixture of cellulolytic enzymes under optimized conditions compared with untreated plant materials [67]. Apart from facilitating the release of phenolic compounds from cell walls, enzymatic treatment also promotes changes in the molecular structure of phenolic compounds by converting phytochemicals into final products with higher bioavailability and antioxidant capacity [68]. Such biotransformation-induced enzymatic treatment was verified by Ruviaro and coworkers in an investigation of the hesperetin content

in citrus juice by-products [69]. Pectinase, tannase, and β-glucosidase were used individually or combined to assess the influence of enzymes on the extraction efficiency of phenolic compounds and the conversion of phenolic glycosides into aglycones. Interestingly, a synergistic effect on aglycones production was observed using a combination of tannase and β-glucosidase.

22.3.6 Hybrid extraction methods

Instead of using the aforementioned extraction methods individually, their combination (hybrid extractions) with the intention of increasing extraction yield or efficiency is a major trend in contemporary phytochemical analysis. UAE is commonly combined with other extraction techniques to facilitate higher mass transfer. Sumere et al. combined ultrasound and PLE to extract phenolic compounds from pomegranate peels (*Punica granatum* L.) [70]. Using water as the extraction solvent, UAE afforded a green and efficient method in which the ultrasonic waves accelerate cell rupture to allow the infiltration of extraction solvent into the inner structures of particles, thus enhancing extraction efficiency. In another study, enzyme-assisted ultrasonic-microwave synergistic extraction (EAUMSE) was employed for the extraction of flavonoids from Chinese water chestnut peel [71]. EAUMSE resulted in a 26.5%, 22.31%, and 12.98% higher extraction yield than solvent extraction, UAE, and MAE, respectively. More importantly, of the 16 flavonoids extracted by EAUMSE, four new flavonoids were isolated for the first time, demonstrating the comprehensiveness of this technique for phytochemical extraction. In addition, sonication can be integrated with conventional hydrodistillation (sono hydrodistillation), which extracted approximately double the yield of essential oil rich in citronellol, linalool, citronellal, and elemol from *Cymbopogon winterianus* [72].

22.3.7 Ionic liquid or natural deep eutectic solvent-assisted extraction

The unique properties of room temperature ILs have prompted interest in their use as extraction solvents and for the fabrication of various IL-based materials for the effective extraction and separation of bioactive compounds from plants [73]. However, NADES is regarded as the fourth generation of ILs with similar characteristic properties but stronger green credentials [74]. An ultrasound-assisted IL extraction technique was used to isolate geraniol from the leaves of Palmarosa [75]. A synergistic effect of combining sonication with ILs was observed resulting in a higher yield of essential oil. A new method based on a pH-dependent IL solvent-based aqueous two-phase system (ATPS) for the simultaneous extraction, transformation, and purification of psoralen from fig leaves (*Ficus carica* L.) was developed [76]. Due to the strong catalytic activity of IL-acid mixtures, the optimized ATPS composition achieving the highest recovery was 1-butyl-3-imidazolium bromide-citric acid.

Chanioti and Tzia studied the extraction efficiency of total phenolic content (TPC) from olive pomace by adding several NADES in different assisted extraction methods, including homogenization (HAE), MAE, UAE, and high hydrostatic pressure [77]. The combination of choline chloride/citric acid and HAE produced the highest yield of TPC and antioxidant activity compared to other NADES and extraction methods. In general, it is critical to find a balance between the improvement in extraction efficiency by addition of IL or NADES and the processing cost and possibility of using these green solvents for large-scale production to extract phytochemicals from plants.

22.3.8 Liquid-phase microextraction

Of the LPME methods, DLLME methods are the most common for phytochemical analysis. An air-assisted vesicle-based microextraction technique derived from the DLLME concept was developed for the simultaneous determination of phenolic acids and flavonoids in *Melissa officinalis* [78]. Diuzheva et al. compared the extraction efficiency of DLLME, ultrasound-assisted DLLME, sugaring-out liquid-liquid extraction, cyclodextrin-based DLLME, salt-aided DLLME, IL-DLLME, and NADES-DLLME, and compared these LPME techniques with MAE for the determination of harpagoside from *Harpagophytum procumbens* [79]. Numerous publications suggest the effectiveness and environmental friendliness of LPME techniques for plant analysis; nonetheless, a scaleup version is necessary if the intention is to realize the practicality of industrial production of phytochemicals from plant materials.

22.4 Conclusion, Research Gap, and Future Prospects

A summary of the extraction methods for analysis of pollutants and natural products in plant materials is shown in Fig. 22.1. LPE remains a frequently used technique in plant analysis due to its efficient, green, and simple experimental procedures. The strengths of LPE are highly dependent on the physicochemical characteristics of the analytes and the objective of the analysis. This chapter highlighted the main LPE techniques for analysis of natural products focused on the beneficial properties that these compounds can offer, particularly their medicinal potential to combat common diseases, including cancer. However, more research into the synergistic effects of multiple phytochemicals or between different classes of bioactive compounds to clarify the mechanisms of actions is required. To achieve this goal, comprehensive extraction methods that are able to simultaneously isolate multiple groups of natural products are needed. Moreover, the enhanced wide-spectrum extraction methods should take into account that extraction conditions do not affect or alter the

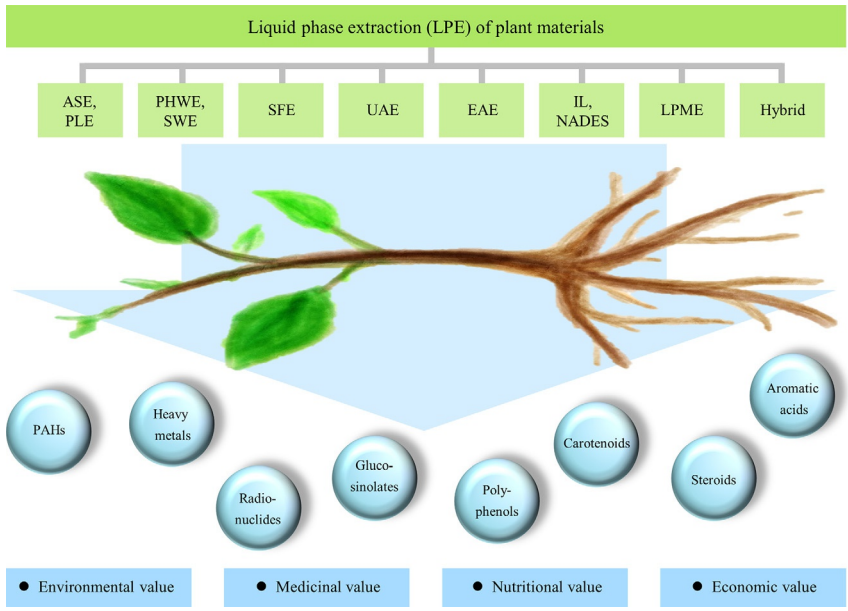

Fig. 22.1 A diagram indicating the types of extraction methods, analytes, and the beneficial values for extracting substances from plant materials. *ASE*, Accelerated solvent extraction; *EAE*, enzyme-assisted extraction; *IL*, ionic liquid; *LPME*, liquid-phase microextraction; *NADES*, natural deep eutectic solvent; *PAHs*, polycyclic aromatic hydrocarbons; *PHWE*, pressurized hot water extraction; *PLE*, pressurized liquid extraction; *SFE*, supercritical fluid extraction; *SWE*, subcritical water extraction; *UAE*, ultrasound-assisted extraction.

antioxidant or therapeutic properties of the extracted compounds. The feasibility for large-scale extraction of natural products from plant by-products should be considered, which help to maximize yield and minimize waste without adverse effects on the environment.

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Chapter 23

Biomedical Applications

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Chapter Outline

Liquid-Phase Extraction. https://doi.org/10.1016/B978-0-12-816911-7.00023-2

23.1 Introduction

The main body of this chapter is divided into two main sections. This first section provides a brief theoretical background for all liquid-phase methods, while selected applications for each method are provided in the second section.

23.1.1 Pressurized-liquid extraction (PLE)

PLE, also referred as accelerated solvent extraction, involves analyte extraction from a solid sample into an extraction phase under increased temperature and pressure. PLE can be run in either static or dynamic mode. In static mode, which is more commonly utilized, the analytes are extracted in consecutive extraction cycles. Usually the sample is pretreated, mixed with an inert agent, and packed into an extraction cell, and the extraction cell is sealed and placed inside an oven. Sample pretreatment includes drying (air-drying, oven-drying, or lyophilization), homogenization (grinding), and particle size distribution (sieving) and enhances the extraction phase/analyte interactions, thus the overall extraction efficiency. Inert dispersing agents can be mixed with the sample to not only further enhance extraction phase/analyte interactions but also reduce the required extraction phase volume while drying agents can be added when water removal is required. Then the extraction cell is preheated, filled with the extraction phase, and subjected to increased temperature and pressure for a given time (up to 200°C temperature, 200 bar pressure, and 15-min extraction time). After the extraction the analyte-containing extraction phase is collected and can be either directly analyzed or further treated for preconcentration and cleanup purposes. Finally the extraction cell is flushed with the extraction solvent and purged with an inert gas between extraction cycles [1]. A typical PLE configuration is shown in Fig. 23.1.

The extraction phase, the temperature and pressure values, the extraction time, and the number of extraction cycles directly affect the extraction efficiency, thus always optimized to achieve the highest possible recoveries. More specifically, temperature and static time are the most important factors in the static mode PLE. Analyte polarity directly affects the extraction solvent selection. Methanol (MeOH) and acetonitrile (ACN) are suitable extraction solvents for polar analytes, while dichloromethane and ethyl acetate (EtOAc) are suitable for less polar analytes. Water can also be employed as the extraction solvent, in which case the technique is called pressurized hot-water extraction. Furthermore, solvent mixtures can be used when the extraction of analytes of different polarity is required. Water-organic solvent

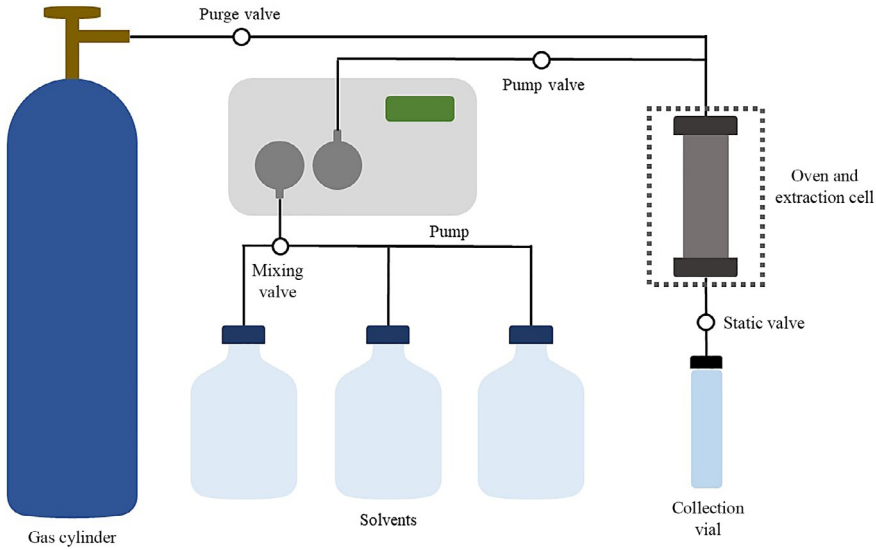

Fig. 23.1 Typical PLE configuration. Basic steps of PLE: (1) The sample is prepared and placed inside the extraction cell. (2) The extraction cell is sealed and transferred inside the oven. (3) The extraction cell is preheated, filled with the extraction solvent, and heated under high pressure. (4) The solvent is collected after the extraction. (5) The extraction cell is flushed and purged between extraction cycles.

mixtures are often used to improve the extraction efficiency. High temperature enhances extraction efficiency by increasing the sample permeability to the extraction phase, disrupting the sample matrix/analyte interactions and enhancing analyte mass transfer. However, extremely high temperature results in the destruction of thermosensitive analytes and the coextraction of sample matrix interferences. The temperature employed in PLE is usually above the boiling point of the extraction solvent; thus high pressure is applied to prevent the liquid solvent from becoming a gas. Similar to high temperature, high pressure increases sample permeability by forcing the extraction phase inside the sample cavities, increases analyte mass transfer by disrupting the sample structure, and reduces interferences caused by trapped air. Static time and the number of extraction cycles are directly related and positively affect the extraction efficiency. Increased static time results in sample soaking and prolonged extraction phase/analyte interactions, while multiple extraction cycles introduce fresh amounts of extraction phase to the sample. Overall, PLE has reduced extraction time and extraction phase requirements. However, multiple extraction cycles result in analyte dilution and the need for an analyte preconcentration step prior to analysis, while the coextraction of sample matrix interferences results in reduced selectivity [2].

23.1.2 QuEChERS (Quick, easy, cheap, effective, rugged and safe) extraction

QuEChERS extraction involves analyte extraction from an aqueous or a solid sample into a water-miscible organic solvent by the salting-out effect of an inorganic salt, followed by a dispersive solid-phase extraction (dSPE) cleanup step. Usually the sample is mixed with an extraction solvent and an inorganic salt, vortex-mixed, and centrifuged. The addition of the inorganic salt results in sample phase/extraction phase separation. Then the organic phase is collected, mixed with a dSPE cleanup sorbent and an inorganic salt, vortex-mixed, and centrifuged. Finally the extraction solvent is collected and analyzed. QuEChERS extraction was originally introduced for the extraction of pesticides from fruit and vegetables with ACN and anhydrous magnesium sulfate ($MgSO_4$) and cleanup with primary secondary amine (PSA) [3]. The basic steps of QuEChERS extraction are outlined in Fig. 23.2.

The extraction solvent, inorganic salt and cleanup sorbent type, and amount directly affect the extraction efficiency of any QuEChERS extraction protocol

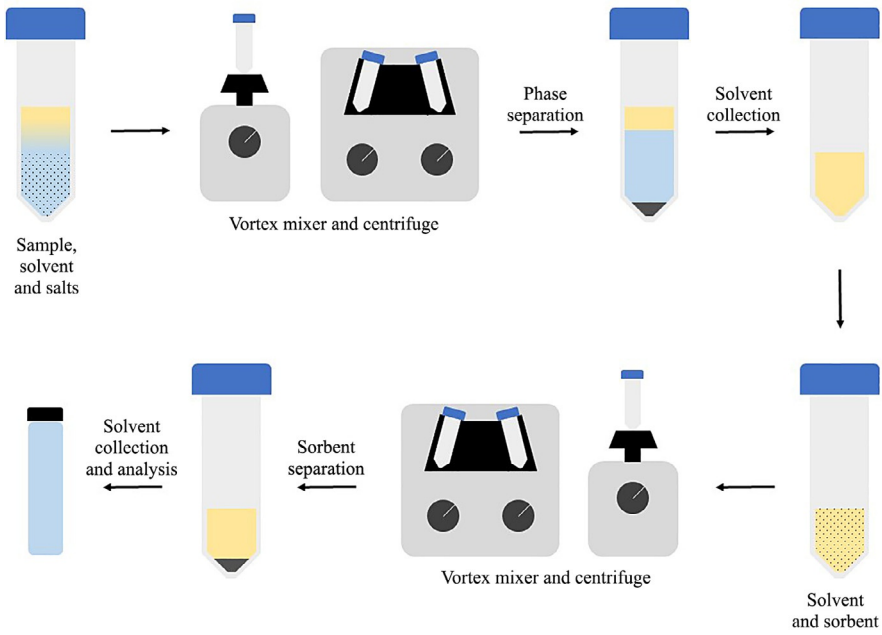

Fig. 23.2 Basic steps of QuEChERS extraction for aqueous samples: (1) The sample is mixed with the solvent and the salt. (2) The mixture is vortex-mixed and centrifuged. (3) The solvent is collected and mixed with the sorbent. (4) The mixture is vortex-mixed and centrifuged. (5) The solvent is collected and analyzed.

and can be modified to extend the application of QuEChERS extraction to a wider range of analytes and sample matrices. Other parameters include the amount of sample and the sample-to-solvent ratio, as well as the use of buffer solution or salts. ACN is the extraction solvent of choice in the majority of QuEChERS applications. ACN is as water-miscible solvent that facilitates analyte extraction from aqueous samples, while it can be effortlessly separated from the sample solution in contrast with solvents such as acetone or ethyl acetate. Additionally, ACN is suitable for analyte extraction from samples with high protein and/or fat content. Anhydrous $MgSO_4$ is the most commonly employed inorganic salt in QuEChERS extraction for phase separation. It provides better sample phase/extraction phase separation and increases the recoveries of polar analytes. An amount of anhydrous $MgSO_4$ is also added in the cleanup step to bind any remaining water from the extraction phase. In addition to $MgSO_4$, sodium chloride is usually added to the sample to enhance phase separation, reduce the coextraction of sample matrix interferences, and increase the extraction selectivity by providing control over the polarity of the extraction solvent. The dSPE cleanup sorbent is selected according to the nature of the coextracted sample matrix interreferences and can be silica-based, ion-exchange, or polymeric solid-phase extraction (SPE) cartridge material. To minimize method errors for samples with increased fat content, an internal standard is always added into the salting-out step or at the end of the extraction. Overall, QuEChERS extraction is a method with reduced time and solvent requirements that offers high sample throughput and recovery values and increased accuracy [4].

23.1.3 Single-drop microextraction (SDME)

SDME involves the extraction of analytes from a sample solution (donor phase) by means of a drop of an extraction phase (acceptor phase). The acceptor phase drop, usually supported by a microsyringe, is introduced to the donor phase and after the extraction is withdrawn and directly injected for analysis. During the extraction the sample solution is continuously stirred and heated. SDME can be run in either two-phase or three-phase mode. In two-phase SDME the analytes are extracted from an aqueous donor phase into an organic acceptor phase drop. The acceptor phase drop can be directly immersed into the donor phase, resulting in direct immersion-single-drop microextraction (DI-SDME). In three-phase SDME, also referred as liquid-liquid-liquid microextraction, the analytes are preextracted from an aqueous donor phase into an intermediate phase, commonly an organic solvent, and back-extracted into an aqueous acceptor phase drop. Alternatively the intermediate phase can be the headspace above the sample solution, resulting in the headspace-single-drop microextraction (HS-SDME) [5]. DI-SDME, HS-SDME, and three-phase SDME modes are illustrated in Fig. 23.3.

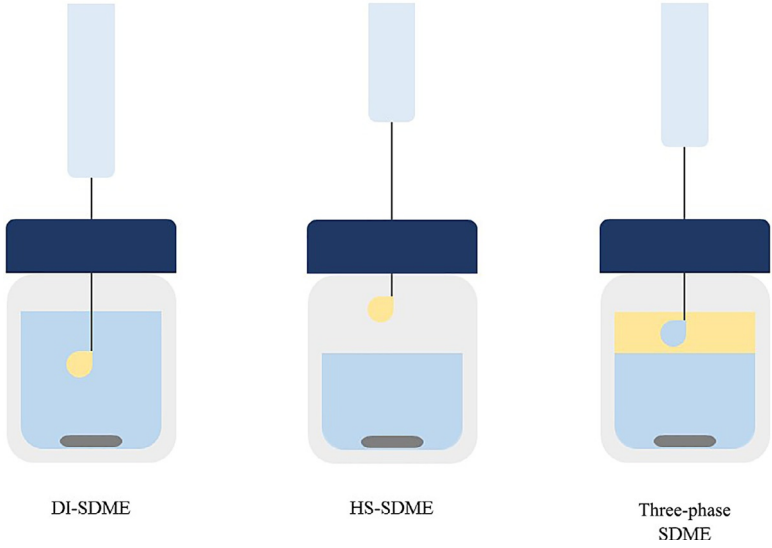

Fig. 23.3 Typical SDME modes. A drop of organic acceptor phase can be either directly immersed into the aqueous donor phase (DI-SDME) or exposed in the headspace above the aqueous donor phase (HS-SDME). In three-phase SDME, a drop of aqueous acceptor phase is introduced into an intermediate organic phase that is in contact with the aqueous donor phase.

Parameters that affect the extraction efficiency of DI-SDME and HS-SDME include donor phase properties, stirring rate, and heating temperature, as well as acceptor phase properties and drop volume. Analyte ionization and polarity and sample solution volatility are properties that determine the application of either DI-SDME or HS-SDME. DI-SDME is suitable for the extraction of less polar and nonpolar analytes of high molecular weight, while HS-SDME is suitable for the extraction of semivolatile and volatile polar and nonpolar analytes of lower molecular weight [6]. In three-phase SDME the analytes are in their neutral form to be preextracted into the intermediate organic solvent phase and then ionized to be extracted into the aqueous acceptor phase. Acidic analytes are extracted from an acidic donor phase into an alkaline acceptor phase, while basic analytes are extracted from an alkaline donor phase into an acidic acceptor phase. Additionally, ionization prevents the coextraction of the nonionized interferences that were initially transferred to the organic phase from the donor phase, thus increasing extraction selectivity in comparison with two-phase SDME [7]. Donor-phase stirring is usually achieved by introducing a magnetic stir bar into the sample solution, at stirring rates ranging between 300 and 600 rpm for DI-SDME and 500–1000 rpm for HS-SDME. Stirring decreases the extraction time and increases the analyte transfer rate; however, higher

stirring rates destabilize the drop in DI-SDME and cause sample solution splashing in HS-SDME. Temperature is a more crucial factor for HS-SDME than DI-SDME. High temperatures result in increased headspace concentration of nonpolar analytes; however, the headspace concentration of polar analytes is decreased at high temperatures due to the increase in analyte water solubility. High temperatures also decrease analyte solubility in the organic acceptor phase. The acceptor phase in SDME should be compatible with the extracted analytes and the analytic instrument to enable direct injection. In the case of DI-SDME, the acceptor phase drop is in direct contact with the donor phase; thus a water-immiscible organic solvent is required. The selected organic solvent should also be sufficiently viscose for easier drop formation. Typical organic solvents used in SDME are 1-octanol, while typical liquid-liquid extraction (LLE) solvents, such as chloroform ($CHCl_3$), EtOAc, and diethyl ether can be employed as modifiers when mixed with water-immiscible solvents, such as toluene. In the case of HS-SDME, the acceptor phase is not in contact with the donor phase solution; thus water immiscibility is not required. The organic solvent should have a boiling point sufficiently high that it does not evaporate at the temperature employed for the extraction. Semivolatile analytes are extracted with organic solvents, such as *o*-xylene, while volatile analytes are extracted with solvents with higher boiling point, such as 1-octanol and tetradecane. Finally, analyte extraction increases with acceptor drop volume. The drop volume at the tip of a microsyringe ranges from 2 to 3 μL. Higher drop volumes result in drop instability in DI-SDME. Also the drop volume in HS-SDME is affected by evaporation and wicking on the microsyringe needle surface [6]. Overall, SDME is a simple and versatile method, with low organic solvent requirements. It successfully combines analyte extraction and preconcentration while enabling direct analysis of the extraction phase.

23.1.4 Dispersive liquid-liquid microextraction (DLLME)

DLLME involves analyte extraction and preconcentration from an aqueous solution into a dispersion of extraction phase droplets. The extraction phase is usually a water-immiscible organic solvent (extraction solvent) and a water-miscible organic solvent (dispersion solvent). The addition of a dispersion solvent enables the dispersion of the extraction solvent in the sample solution. Usually the extraction phase is injected rapidly into the sample solution, and a cloudy solution of finely dispersed extraction phase droplets is formed via manual or mechanical agitation. After the extraction the organic phase is separated from the aqueous sample via centrifugation and collected by means of a microsyringe. The extraction phase can be either further treated or directly analyzed. In general, DLLME is a simple method with low solvent consumption and sample requirements that offers high recovery values. More importantly the formation of finely dispersed extraction phase droplets in the aqueous sample

solution results in increased analyte transfer from the aqueous sample into the extraction solvent, thus significantly reducing the extraction time [8].

Important parameters that are optimized during the development of a DLLME protocol are the selection of the extraction and the dispersion solvent type and volume, the sample solution pH, and the addition of a salting-out agent. The selected extraction solvent must be water-immiscible, so that it does not form a solution with the aqueous phase and can be fully separated from the sample solution. Also, it must be fully dispersible after agitation, either with or without the addition of the dispersion solvent, and compatible with the extracted analytes and the analytic instrument to enable direct injection. Most common extraction solvents for DLLME are high-density chlorinated solvents, such as $CHCl_3$ and carbon tetrachloride (CCl_4), while solvents of low density, such as toluene, 1-octanol, 1-dodecanol, and 1-udecanol, constitute more environmentally friendly alternatives. The selected dispersion solvent assists the dispersion of the extraction solvent droplets throughout the aqueous sample solution; thus it should be both water-miscible and miscible in the extraction solvent. Also, it must be compatible with the extraction solvent in both dispersion and separation steps. ACN, MeOH, acetone, ethanol, and isopropanol are commonly employed as dispersion solvents. Typical extraction solvent volumes range from 10 to 250 μL and dispersion solvent volumes from 0.15 to 2.5 mL. Similar to SDME the sample pH is adjusted by the addition of an acidic or an alkaline solution so that the analytes are in their neutral form and effectively extracted into the extraction solvent. The salting-out effect can be exploited for improved extraction efficiency in DLLME as well. Furthermore, sample pretreatment can be beneficial to the overall extraction. Common practices are sample dilution prior to pH adjustment and protein precipitation for biological samples high in proteins, such as whole blood, plasma, and serum. In the case of protein precipitation, the organic solvent selected to precipitate the sample proteins can also act as the dispersion solvent for the DLLME step. Biological samples low in proteins, such as urine and saliva, usually do not require sample pretreatment and can be directly employed in DLLME [8, 9].

Various DLLME modes have been reported that depend on the selected extraction solvent density or the extraction phase dispersion approach. An organic solvent with density higher than water ($CHCl_3$ and CCl_4) is usually selected as extraction solvent in conventional DLLME applications and along with the dispersive solvent is injected rapidly into the sample solution. The mixture is manually agitated to achieve extraction solvent dispersion, and after centrifugation the extraction phase is collected with a microsyringe from the bottom of the extraction vessel. Prior to analysis the extraction solvent is evaporated, and the dry residue is reconstituted with an instrument compatible solvent. However, the evaporation/reconstitution step is a disadvantage of conventional DLLME. Low-density solvent-dispersive liquid-liquid microextraction (LDS-DLLME) overcomes the previously mentioned disadvantage by employing organic solvents with a lower density than water (toluene and

1-octanol) as extraction solvents. The lighter extraction solvent is now collected from the top of the sample solution with a microsyringe and directly analyzed. However, extraction solvent separation is not always satisfactory in LDS-DLLME, and dispersive liquid-liquid microextraction-solidification of floating organic droplet (DLLME-SFOD) can be utilized as an alternative. In DLLME-SFOD, solvents with density lower than water and low melting points (1-dodecanol and 1-undecanol) are selected as extraction solvents. After analyte extraction and organic phase separation, the extraction vessel is placed in an ice bath, and the extraction solvent is solidified and collected. While DLLME-SFOD employs less toxic extraction solvents and does not require agitation for extraction solvent dispersion in comparison with conventional DLLME, it is limited by the melting point of the organic solvents and provides lower extraction rates [9, 10]. Ionic liquid-dispersive liquid-liquid microextraction (IL-DLLME) is an alternative DLLME mode that employs ionic liquids as extraction solvents, replacing the toxic chlorinated organic solvents. Ionic liquids have, or presumed to have, lower toxicity than traditional chlorinated organic solvents. Ionic liquids such as $[C_4MIM][PF_6]$ (1-butyl-3-methylimidazolium hexafluorophosphate) and $[C_8MIM][PF_6]$ (1-octyl-3-methylimidazolium hexafluorophosphate) are interesting alternative extraction solvent for DLLME [8]. Finally, sonication for a few minutes is a superior alternative to manual agitation employed in conventional DLLME for dispersion of the extraction phase. Ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME), also referred to as ultrasound-assisted emulsification microextraction, provides improved analyte extraction rates and in some cases eliminates the use of a dispersion solvent; however, extended sonication may cause analyte decomposition [9]. The basic steps of conventional DLLME, LDS-DLLME, and DLLME-SFOD are given in Fig. 23.4.

23.2 Biomedical Applications

23.2.1 PLE applications

Human hair is the most common solid sample employed for bioanalytical purposes. A pressurized liquid extraction-liquid chromatography-tandem mass spectrometry (PLE-LC-MS/MS) and two pressurized liquid extraction-liquid chromatography-high-resolution mass spectrometry (PLE-LC-HRMS) methods were developed by the same team for the determination of illicit psychotropic drugs and their metabolites in human hair for drug abuse testing purposes. The PLE-LC-MS/MS method was developed for the determination of amphetamines, cocaine, hallucinogens, and opiates. Decontaminated hair samples (1–2 mm; 100 mg) were mixed with diatomaceous earth in a mortar and pestle, and the mixture was placed and sealed inside an extraction cell. The void volume inside the cell was filled with diatomaceous earth.

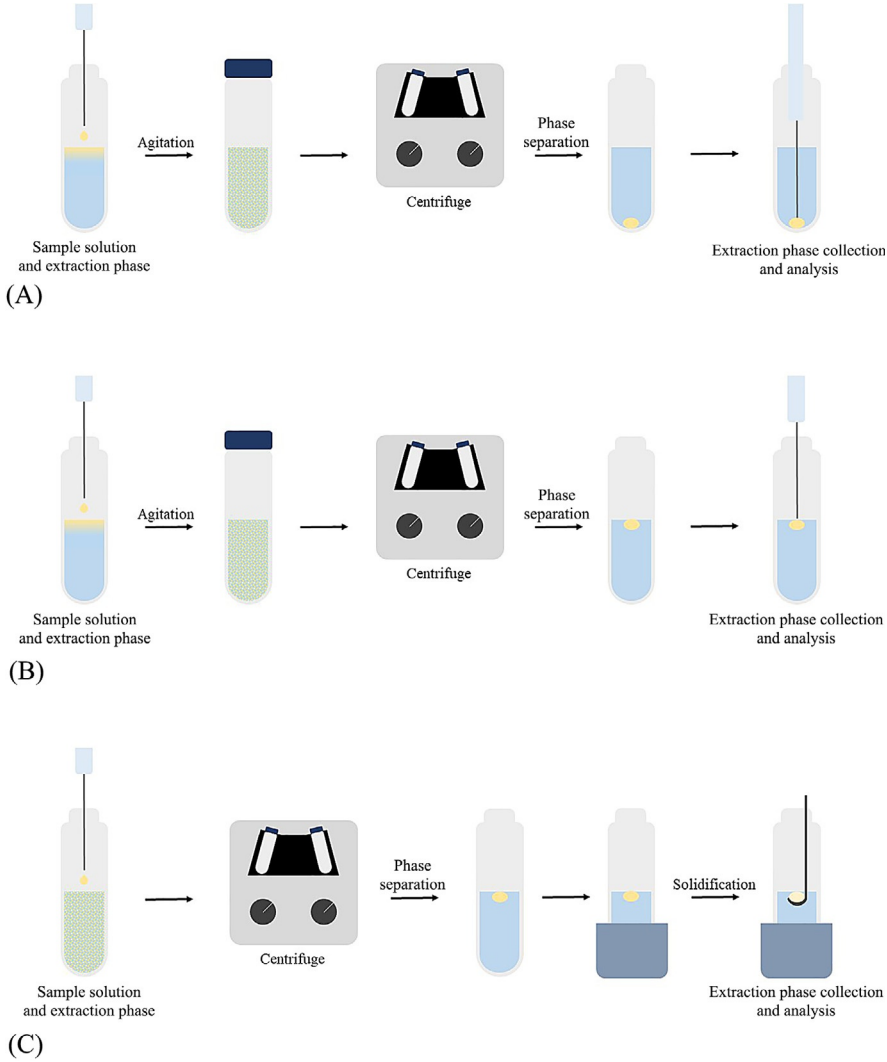

Fig. 23.4 Basic steps of (A) conventional DLLME, (B) LDS-DLLME, and (C) DLLME-SFOD. In all DLLME modes the extraction phase is dispersed into the sample solution, with or without agitation, and after centrifugation, it is collected from the bottom of the extraction vessel in conventional DLLME or the top of the aqueous solution in LDS-DLLME. In DLLME-SFOD the extraction phase is solidified by means of an ice bath and is collected. Sonication can be applied in all modes to improve extraction phase dispersion.

The extraction cell was preheated (1 min), filled with water-MeOH (80:20, v/v), and heated (150°C, 7 min). PLE was performed at 100 bar pressure for 5 min. The extraction phase (6–8 mL) was collected and loaded into a SPE cartridge preconditioned with MeOH (1 mL) and water-MeOH (80:20, v/v; 1 mL). The loaded cartridge was washed with ultrapure water (1 mL) and dried under vacuum and analyte elution achieved with formic acid in MeOH (0.01 M; 0.5 mL) [11]. A similar extraction protocol was applied in the two PLE-LC-HRMS methods. Decontaminated hair sample (1–2 mm; 50 mg) and extraction phase of water-MeOH (90:10, v/v) were employed in the first PLE-LC-HRMS method for the determination of cannabinoids [12], while decontaminated dried hair (1–2 mm; 20 mg), water-MeOH (70:30, v/v), and 125°C were employed in the second PLE-LC-HRMS method [13]. Decontamination was achieved by washing the hair samples sequentially with either phosphate buffer (0.1 M, pH 6; 5 mL), isopropanol (5 mL) and dichloromethane (5 mL) [11, 12], or water (5 mL) and MeOH (2 × 5 mL) [13]. All PLE applications are summarized in Table 23.1.

23.2.2 QuEChERS EXTRACTION APPLICATIONS

23.2.2.1 QuEChERS Extraction for Whole Blood Samples

A typical QuEChERS extraction-gas chromatography-tandem mass spectrometry (GC-MS/MS) method was developed for the determination of Δ^9tetrahydrocannabinol and two metabolites. Whole blood (350 mg) was mixed with $MgSO_4$ (150 mg) and NaCl (80 mg) and vortex-mixed (1 min). ACN (0.65 mL) was added, and the mixture was vortex-mixed (5 min) and centrifuged (13,750 rpm, 10 min). The extraction phase (0.6 mL) was collected, mixed with C_{18} (octadecylsiloxane-bonded silica), vortex-mixed, and centrifuged. The supernatant was collected and evaporated (N_2 stream) and the dry residue reconstituted with derivatization mixture prior to analysis [14]. Another typical QuEChERS—LC-MS/MS method—was developed for the determination of 90 drugs of forensic interest. Diluted whole blood samples (0.5 mL) were mixed with $MgSO_4$ (6 g), sodium acetate (1.5 g), and ACN internal standard solution (1 mL), and the mixture was shaken (30 s) and centrifuged (4400 rpm, 5 min). The extraction phase (0.6 mL) was collected and mixed with PSA (25 mg), C_{18} (25 mg), and $MgSO_4$ (150 mg), and the mixture was shaken (10 s) and centrifuged (4400 rpm, 1 min). The supernatant was collected and analyzed [15].

A modified QuEChERS extraction—LC-MS/MS method was developed for the determination of benzodiazepines, amphetamines, cocaine, lysergic acid diethylamide and opiates. Pretreated blood (1 mL) was mixed with saturated carbonate buffer (0.1 mL), PSA (25 mg), $MgSO_4$ (150 mg), and EtOAc (3 mL), and the mixture was shaken (10 min) and centrifuged (4000 rpm, 8 min). The extraction phase

TABLE 23.1 PLE and QuEChERS Extraction Applications

Bioanalytical Sample Sample Size	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Hair 100 mg	14 Illicit psychotropic drugs and metabolites (amphetamines, cocaine, hallucinogens, and opiates)	PLE	Pretreatment: decontamination with phosphate buffer (0.1 M, pH 6, 5 mL), isopropanol (5 mL) and dichloromethane (5 mL); extraction phase: H_2O/MeOH (80:20, v/v); temperature: 150°C; pressure: 100 bar; static time: 5 min; cycles: 1; cleanup: SPE	LC-MS/MS 10 min	LOD (μg/kg): 0.5–4.7 LOQ (μg/kg): 1.8–16	50%–100% ≤13%	[11]
Hair 50 mg	4 Illicit psychotropic drugs and metabolites (cannabidiol, cannabinol, Δ^9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol)	PLE	Pretreatment: decontamination with phosphate buffer (0.1 M, pH 6, 5 mL), isopropanol (5 mL) and dichloromethane (5 mL); extraction phase: H_2O/MeOH (90:10, v/v); temperature: 150°C; pressure: 100 bar; static time: 5 min; cycles: 1; cleanup: SPE	HPLC-HRMS 4 min	LOD (μg/kg): 0.03–0.8 LOQ (μg/kg): 0.1–2	86%–100% ≤7%	[12]

Hair 20 mg	16 Illicit psychotropic drugs and metabolites	PLE	Pretreatment: decontamination with H_2O (5 mL) and MeOH (2 × 5 mL); extraction phase: H_2O/MeOH (70:30, v/v); temperature: 125°C; pressure: 100 bar; static time: 5 min; cycles: 1; cleanup: SPE	HPLC-HRMS 10 min	LOD (μg/kg): 0.5–10 LOQ (μg/kg): 8–50	91%–108% ≤11%	[13]
Whole blood 350 mg	3 Cannabinoids and metabolites (Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol)	QuEChERS extraction	Extraction solvent: ACN (0.65 mL); salting-out: $MgSO_4$ (150 mg) and NaCl (80 mg); dSPE: C18 (12.5 mg)	GC-MS/MS ~33 min	LOD (μg/kg): 0.011–0.13 LOQ (μg/kg): 0.033–0.43	94.2%–102% <7%	[14]
Whole blood 0.5 mL	90 Drugs	QuEChERS extraction	Extraction solvent: ACN; salting-out: $MgSO_4$ (6 g) and NaOAC (1.5 g); dSPE: PSA (25 mg), C18 (25 mg) and $MgSO_4$ (150 mg)	LC-MS/MS 30 min	LOD (ng/L): 0.12–4.43 LOQ (μg/L): 1–14.7	39%–127% ≤16.6%	[15]
Blood 1 mL	47 Psychotropic drugs (benzodiazepines, amphetamines, cocaine, LSDs, and opiates)	Modified QuEChERS extraction	Extraction solvent: EtOAc (3 mL); salting-out: saturated carbonate buffer (0.1 mL) of $NaHCO_3$ (45 g) and Na_2CO_3 (30 g) in distilled H_2O; dSPE: PSA (25 mg) and $MgSO_4$ (150 mg)	UHPLC-MS/MS <20 min	LOD (μg/L): 0.5–2 (benzodiazepines) and 0.05–2 (other drugs) LOQ (μg/L): 2–20 (benzodiazepines) and 0.2–2 (other drugs)	20%–99% N/A[c]	[16]

Continued

TABLE 23.1 PLE and QuEChERS Extraction Applications—cont'd

Bioanalytical Sample Sample Size	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Whole blood 0.1 mL	35 Illicit psychotropic drugs and metabolites (amphetamines, cocaine, and opiates)	Modified QuEChERS extraction	Extraction solvent: ACN (0.2 mL); salting-out: $MgSO_4$ (4 g), NaCl (1 g), $Na_3C_6H_5O_7$ (1 g), and $Na_2C_6H_6O_7$ (500 mg); dSPE: not applied	LC-MS/MS 5.3 min	LOD (μg/L): 3 LOQ[d] (μg/L): 5	34.5%–106% <20%	[17]
Whole blood 0.1 mL	13 Psychotropic drugs and metabolites (amphetamines and benzodiazepines)	Modified "caking" QuEChERS extraction	Extraction solvent: CH_3COOH in ACN (0.2%; 0.5 mL); salting-out: $MgSO_4$ and NaCl (150 mg); dSPE: not applied	GC-MS LC-MS <20 min	LOD (μg/L): 1 (GC) and 2–100 (LC) LOQ (μg/L): N/A	58.5%–92.9% <11%	[18]
Postmortem blood and serum 0.1 mL	15 Psychotropic drugs (antidepressant, antipsychotic, and hypnotic drugs)	Modified mini-QuEChERS extraction	Extraction solvent: ACN (0.6 mL); salting-out: $MgSO_4$ (150 mg) and K_2CO_3 (5 mg); dSPE: not applied	UPLC-MS/MS 10.5 min	LOD (μg/L): 0.3–17 LOQ (μg/L): 1–50	87.3%–113.6% ≤13.2%	[19]
Plasma 0.2 mL	1 Antiepileptic drug (valproate)	QuEChERS extraction	Extraction solvent: ACN (1 mL); salting-out: $MgSO_4$ (400 mg) and NaOAC (100 mg); dSPE: C_{18} (50 mg) and $MgSO_4$ (150 mg)	GC-MS/MS ~11 min	LOD (μg/L): 10 LOQ (μg/L): 50	85.3%–108% <18.5%	[20]

Plasma 1.5 mL	1 Antiretroviral drug (nevirapine)	QuEChERS extraction	Extraction solvent: CH_3COOH in ACN (0.1%; 4 mL for GC, 3 mL for LC); salting-out: $MgSO_4$, NaCl, $Na_3C_6H_5O_7$, and $Na_2C_6H_6O_7$ (2.6 g for GC, 1.95 g for LC); dSPE: C_{18}, PSA and $MgSO_4$ (150 mg)	GC-MS 12 min LC-MS HPLC-UV <5 min	MDL (μg/L): 11.1–29.8 and 13.7–36.0 (GC), 3.14–47.1 (LC) LOQ (μg/L): 16.5–66.7 and 28.4–98.7 (GC), 2.85–90 (LC)	83% 4.6% (GC)	[21]
Plasma 5 mL	5 Antiretroviral drugs (efavirenz, emtricitabine, lopinavir, ritonavir, and tenofovir)	QuEChERS extraction	Extraction solvent: CH_3COOH in ACN (0.1%; 5 mL); salting-out: $MgSO_4$, NaCl, $Na_3C_6H_5O_7$, and $Na_2C_6H_6O_7$ (2.16 g); dSPE: C_{18}, PSA and $MgSO_4$ (150 mg)	LC-MS 9 min	LOD (μg/L): 14.6–56.2 LOQ (μg/L): 29.3–112.5	60.1%– 83.2% <8%	[22]
Urine 2 mL	4 Antidepressant drugs and metabolites (fluoxetine, norfluoxetine, clomipramine, and desmethylclomipramine)	QuEChERS extraction	Extraction solvent: EtOAc (2 mL); salting-out: $MgSO_4$ (800 mg), NaCl (200 mg), $Na_3C_6H_5O_7$ (200 mg), and $Na_2C_6H_6O_7$ (100 mg); dSPE: PSA (25 mg), C_{18} (25 mg), and $MgSO_4$ (150 mg)	UHPLC-PDA 10 min	LOD (μg/L): 60–92 LOQ (μg/L): 100	86.1%– 108.8% <10%	[23]
Urine 10 mL	3 Psychotropic drugs (diazepam, flunitrazepam, and medazepam)	QuEChERS extraction	Extraction solvent: ACN (10 mL); salting-out: $MgSO_4$ (4 g) and NaCl (1 g); dSPE: GCB (25 mg) and $MgSO_4$ (150 mg)	GC-MS <20 min	N/A	55%– 128% N/A	[24]

Continued

TABLE 23.1 PLE and QuEChERS Extraction Applications—cont'd

Bioanalytical Sample Sample Size	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Urine 0.1 mL	2 Illicit psychotropic drug metabolites (synthetic cannabinoid)	Modified QuEChERS extraction/ filtration	Extraction solvent: ACN (1 mL); salting-out: not applied; dSPE: C_{18} (25 mg) and $MgSO_4$ (150 mg)	LC-MS/MS 10 min	LOD (μg/L): 1 LOQ (μg/L): 5	81.2%–104% ≤15.3%	[25]
Hair 50 mg	50 Psychotropic drugs and metabolites (analgesic, antiepileptic, antidepressant, antipsychotics, and hypnotic drugs)	QuEChERS extraction	Extraction solvent: MeOH (2 mL); salting-out: not applied; dSPE: PSA (50 mg), C_{18} (50 mg), and $MgSO_4$ (150 mg)	LC-MS/MS 30 min	LOD (μg/L): 0.002–0.02 LOQ (μg/L): 0.005–0.05	32.4%–99.5% <14%	[26]

[a] LOD, limit of detection; LOQ, limit of quantification.
[b] Extraction recovery refers to either absolute or relative recovery, depending on which one is provided by the authors.
[c] Not available information.
[d] Lower limit of quantification.

was collected and evaporated (N_2 stream) and the dry residue reconstituted with 0.1% formic acid aqueous solution (0.1 mL) [16]. A modified "one-step" QuEChERS extraction—LC-MS/MS method was developed for the determination of amphetamines, cocaine, and opiates. Whole blood (0.1 mL) was mixed with ethylenediaminetetraacetic acid (EDTA) and ACN (0.2 mL) and the mixture vortexed (30 s). $MgSO_4$ (4 g), NaCl (1 g), $Na_3C_6H_5O_7$ (trisodium citrate) (1 g), and $Na_2C_6H_6O_7$ (disodium citrate) (500 mg) were added, and the mixture was vortex-mixed and centrifuged (18,200 g, 10 min). The extraction phase (0.05 mL) was collected and diluted with ammonium formate-formic acid buffer prior to analysis [17]. A modified "one-pot" QuEChERS extraction—GC-MS method was developed for the determination of amphetamines and benzodiazepines. $MgSO_4$ and NaCl (150 mg in total) were mixed with 0.2% (v/v) acetic acid in ACN (0.5 mL), and the mixture was vortex-mixed (10 s). Blood (0.1 mL) was added and the mixture vortex-mixed (10 s) and centrifuged (7300 g, 2 min). The extraction phase was collected by means of a pipette. A second portion of 0.2% (v/v) acetic acid in ACN (0.5 mL) was added to the blood/inorganic salts "cake" mass and the mixture vortex-mixed and decanted. Both extraction phases were combined and evaporated (N_2 stream), and the dry residue was reconstituted with mobile phase (0.1 mL) [18]. A modified "one-step" mini-QuEChERS extraction LC-MS/MS method was developed for the determination of antidepressant, antipsychotic, and hypnotic drugs in postmortem blood and serum samples. Blood or serum (0.1 mL) was mixed with ACN (0.6 mL), $MgSO_4$ (150 mg), and potassium carbonate (5 mg) and the mixture vortex-mixed (5 min) and centrifuged (6300 g). The extraction phase was collected and evaporated (N_2 stream) and the dry residue reconstituted in internal standard solution (0.1 mL) [19].

23.2.2.2 QuEChERS Extraction for Plasma Samples

A typical QuEChERS extraction—GC-MS/MS method was developed for the determination of the antiepileptic drug valproate for clinical drug monitoring and forensic toxicology. Plasma (0.2 mL) was diluted with distilled water (1.3 mL) and centrifuged (19,600 g, 3 min). The supernatant was collected and mixed with ACN (1 mL), $MgSO_4$ (400 mg), and sodium acetate (100 mg); shaken (30 s); and centrifuged (2000 g, 10 min). The extraction phase was collected and mixed with C_{18} (50 mg) and $MgSO_4$ (150 mg) and the mixture vortex-mixed (2 min) and centrifuged (3000 g, 5 min). The supernatant was collected and evaporated (N_2 stream) and the dry residue reconstituted with ethyl acetate (0.04 mL) [20]. The same team developed a QuEChERS extraction protocol for the determination of antiretroviral drugs nevirapine [21] and efavirenz, emtricitabine, lopinavir, ritonavir, and tenofovir [22] in human plasma. Plasma (1.5 mL) was mixed with 0.1% (v/v) acetic acid in

ACN (4 mL) and $MgSO_4$, NaCl, $Na_3C_6H_5O_7$, and $Na_2C_6H_6O_7$ (2.6 g in total); the mixture was shaken (4 min) and centrifuged (4500 rpm, 5 min). The extraction phase was collected and mixed with C_{18}, PSA, and $MgSO_4$ (150 mg); shaken (2 min); and centrifuged (4500 rpm, 2 min). The supernatant was collected, filtered (0.45 μm), and evaporated (N_2 stream), and the dry residue reconstituted with acetone for analysis by GC-MS. The same steps were taken with 0.1% (v/v) acetic acid in ACN (3 mL) and $MgSO_4$, NaCl, $Na_3C_6H_5O_7$, and $Na_2C_6H_6O_7$ (1.95 g in total) and direct injection of the supernatant for analysis by LC-MS and liquid chromatography-ultraviolet absorption detection (LC-UV) [21]. A similar QuEChERS extraction protocol, for plasma (5 mL); 0.1% (v/v) acetic acid in ACN (5 mL); and $MgSO_4$, NaCl, $Na_3C_6H_5O_7$, and $Na_2C_6H_6O_7$ (2.16 g in total), was developed in a second report. Analysis was carried out by LC-MS. QuEChERS extraction/liquid extraction and QuEChERS extraction/protein precipitation combination were also tested, but QuEChERS extraction without any further sample treatment provided the highest recoveries for all analytes [22].

23.2.2.3 QuEChERS Extraction for Urine Samples

A typical QuEChERS extraction—LC-UV method was developed for the determination of fluoxetine, norfluoxetine, clomipramine, and desmethylclomipramine in urine. Urine (2 mL) was mixed with EtOAc (2 mL) and the mixture shaken (15 s) and vortex-mixed (1 min). $MgSO_4$ (800 mg), NaCl (200 mg), $Na_3C_6H_5O_7$ (200 mg), and $Na_2C_6H_6O_7$ (100 mg) were added, and the mixture was shaken (15 s), vortex-mixed (1 min), and centrifuged (3000 rpm, 5 min). The extraction phase was collected and mixed with PSA (25 mg), C_{18} (25 mg), and $MgSO_4$ (150 mg) and the mixture vortex-mixed and centrifuged (5000 rpm, 3 min). The supernatant was collected and evaporated (N_2 stream) and the dry residue reconstituted with mobile phase (100 μL) [23]. Another QuEChERS extraction—GC-MS method was developed for the determination of diazepam, flunitrazepam, and medazepam. Urine (10 mL) was mixed with ACN (10 mL) and the mixture vortex-mixed (1 min). $MgSO_4$ (4 g) and NaCl (1 g) were added, and the mixture was vortex-mixed (1 min) and centrifuged (3000 rpm, 5 min). The extraction phase (1 mL) was collected, mixed with GCB (graphitized carbon black) (25 mg) and $MgSO_4$ (150 mg), vortex-mixed (30 s), and centrifuged (5000 rpm, 3 min). The supernatant was collected and directly analyzed [24]. A modified QuEChERS extraction combined with filtration—LC-MS/MS method was developed for the determination of two synthetic cannabinoid metabolites. Urine (0.1 mL) was mixed with ACN (1 mL) and shaken. C_{18} (25 mg) and $MgSO_4$ (150 mg) were added, and the mixture was vortex-mixed (30 s) and centrifuged (10,000 rpm, 2 min). The extraction phase was collected and filtered prior to analysis [25].

23.2.2.4 QuEChERS Extraction for Hair Samples

While QuEChERS extraction is suitable for liquid samples, it is also suitable for the extraction of analgesic, antiepileptic, antidepressant, antipsychotic, and hypnotic drugs from human hair for clinical purposes. Pulverized hair sample (1–2 mm; 50 mg), decontaminated with acetone (2 × 5 mL) and n-hexane (2 × 5 mL), was mixed with MeOH (2 mL) and the mixture sonicated overnight at 45°C. PSA (50 mg), C_{18} (50 mg), and $MgSO_4$ (150 mg) were added, and the mixture was vortex-mixed (1 min) and centrifuged (5000 rpm, 4 min). The extraction phase was collected and evaporated and the dry residue reconstituted with mobile phase (0.2 mL). Analysis was carried out by LC-MS/MS [26].

23.2.3 SDME APPLICATIONS

23.2.3.1 DI-SDME and HS-SDME

A DI-SDME—LC-UV method was developed for the determination of lidocaine, bupivacaine, and tetracaine in human urine. A drop of *o*-dibutyl phthalate (1 μL), supported on a microsyringe needle, was immersed into the sample solution (pH 11; 6 mL) for 30 min with stirring (160 rpm) at 30°C. After the extraction the acceptor phase was withdrawn and directly injected for analysis. The microsyringe was washed with MeOH, water, and o-dibutyl phthalate between extractions [27]. A HS-SDME—GC-FID (gas chromatography-flame ionization detector) method was developed for the determination of valproic acid in human serum. A drop of *n*-dodecane (2 μL) was suspended in the headspace of the sample solution (pH 1.5; 1 mL) for 20 min, while the solution was continuously stirred (400 rpm) and heated (45°C). After the extraction the acceptor phase was withdrawn and directly injected for analysis [28]. Finally a "water drop-based" HS-SDME—LC-UV method was developed for the determination of methamphetamine and amphetamine in human urine. A drop of phosphoric acid solution (0.05 M; 5 μL) was suspended in the headspace of the sample solution (5 mL) for 20 min, while the solution was stirred (1200 rpm) and heated (80°C). After the extraction the acceptor phase was withdrawn and directly injected for analysis [29].

23.2.3.2 Three-Phase SDME

A three-phase SDME—LC-UV method was developed for the determination of methadone in human plasma and urine. The analyte was preextracted from the sample solution (pH 9.8; 3.5 mL) with dibutyl ether (0.08 mL) with stirring (1000 rpm) and heating (60°C) for 27 min. Then a drop of phosphoric acid-monosodium phosphate solution (0.01 M, pH 2.7; 7 μL) was suspended in the organic phase and the

analyte back-extracted for 9.5 min. The acceptor phase was withdrawn and directly injected for analysis [30]. The same team developed another three-phase SDME—LC-UV method for the determination of fentanyl in human plasma and urine. The analyte was preextracted from the sample solution (3.5 mL) with *n*-octane (0.1 mL) with stirring (1000 rpm) and heating (30°C) for 30 min. Then a drop of perchloric acid solution (0.001 M; 5 μL) was suspended in the organic phase and the analyte back-extracted for 20 min with stirring (700 rpm). The acceptor phase was withdrawn and directly injected for analysis [31]. Two three-phase SDME protocols were developed for the determination of methamphetamine and amphetamine [32] and ephedrine, morphine, and pethidine [33] in human urine. In the first protocol the analytes were preextracted from the sample solution (6 mL) with *n*-hexane (0.4 mL) with stirring (1200 rpm) for 40 min. Then a drop of phosphoric acid solution (0.02 M; 5 μL) was suspended in the organic phase, and the analytes were back-extracted for 40 min. The acceptor phase was withdrawn and directly injected for LC-UV analysis [32]. In the second "carrier mediated" three-phase SDME protocol, the analytes were extracted by a drop of hydrochloric acid solution (0.2 M; 1.5 μL) suspended in the organic layer containing tricaprylmethylammonium chloride-toluene (0.1 M; 0.3 mL) on the surface of the sample solution (pH 11.5; 4.9 mL). The extraction was achieved under continuous stirring (400 rpm) for 15 min, and the acceptor phase was withdrawn and directly injected for LC-UV analysis [33]. Finally a "directly suspended droplet" three-phase SDME—LC-UV method was developed for the determination of ecstasy in human hair. Before the extraction, hair (2 g) was washed with dichloromethane (20 mL), acetone (15 mL), and MeOH (15 and 10 mL) and dried at room temperature. Then the hair sample (50 mg) was cut and mixed with MeOH (2 mL) and the mixture adjusted to pH 7.4 and digested (50°C, 5 h). The sample solution was collected and filtered. The hair was rinsed with ethanol (0.5 mL) and combined with the sample solution. Ecstasy was extracted from the sample solution (pH 11; 5 mL) with 1-octanol (0.35 mL) with stirring (1000 rpm) for 3 min. Then a drop of acidified deionized water (pH 5; 10 μL) was suspended in the organic phase and the ecstasy back-extracted for 20 min. The acceptor phase was withdrawn and directly injected for analysis [34]. All SDME applications are summarized in Table 23.2.

23.2.4 DLLME APPLICATIONS

23.2.4.1 Conventional DLLME and LDS-DLLME

A conventional DLLME—LC-UV method was developed for the determination of methadone in human plasma, saliva, sweat, and urine for drug of abuse detection purposes. Chloroform (250 μL) and MeOH (2.5 mL) were injected rapidly into the sample solution (pH 10; 10 mL) and the cloudy solution formed centrifuged

TABLE 23.2 SDME and DLLME Applications

Bioanalytical Sample	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Urine	3 Anesthetic drugs (bupivacaine, lidocaine, and tetracaine)	DI-SDME	Aqueous donor phase: pH 11 (6 mL); organic acceptor phase drop: *o*-dibutyl phthalate (1 μL); stir rate: 160 rpm; temperature: 30°C; extraction time: 30 min	HPLC-UV 30 min	LOD (μg/L): 30–50 LOQ (μg/L): N/A	68.6%–99% ≤5.5%	[27]
Serum	1 Antiepileptic drug (valproic acid)	HS-SDME	Aqueous donor phase: pH 1.5 (1 mL); organic acceptor phase drop: *n*-dodecane (2 μL); stir rate: 400 rpm; temperature: 45°C; extraction time: 20 min	GC-FID <10 min	LOD (μg/L): 800 LOQ (μg/L): N/A	N/A[c] ≤9.8%	[28]
Urine	1 Illicit psychotropic drug and metabolite (methamphetamine and amphetamine)	HS-SDME	Aqueous donor phase: alkaline (5 mL); aqueous acceptor phase drop: H_3PO_4 solution (0.05 M; 5 μL); stir rate: 1200 rpm; temperature: 80°C; extraction time: 20 min	HPLC-UV <7 min	LOD (μg/L): 0.3 LOQ (μg/L): N/A	89.3%–105% ≤16.5%	[29]

Continued

TABLE 23.2 SDME and DLLME Applications—cont'd

Bioanalytical Sample	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Plasma and urine	1 Psychotropic drug (methadone)	Three-phase SDME	Aqueous donor phase: pH 9.8 (3.5 mL); aqueous acceptor phase drop: H_3PO_4/NaH_2PO_4 solution (0.01 M, pH 2.7; 7 μL); organic phase: dibutyl ether (0.08 mL); stir rate: 1000 rpm; temperature: 60°C; preextraction time: 27 min; back-extraction time: 9.5 min	HPLC-UV ~10 min	LOD (μg/L): 0.2 LOQ (μg/L): N/A	77% <8.1%	[30]
Plasma and urine	1 Analgesic drug (fentanyl)	Three-phase SDME	Aqueous donor phase: alkaline (3.6 mL); aqueous acceptor phase drop: $HClO_4$ solution (0.001 M; 5 μL); organic phase: *n*-octane (0.1 mL); stir rate: 1000 and 700 rpm; temperature: 30°C; preextraction time: 30 min; back-extraction time: 20 min	HPLC-UV <8 min	LOD (μg/L): 0.1 LOQ (μg/L): 0.3	49% <9%	[31]

Urine	1 Illicit psychotropic drug and metabolite (methamphetamine and amphetamine)	Three-phase SDME	Aqueous donor phase: alkaline (6 mL); aqueous acceptor phase drop: H_3PO_4 solution (0.02 M; 5 μL); organic phase: *n*-hexane (0.4 mL); stir rate: 1200 rpm; temperature: N/A; preextraction time: 40 min; back-extraction time: 40 min	HPLC-UV 6 min	LOD (μg/L): 0.5 LOQ (μg/L): N/A	95.1%–107.9% ≤5.9%	[32]
Urine	3 Illicit drugs (ephedrine, morphine, and pethidine)	Three-phase SDME	Aqueous donor phase: pH 11.5 (4.9 mL); aqueous acceptor phase drop: HCl solution (0.2 M; 1.5 μL); organic phase: Aliquat 336/toluene (0.1 M; 0.3 mL); stir rate: 400 rpm; temperature: N/A; preextraction time: N/A; back-extraction time: 15 min	HPLC-DAD 25 min	LOD (μg/L): 0.02–0.05 LOQ (μg/L): N/A	90.8%–104.8% ≤9.8%	[33]
Hair	1 Illicit psychotropic drug (ecstasy)	Three-phase SDME (directly suspended drop)	Aqueous donor phase: pH 11 (5 mL); aqueous acceptor phase drop: acidified deionized H_2O (pH 5; 10 μL); organic phase: 1-octanol (0.35 mL); stir rate: 1000 and 600 rpm; temperature: N/A; preextraction time: 3 min; back-extraction time: 20 min	HPLC-DAD <7 min	LOD (μg/L): 0.1 LOQ (μg/L): 1	65.4% ~5.4%	[34]

Continued

TABLE 23.2 SDME and DLLME Applications—cont'd

Bioanalytical Sample	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Plasma, saliva, sweat, and urine	1 Psychotropic drug (methadone)	DLLME	Pretreatment: centrifugation (5000 rpm, 10 min), filtration (0.45 μm), and dilution (from 0.5 to 10 mL); sample solution: pH 10 (10 mL); extraction solvent: $CHCl_3$ (250 μL); dispersion solvent: MeOH (2.5 mL); salt: no addition; extraction time: N/A	HPLC-UV <15 min	LOD (μg/L): 4.90–25.12 LOQ (μg/L): 16.32–83.65	98.26%–100.34% ≤2.26%	[35]
Blood and urine	1 Analgesic drug (tramadol)	DLLME	Pretreatment: dilution (fivefold with distilled H_2O); sample solution: pH 12 (5 mL); extraction solvent: CCl_4 (30 μL); dispersion solvent: EtOH (1 mL); salt: no addition; extraction time: 3 min	GC-MS <30 min	LOD (μg/L): 0.08 LOQ (μg/L): 0.26	99.2% 3.6%	[36]
Urine	1 Analgesic drug (tramadol)	DLLME	Pretreatment: centrifugation (1133 g, 10 min); sample solution: pH 10 (5 mL); extraction solvent: $CHCl_3$ (70 μL) and EtOAc (30 μL); dispersion solvent: Ace (0.6 mL); salt: NaCl (7.5%, w/v); extraction time: N/A	HPLC-FLD <7 min	LOD (μg/L): 0.2 LOQ (μg/L): 0.9	95.6%–99.6% 4.1%	[37]

Urine	1 Antibacterial drug (ceftazidime)	DLLME	Sample solution: 2 mL; extraction solvent: 1,2-dichloroethane (150 μL); dispersion solvent: Ace (300 μL); salt: no addition; extraction time: 2 min	HPLC-UV <8 min	LOD (μg/L): 0.17 LOQ (μg/L): 0.51	82% 4.2%	[38]
Plasma	5 Antiarrhythmic drugs (carvedilol, diltiazem, metoprolol, propranolol, and verapamil)	DLLME	Pretreatment: protein precipitation with ACN (1.34 mL), vortex mixing (1 min) and centrifugation (8000 rpm, 5 min); sample solution: pH 11.5 (5 mL); extraction solvent: dichloromethane (100 μL); dispersion solvent: no addition; salt: NaCl (1%, w/v); extraction time: N/A	HPLC-UV 7 min	LOD (μg/L): 2–6 LOQ (μg/L): 7–19	90%–104% ≤13%	[39]
Plasma and urine	1 Antiviral/antiparkinsonian drug (amantadine)	DLLME	Pretreatment: plasma protein precipitation with MeOH (2 mL), shaking (5 min), centrifugation (4472 g, 10 min) and dilution with carbonate buffer (0.5 M, pH 10; 4 mL), and urine centrifugation (1118 g, 5 min) and dilution (fivefold with carbonate buffer); sample solution: pH 10 (5 mL); extraction solvent: 1,2-dibromoethane (10 μL); dispersion solvent: MeOH (750 μL); salt: N/A; extraction time: 5 min	GC-FID <20 min	LOD (μg/L): 2.7–4.2 LOQ (μg/L): 8.7–14	72%–93% ≤6%	[40]

Continued

TABLE 23.2 SDME and DLLME Applications—cont'd

Bioanalytical Sample	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Plasma	1 Anticoagulant drug (warfarin)	LDS-DLLME	Pretreatment: with trichloroacetic acid solution (10%, w/v; 5 mL), vortex mixing (20s), refrigeration (4°C, 20 min), centrifugation (3000 rpm, 10 min), and dilution (deionized H_2O); sample solution: pH 2.3 (11 mL); extraction solvent: 1-octanol (150 μL); dispersion solvent: MeOH (150 μL); salt: no addition; extraction time: 2 min	HPLC-UV ~12 min	LOD (μg/L): 5 LOQ (μg/L): N/A	91% 3.8%	[41]
Plasma and urine	1 Antidepressant drug (fluoxetine)	LDS-DLLME	Pretreatment: plasma centrifugation (8000 rpm, 15 min) and filtration (0.45 μm) and urine centrifugation (3000 rpm, 10 min), dilution (distilled H_2O), and filtration (0.45 μm); sample solution: pH 11.3 (20 mL); extraction solvent: 1-octanol (172 μL); dispersion solvent: MeOH (400 μL); salt: no addition; extraction time: 1 min	HPLC-UV <10 min	LOD (μg/L): 3–4.2 LOQ (μg/L): N/A	89%–90.15% <7%	[42]

Urine	1 Antihypertensive drug (valsartan)	DLLME-SFOD	Pretreatment: centrifugation (5000 rpm, 10 min), filtration (0.45 μm), and dilution (threefold); sample solution: pH 3.8 (5 mL); extraction solvent: 1-dodecanol (65 μL); dispersion solvent: EtOH (250 μL); salt: NaCl (4%, w/v); extraction time: 1 min	HPLC-UV ~5 min	LOD (μg/L): 4 LOQ (μg/L): 13	95% <5%	[43]
Urine	5 Macrolide antibiotics (azithromycin, clarithromycin, dirithromycin, erythromycin, and roxithromycin)	DLLME-SFOD	Sample solution: alkaline (5 mL); extraction solvent: 1-dodecanol (60 μL); dispersion solvent: MeOH (440 μL); salt: NaCl (9%, w/v); extraction time: 2 min	LC-CAD ~20 min	LOD (μg/L): 10–40 LOQ (μg/L): 25–100	94.6%–118.4% ≤12.6%	[44]
Plasma	4 Illicit drugs (codeine, morphine, noscapine, and papaverine)	DLLME-SFOD	Pretreatment: plasma protein precipitation with 15% $ZnSO_4$ solution/ACN mixture (50:40, v/v; 0.9–1 mL), vortex mixing (20 min), refrigeration (4°C, 20 min), centrifugation (4000 rpm, 5 min), and dilution (H_2O to 5 mL); sample solution: pH 9 (5 mL); extraction solvent: 1-undecanol (30 μL); dispersion solvent: Ace (470 μL); salt: NaCl (1%, w/v); extraction time: 0.5 min	HPLC-UV <15 min	LOD (μg/L): 0.05–5 LOQ (μg/L): N/A	93.7%–110.5% ≤7.4%	[45]

Continued

TABLE 23.2 SDME and DLLME Applications—cont'd

Bioanalytical Sample	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Serum and urine	1 Psychotropic drug (methadone)	DLLME-SFOD	Pretreatment: plasma protein precipitation with 15% $ZnSO_4$ solution/ACN mixture (50:40, v/v; 0.9–1 mL), vortex mixing (20 min), refrigeration (4° C, 20 min), centrifugation (4000 rpm, 5 min), and dilution (H_2O to 5 mL) and urine vortex mixing, centrifugation (5000 rpm, 10 min), and dilution (H_2O to 5 mL); sample solution: pH 8.9 (5 mL); extraction solvent: 1-undecanol (58 μL); dispersion solvent: MeOH (580 μL); salt: NaCl (1.3%, w/v); extraction time: N/A	HPLC-UV 7 min	LOD (μg/L): 1.67–3.34 LOQ (μg/L): N/A	91.7%–101.5% ≤12.9%	[46]

Plasma	1 Antidepressant drug (duloxetine)	DLLME-SFOD	Pretreatment: plasma protein precipitation with 15% $ZnSO_4$ solution/ACN mixture (50:40, v/v; 0.9–1 mL), vortex mixing (20 min), refrigeration (4° C, 20 min), centrifugation (4000 rpm, 5 min), and dilution (H_2O to 5 mL); sample solution: alkaline (1 mL); extraction solvent: 1-undecanol (50 μL); dispersion solvent: no addition; salt: no addition; extraction time: 1 min	HPLC-FLD ~10 min	LOD (μg/L): N/A LOQ (μg/L): 2.5	59.6%–65.5% ≤11.1%	[47]
Dried blood spot	1 Antiasthmatic drug (salmeterol)	IL-DLLME	Sample solution: pH 12; extraction solvent: $[C_4MIM][PF_6]$ (54 μL); dispersion solvent: MeOH (500 μL); salt: NaCl (10%, w/v); extraction time: N/A	HPLC-FLD <10 min	LOD (μg/L): 0.3 LOQ (μg/L): 1	91.2%–94% ≤8.4%	[48]
Whole blood	28 Hypnotic drugs	IL-DLLME	sample solution: pH 8 (2 mL); extraction solvent: $[C_4MIM][PF_6]$ (60 μL); dispersion solvent: no addition; salt: N/A; extraction time: 5 min	LC-MS/MS ~13 min	LOD (μg/L): 0.003–4.74 LOQ (μg/L): 2–50	24.7%–127.2% <17%	[49]
Urine	4 Nonsteroidal antiinflammatory drugs (flurbiprofen, indomethacin, ketoprofen and naproxen)	One-step in-syringe IL-DLLME	Sample solution: pH 3 (10 mL); extraction solvent: $[C_4MIM][PF_6]$ (280 μL); dispersion solvent: MeOH (720 μL); salt: N/A; extraction time: <5 min	HPLC-UV 20 min	LOD (μg/L): 8.3–32 LOQ (μg/L): N/A	99.6%–107% ≤8.6%	[50]

Continued

TABLE 23.2 SDME and DLLME Applications—cont'd

Bioanalytical Sample	Studied Analytes	Extraction Method	Extraction Conditions	Analytic Instrument Run-Time	LOD LOQ[a]	Extraction Recovery[b] %RSD	References
Breast milk	2 Antichagasic drugs (benznidazole and nifurtimox)	IL-DLLME	Pretreatment: protein precipitation with $HClO_4/H_3PO_4$/MeOH mixture, vortex mixing (2 min), incubation (80°C, 60 min), centrifugation (10,000 rpm, 20 min), and filtration (0.22 μm); sample solution: alkaline; extraction solvent: $[C_8MIM][PF_6]$ (42 μL); dispersion solvent: MeOH (101 μL for benznidazole, 80 μL for nifurtimox); salt: KCl (30%, w/v); extraction time: 6 min	HPLC-UV <7 min	LOD (μg/L): 90 (benznidazole), 60 (nifurtimox) LOQ (μg/L): 300 (benznidazole), 200 (nifurtimox)	89.7% (benznidazole), 77.5% (nifurtimox) ≤6.25%	[51]
Plasma	3 Phosphodiesterase inhibitors (aildenafil, sildenafil, and vardenafil)	Back-extraction IL-DLLME	Sample solution: acidic (0.96 mL); extraction solvent: $[C_8MIM][PF_6]$ (20 μL); dispersion solvent: MeOH (20 μL); salt: NaCl (300 mg/ml); extraction time: N/A	HPLC-UV ~20 min	LOD (μg/L): 0.92–2.69 LOQ (μg/L): N/A	100.4%–103.9% <10%	[52]
Urine	1 Antiepileptic drug (valproic acid)	UA-DLLME	Sample solution: pH 2 (5 mL); extraction solvent: trichloroethylene (20 μL); dispersion solvent: MeOH (200 μL); salt: no addition; sonication time: 1 min	GC-MS/MS <10 min	LOD (μg/L): 0.4 LOQ (μg/L): 1.4	80%–92% <8%	[53]

Whole blood	12 Antidepressant and 2 antipsychotic drugs	UA-LDS-DLLME	Sample solution: pH 12 (0.5 mL); extraction solvent: toluene (100 μL); dispersion solvent: no addition; salt: NaCl (10 mg); sonication time: 3 min	GC-MS 25 min	LOD (μg/L): N/A LLOQ[d] (μg/L): 5–15	30.3%–99.4% ≤13.2%	[54]
Plasma	2 Antihypertensive drugs (amlodipine and nifedipine)	In-syringe UA-LDS-DLLME	Sample solution: pH 12 (10 mL); extraction solvent: 1-octanol (45 μL); dispersion solvent: no addition; salt: NaCl (18.95%, w/v); sonication time: 2.58 min	HPLC-UV 10 min	LOD (μg/L): 0.17 (amlodipine), 0.15 (nifedipine) LOQ (μg/L): 0.569 (amlodipine), 0.502 (nifedipine)	93.6% (amlodipine), 96% (nifedipine) <7%	[55]
Plasma and urine	3 Antidepressant drugs (citalopram, fluoxetine, and venlafaxine)	UA-DLLME-SFOD	Sample solution: alkaline (5 mL); extraction solvent: 1-undecanol (30 μL); dispersion solvent: no addition; salt: NaCl (5%, w/v); sonication time: 20 min	HPLC-UV <15 min	LOD (μg/L): 3 LOQ (μg/L): N/A	91.4%–109% <14%	[56]
Serum	1 Contraceptive drug (ulipristal acetate)	UA-IL-DLLME	Sample solution: pH 8 (10 mL); extraction solvent: $[C_8MIM][PF_6]$ (50 μL); dispersion solvent: no addition; salt: no addition; sonication time: 10 min	HPLC-PDA ~5 min	LOD (μg/L): 9.3 LOQ (μg/L): N/A	95% ≤5.5%	[57]

[a] LOD, limit of detection; LOQ, limit of quantification.
[b] Extraction recovery refers to either absolute or relative recovery, depending on which one is provided by the authors.
[c] Not available information.
[d] Lower limit of quantification.

(3000 rpm, 3 min). The extraction solvent was collected and evaporated (N_2 stream) and the dry residue reconstituted with MeOH (50 μL) prior to analysis [35]. A conventional DLLME—GC-MS method [36] and a "binary solvent" DLLME—liquid chromatography-fluorescence detector (LC-FLD) [37] were developed for the determination of tramadol in human blood and urine for pharmacokinetic studies and tramadol abuse detection purposes. For the first method, carbon tetrachloride (30 μL) and ethanol (1 mL) were injected rapidly into the sample solution (pH 12; 5 mL). The cloudy solution formed was left for 3 min and then centrifuged (5000 rpm, 3 min). The extraction solvent was collected and directly analyzed [36]. For the second method, chloroform (70 μL), ethyl acetate (30 μL), and acetone (0.6 mL) were injected rapidly into the sample solution (pH 10; 5 mL). The cloudy solution formed was centrifuged (1133 g, 10 min) and the extraction solvent collected and evaporated (N_2 stream). The dry residue was reconstituted with mobile phase and analyzed [37].

Less toxic chlorinated or brominated organic solvents can be employed as alternative extraction solvents in DLLME. A DLLME—LC-UV method with 1,2-dichloroethane as the extraction solvent was developed for the determination of ceftazidime in human urine. Acetone (300 μL) and 1,2-dichloroethane (150 μL) were injected rapidly into the sample solution (2 mL), and the mixture was shaken. The cloudy solution formed was left for 2 min and centrifuged (2800 rpm, 10 min). The extraction solvent was collected and directly analyzed [38]. Dichloromethane was employed as the extraction solvent for the determination of carvedilol, diltiazem, metoprolol, propranolol, and verapamil in human plasma. The sample solution was injected with dichloromethane (100 μL), and the cloudy solution formed was centrifuged (4000 rpm, 5 min). The extraction solvent was collected and evaporated (N_2 stream, 35°C), and the dry residue reconstituted with acidified water (pH 3; 50 μL), vortex-mixed (1 min), and analyzed by LC-UV. In this application, ACN was employed for both sample pretreatment and extraction solvent dispersion [39]. Finally, 1.2-dibromoethane was employed as the extraction solvent for the determination of amantadine in human plasma and urine. MeOH (750 μL) and 1,2-dibromoethane (10 mL) were injected rapidly into the sample solution (pH 10; 5 mL). The cloudy solution formed was left for 5 min and centrifuged (1118 g, 3 min) and the extraction phase collected and analyzed by GC-FID [40].

In the case of LDS-DLLME, 1-octanol and MeOH were employed for the extraction of warfarin [41] and fluoxetine [42] in human plasma and urine. For warfarin the sample solution (pH 2.3; 11 mL) was mixed with 1-octanol (150 μL) and MeOH (150 μL) and the mixture stirred (1000 rpm). The cloudy solution formed was left for 2 min and centrifuged (3500 rpm, 5 min). The extraction phase was collected from the surface of the sample solution and analyzed by LC-UV [41]. For fluoxetine, MeOH (400 μL) and 1-octanol (172 μL) were injected rapidly into the sample solution (pH 11.3; 20 mL), and the cloudy solution formed was centrifuged (3500 rpm, 5 min). Analysis was carried out by LC-UV [42].

23.2.4.2 DLLME-SFOD

A DLLME-SFOD—LC-UV method was developed for the determination of valsartan in human urine. 1-Dodecanol (65 μL) and ethanol (250 μL) were injected rapidly into the sample solution (pH 3.8; 5 mL), and the mixture was shaken (1 min). The cloudy solution formed was centrifuged (5000 rpm, 4 min) and placed into an ice bath. The solidified extraction solvent was collected, left to melt at room temperature, and diluted with mobile phase to a final volume of 100 μL prior to analysis [43]. A second DLLME-SFOD—liquid chromatography-charged aerosol detector (LC-CAD) method with 1-dodecanol as the extraction solvent and MeOH as the dispersion solvent was developed for the determination of azithromycin, clarithromycin, dirithromycin, erythromycin, and roxithromycin in human urine. MeOH (440 μL) and 1-dodecanol (60 μL) were injected rapidly into the sample solution (5 mL), and the cloudy solution formed was centrifuged (3500 rpm, 5 min) and placed into an ice bath for 5 min. The solidified extraction solvent was collected, thawed, and centrifuged (3500 rpm, 3 min). The supernatant (40 μL) was diluted with MeOH to a final volume of 120 μL and analyzed [44].

The same team developed two DLLME-SFOD protocols with 1-undecanol as the extraction solvent for the determination of codeine, morphine, noscapine, and papaverine in human plasma [45] and methadone in human serum and urine [46] for clinical and forensic purposes. For the first protocol, 1-undecanol (30 μL) and acetone (470 μL) were injected rapidly into the sample solution (pH 9; 5 mL), and the cloudy solution formed was centrifuged (4000 rpm, 5 min) and placed into an ice bath for 5 min. The solidified extraction solvent was collected, thawed at room temperature, and analyzed by LC-UV [45]. Similar steps with MeOH (580 μL) as the dispersion solvent were employed in the second protocol, and analysis was carried out with LC-UV [46]. Finally a DLLME-SFOD—LC-FLD method with no dispersion solvent addition was developed for the determination of duloxetine in human plasma. A volume of 1-undecanol (50 μL) was injected rapidly into the sample solution (1 mL), and the mixture was vortex-mixed. The cloudy solution formed was centrifuged (4000 rpm, 5 min) and placed into an ice bath for 5 min. The solidified extraction solvent was collected, thawed at room temperature, and diluted with MeOH (150 μL) prior to analysis. In this application, ACN was employed for both sample pretreatment and extraction solvent dispersion [47].

23.2.4.3 IL-DLLME

An IL-DLLME—HPLC-FLD method was developed for the determination of salmeterol in dried human blood spots. $[C_4MIM][PF_6]$ (54 μL) and MeOH (500 μL) were injected into prepared filter paper disks that contained the dried blood spots, and the mixture was sonicated (10 min) and centrifuged (3000 rpm, 5 min). The supernatant

was collected, salinized/alkalized, and centrifuged (3000 rpm, 5 min). The ionic liquid was collected and directly analyzed [48]. A second IL-DLLME—LC-MS/MS method was developed for the determination of 28 hypnotic drugs in human whole blood. [C_4MIM][PF_6] (60 μL) was injected rapidly into the sample solution (pH 8; 2 mL), and the mixture was vortex-mixed (5 min). The cloudy solution formed was centrifuged (3500 rpm, 6 min), and the ionic liquid was collected and diluted 10-fold with MeOH [49]. A "one-step in-syringe" IL-DLLME—LC-UV method was developed for the determination of flurbiprofen, indomethacin, ketoprofen, and naproxen in human urine. The sample solution (pH 3; 10 mL) was withdrawn into a plastic syringe (10 mL volume) and injected with [C_4MIM][PF_6] (280 μL) and MeOH (0.72 mL). The cloudy solution formed was left to sediment and the ionic liquid collected from the syringe tip by moving the syringe plunger to the initial position and diluted in mobile phase. This extraction protocol eliminated the centrifugation step required for the collection of the extraction solvent in DLLME applications [50]. An IL-DLLME—LC-UV method was developed for the determination of benznidazole and nifurtimox in human breast milk. [C_8MIM][PF_6] (42 μL) and MeOH (101 μL for benznidazole or 80 μL for nifurtimox) were injected rapidly into the sample solution, and the mixture was vortex-mixed (6 min). The cloudy solution formed was centrifuged (10,000 rpm, 20 min), and the ionic liquid was collected and directly analyzed [51]. Finally a back-extraction IL-DLLME—LC-UV method was developed for the determination of sildenafil, vardenafil, and aildenafil in human plasma. [C_8MIM][PF_6] (20 μL) and MeOH (20 μL) were injected rapidly into the sample solution (0.96 mL), and the mixture was shaken. The cloudy solution formed was centrifuged (104 rpm, 5 min) and the ionic liquid collected and mixed with acetic acid solution (40 μL); the mixture was vortex-mixed (5 min) and centrifuged (104 rpm, 5 min). The aqueous supernatant was collected and analyzed [52].

23.2.4.4 UA-DLLME

Sonication can be applied in all the previously mentioned DLLME modes to improve extraction efficiency. An UA-DLLME—GC-MS/MS method was developed for the determination of valproic acid in human urine with trichloroethylene (20 μL) and MeOH (200 μL) as the extraction phase and sonication (1 min) [53]. An ultrasound-assisted-low-density solvent-dispersive liquid-liquid microextraction (UA-LDS-DLLME)—GC-MS method was developed for the determination of 12 antidepressant and 2 antipsychotic drugs in human whole blood with toluene (100 μL) as the extraction solvent with no dispersion solvent and sonication for 3 min [54]. An in-syringe UA-LDS-DLLME—LC-UV method was developed for the determination of amlodipine and nifedipine in human plasma by employing 1-octanol (45 μL) as the extraction solvent, with no dispersion solvent, and sonication for 2.58 min [55]. An ultrasound-assisted-dispersive liquid-liquid microextraction-solidification of floating organic droplet (UA-DLLME-SFOD)—LC-UV method

was developed for the determination of citalopram, fluoxetine, and venlafaxine in human plasma and urine by employing 1-undecanol (30 μL) as the extraction solvent, with no dispersion solvent, and sonication for 20 min [56]. Finally an UA-IL-DLLME—LC-UV method was developed for the determination of ulipristal acetate in human by employing $[C_8MIM][PF_6]$ (50 μL) as the extraction solvent, with no dispersion solvent, and sonication for 10 min [57].

23.3 Discussion

23.3.1 PLE

In all PLE applications the human hair samples were decontaminated to remove polar and nonpolar interferences such as hair skin secretions, personal care products, and external contamination. The optimum temperatures were chosen to avoid analyte decomposition and achieve higher extraction recoveries, while MeOH was added in the extraction solvent to improve the extraction efficiency. An additional SPE cleanup step was essential for removal of matrix interference and analyte preconcentration. The developed PLE/SPE cleanup protocols were superior compared with hydrochloric acid extraction and sodium hydroxide digestion-LLE cleanup reference protocols in terms of analyte stability and extraction recoveries.

23.3.2 QuEChERS extraction

QuEChERS extraction was initially developed for the extraction of pesticides from vegetables; however, its applicability can be extended to the extraction of drugs from biological samples of human origin. QuEChERS extraction can be used for human whole blood [14], plasma [20], urine [24], and hair [26] for the extraction of a large number of pharmaceuticals [15]. ACN is the most common extraction solvent for QuEChERS extraction; acidified ACN [21], MeOH [26], and ethyl acetate, which increase the extraction efficiency of nonpolar compounds [23], can also be reported as alternatives. In the case of the inorganic salts employed for phase separation, combinations of $MgSO_4$-sodium acetate [15], $MgSO_4$-potassium carbonate [19], $MgSO_4$/NaCl/$Na_3C_6H_5O_7$/$Na_2C_6H_6O_7$ [22], and sodium bicarbonate-sodium carbonate saturated buffer [16] have been used as alternatives to the typical $MgSO_4$-NaCl combination. The typical dSPE cleanup step in QuEChERS extraction is usually achieved by $MgSO_4$, PSA, and/or C_{18} combinations; other sorbents such as GCB [24] constitute novel alternatives.

Modifications can be utilized in QuEChERS applications. The salting-out [25] or the dSPE cleanup step can be omitted [17] in modified one-step QuEChERS extraction protocols, reducing the extraction time without any significant reduction in

extraction efficiency. Bioanalytical samples are usually high in proteins and lipids that cause a variety of problems in sample handling. For this reason, dSPE cleanup can be replaced by filtration of the extraction phase with special filters/cartridges for protein precipitation [25]. Furthermore a reported QuEChERS application introduced the "caking" of an excess amount of inorganic salts that absorbs the aqueous phase of the bioanalytical samples and reduces the lipid content in the extraction phase, also eliminates the dSPE cleanup step, and reduces the overall extraction time [18]. Finally a mini-QuEChERS protocol that required a few microliters of the sample [19] goes in line with method miniaturization that is an essential modification in modern analytical chemistry.

23.3.3 SDME

SDME can be applied for the extraction of drugs from human plasma, serum, urine, and hair samples. The crucial parameters were optimized in all of the SDME biomedical applications. The biological samples were diluted prior to SDME extraction to reduce the matrix interferences, and the sample pH was adjusted with hydrochloric acid or sodium hydroxide to improve analyte transfer into the acceptor phase or the intermediate organic phase. Regarding the selected acceptor phases, o-dibutyl phthalate was selected for DI-SDME protocol due to water immiscibility and compatibility with the extracted analytes and the analytic instrument [27], *n*-dodecane was selected for a HS-SDME protocol [28], and aqueous phosphoric acid solution was selected as the acceptor phase in both HS-SDME [29] and three-phase SDME [32] protocols. Basic analytes are protonated when they come into contact with phosphoric acid and their solubility increases; thus their extraction into the acceptor phase is enhanced. In three-phase SDME applications, phosphate buffer solution [30], perchloric acid solution [31], hydrochloric acid solution [33], and acidified deionized water [34] were selected as acceptor phases for the extraction of basic drugs, while 1-octanol [34], dibutyl ether [30], toluene [33], *n*-hexane [32], and *n*-octane [31] were selected as intermediate organic phases. To further improve analyte extraction, some authors utilized the salting-out effect by adding sodium chloride [28] or the nonionic surfactant Triton X-100 [34] to the sample solutions. These "additives" decrease analyte solubility in the donor phase and favor analyte transfer to the intermediate organic phase or the acceptor phase. Additionally an excessive concentration of sodium hydroxide in the sample solution results in the same salting-out effect as sodium chloride [29, 32].

23.3.4 DLLME

Traditional DLLME and DLMME modes can be used for the extraction of drugs from human whole blood [49, 54], plasma [39, 41, 45], serum [46, 57], and urine

[37, 43, 53], as well as human saliva and sweat [35], dried human blood spots [48], and breast milk [51]. Extraction solvents such as chloroform [35] and carbon tetrachloride [36] and less toxic alternatives such as 1,2-dichloroethane [38], dichloromethane [39], trichloroethylene [53], and 1,2-dibromoethane [40] were applied in traditional DLLME protocols, while low-density solvents such as 1-octanol [41, 42] and toluene [54] were used in LDS-DLLME protocols. In the case of DLLME-SFOD applications, 1-dodecanol [43, 44] and 1-undecanol [45, 46] were the most common extraction solvents, while $[C_4MIM][PF_6]$-MeOH [48, 49] and $[C_8MIM][PF_6]$ [51, 57] were used for IL-DLLME protocols. MeOH was the most common dispersion solvent in all DLLME modes, followed by ethanol and acetone. Prior to extraction, human plasma was treated with ACN [39], MeOH [40], trichloroacetic acid [41], or zinc sulfate ($ZnSO_4$) solution-ACN mixture [45] to precipitate proteins followed by centrifugation and dilution, while urine samples were usually centrifuged, filtrated, and diluted. ACN can be employed for both protein precipitation and extraction solvent dispersion, thus eliminating the need for an additional dispersion solvent [39, 47]. Human breast milk was treated with perchloric acid-phosphoric acid-MeOH to precipitate proteins followed by sequential vortex mixing, incubation, centrifugation, and filtration [51]. Then the pretreated sample solutions were alkalized (pH 8–12) or acidified (pH 2–3) to enhance analyte extraction, while some authors employed the salting-out effect by adding sodium chloride (1%–10%, w/v) or potassium chloride. Finally, sonication was applied in conventional DLLE [53], LDS-DLLME [54], DLLME-SFOD [56], and IL-DLLME [57] protocols to increase analyte extraction efficiency, while some authors employed a plastic syringe as the extraction vessel to eliminate the centrifugation step required for the collection of the extraction solvent and to simplify the whole extraction procedure [50, 55].

23.4 Conclusions

This chapter provides a brief theoretical background for the principal liquid-phase extraction methods and selected biomedical applications. We hope that this chapter will be educational to new bioanalytical scientists and informative to analytic chemists working in either academia or industry and enlightening to professionals of all disciplines outside of separation science.

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Chapter 24

Solvent Extraction for Nuclear Power

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Chapter Outline

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24.1 Introduction

The need to produce large quantities of fissile actinide isotopes, ^{235}U and ^{239}Pu, for nuclear weapons was the cause of the rapid development of solvent extraction methods for the separation of metal ions within the US Manhattan Project [1] in the 1940s. Many tons of raw uranium nitrate were purified from admixtures of other metals, including the radioactive decay products, using diethyl ether as the extractant. The purified material was then enriched in the fissile ^{235}U, using various isotope separation processes. The next task was to irradiate the uranium fuel in nuclear reactors built as a part of the project and then separate the pure plutonium from the fuel. Initially the separation of plutonium was carried out by precipitation (bismuth phosphate process). Soon after a solvent extraction process was developed that allowed the separation of pure plutonium from the fuel solution in nitric acid using a new extractant—tributyl phosphate [2]. This process, Plutonium Uranium Redox EXtraction (PUREX), is still (with minor modifications) the standard method of reprocessing spent nuclear fuel for the recovery of uranium and plutonium all over the world [3]. The goal was to improve the low efficiency of the open nuclear fuel cycle (no reprocessing), which uses only a few percent of the energy contained in uranium. However, this efficiency can be further improved through multirecycling strategies that anticipate the use of fast-neutron reactors. Solvent extraction techniques for the separation of actinides from fission products are currently the most important and actively studied methods of reprocessing spent nuclear fuel and recycling the actinides. The closing—in this way—of the nuclear fuel cycle will not only allow more effective use of global uranium resources but also drastically reduce the long-term threat to the environment by nuclear waste, contributing to the safety of nuclear power and to the sustainable development of the world.

24.2 Spent Nuclear Fuel (SNF)

Spent nuclear fuel, also called *used nuclear fuel*, is the fuel that has undergone a reactor campaign and is no longer useful for sustaining the nuclear fission chain reaction in a thermal reactor. This apparent burnout is due to the accumulation in the fuel of large amounts of fission products (several lanthanide isotopes, in particular Sm, Gd, and Eu) that have high cross sections for thermal neutron capture and are classified as *reactor poisons*. In the case of the most popular power reactors moderated with light water, the reactor is loaded with uranium fuel enriched up to 3%–5% of fissile ^{235}U, the rest being the fertile ^{238}U isotope. On an average 18-month fuel cycle, approximately one-third of the fuel must be replaced. The fuel discharged from the reactor is highly radioactive (primarily β^- and γ radiation) and strongly generates heat. After 1 year in storage, the heat load drops to 10.8 W/kg, 95% derived from fission product decay [4]. One ton of the spent nuclear fuel irradiated in a typical operational cycle

contains approximately 10 kg of plutonium isotopes; 0.5 kg of ^{237}Np; 40 kg of fission products; and small amounts of transplutonium elements, mainly americium and curium (equivalent to ca 2% of the amount of lanthanide fission products) [4, 5]. The transuranium elements are formed in a series of consecutive nuclear reactions, mainly with ^{238}U: the capture of a thermal neutron; followed by β-decay of the heavier isotope, $^{238}U(n,\gamma)^{239}U \rightarrow {}^{239}Np + \beta^-$, etc.; the reaction with fast fission neutrons, $^{238}U(n,2n)^{237}U \rightarrow {}^{237}Np + \beta^-$; and others.

There are two general approaches used to manage spent nuclear fuel. In an open (or once-through) fuel cycle, the spent fuel elements are either ultimately or temporarily stored as highly radioactive nuclear waste. In a partially closed fuel cycle, spent nuclear fuel is reprocessed to reduce the long-term threat to people and the environment, caused by this radiotoxic material, and to recover uranium and plutonium to be converted into a new fuel. Less useful long-lived radionuclides are removed as nuclear waste that must be stored in geologic repositories and isolated from the biosphere through solidification and multibarrier protection against migration of the radionuclides into the environment [6, 7].

Nowadays, around 90% of nuclear reactors operate in the once-through fuel cycle mode. The dominant part of SFN is temporarily stored until a final solution to the problem is found. This is expected to be possible thanks to the fourth generation of fast-neutron reactors, which ensure efficient burning of plutonium and minor actinides (neptunium, americium, curium, etc.) that are the fuel for these reactors. At present, however, only a small portion of SFN is being reprocessed. In addition to hydrometallurgical technologies commonly used for this purpose, pyrochemical separation processes are also being developed as an alternative to aqueous reprocessing, especially for reprocessing fuels from molten salt reactors [7–9]. Also, spent thorium fuels that contain thorium(IV), fissile ^{233}U, and fission products are subjected to reprocessing [3, 5, 8, 10]. The scope of this chapter is limited to the issues of reprocessing spent uranium and uranium-plutonium fuels, using technologies based on solvent extraction processes.

24.3 Classical and Advanced PUREX Processes

The PUREX process was developed in the early 1950s to support the production of plutonium for nuclear weapons [2, 3]. It was based on solvent extraction of U(VI) and Pu(IV) from strongly acidic nitrate solutions of SNF, using tributyl phosphate (TBP, Fig. 24.1) dissolved in an inert aliphatic diluent, typically 30% TBP in kerosene.

Upon contacting these two immiscible solutions, the TBP forms complexes (solvated salts) of the two metals, readily extracted by the organic phase:

$$UO_2^{2+} + 2NO_3^- + 2TBP_{org} \rightarrow \left[UO_2(NO_3)_2(TBP)_2\right]_{org} \qquad (24.1)$$

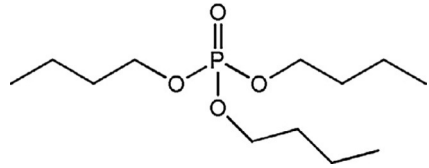

Fig. 24.1 Molecular formula of the tributyl phosphate (TBP) extractant

$$Pu^{4+} + 4NO_3^- + 2TBP_{org} \rightarrow \left[Pu(NO_3)_4(TBP)_2\right]_{org} \tag{24.2}$$

The TBP extractant selectively and nearly quantitatively transfers the tetra- and hexavalent actinides to the organic phase, while the trivalent actinides (mainly americium and curium); pentavalent neptunium; and mono-, di-, and trivalent fission products remain in the highly active aqueous phase. However, easy changes of the oxidation states of neptunium in the nitric acid solutions [10, 11] result in the extraction of the major part of the neptunium by TBP from the 3-4-M HNO_3. To separate plutonium the loaded organic phase is contacted with a fresh aqueous phase containing a reducing agent. The extracted Pu(IV) is reduced to the nonextractable Pu(III) easily stripped into the aqueous phase to convert it into pure solid compounds. The remaining organic phase is then contacted at elevated temperature with a dilute HNO_3 solution, which results in uranium stripping. To obtain a pure uranium product, the loaded aqueous phase should be purified from the accompanying neptunium, traces of plutonium, and some fission products. The specificity of the SNF reprocessing lies in the fact that the extraction takes place in the presence of high-intensity ionizing radiation mainly from the fission products, which results in a significant radiolytic degradation (see Section 24.6.2) of the extractant and diluent. As some degradation products strongly compete with TBP in the complexation of the separated metal ions, the regeneration of the TBP solvent prior to its reuse is an important factor in the PUREX process [5].

During the use for decades, the PUREX process underwent several optimizations. Plutonium and uranium are now commercially recovered from SNF from nuclear power plants and recycled as a fresh mixed-oxide (MOX) fuel containing UO_2 and PuO_2, to be used in conventional pressurized water reactors. Recent modifications to the PUREX process have made possible the separation of neptunium and some long-lived fission products [10, 12]. However, the initial focus of SNF reprocessing solely on obtaining pure plutonium (and uranium) is already insufficient. The most important goal of the SNF reprocessing now is to optimize the use of natural uranium resources and minimize the long-term hazard from high-level nuclear waste. The PUREX reprocessing really extends the nuclear fuel supply and significantly reduces the volume of nuclear waste to be disposed. Unfortunately the minor actinides left in the waste, in particular the long-lived ^{241}Am and ^{243}Am isotopes, do

not allow a significant reduction in the long-term radiotoxicity of the waste [7]. Another current requirement is to provide increased safeguards for the recycled plutonium to prevent its illicit use for the production of weapons. To increase the proliferation resistance of the fuel cycle, modifications have been introduced to the PUREX process, preventing the separation of pure plutonium. For example, the CO-EXtraction of uranium and plutonium (COEX) process leaves certain amounts of recovered uranium with the plutonium that is useful for MOX fuel fabrication [3, 13]. Some subtle changes in the PUREX chemistry result in costripping of small amounts of processed uranium together with the plutonium from the loaded organic phase. The further coprecipitation of uranium and plutonium as a mixed oxide, (U, Pu)O_2, (in addition to the pure uranium stream) eliminates any separation of plutonium alone. However, the trivalent minor actinides still remain in the highly active aqueous phase—raffinate, routed to nuclear waste.

The similarity of the chemical properties of trivalent actinides and the lanthanide fission products does not allow direct selective removal of the former from the PUREX raffinate. Usually, these two groups of metal ions are first separated from the rest of the fission products, and the separation between the two groups can be done in a subsequent step. The separation requires the application of a different class of reagents, in particular ligands that differ in the strength of their interaction with trivalent actinides and lanthanides. This is the case of solvent extraction systems containing two competing ligands: lipophilic (extractant) and hydrophilic (complexant) with hard and soft (according to Pearson's HSAB concept) donor atoms, respectively. In the old Trivalent Actinide-Lanthanide Separation with Phosphorus-reagent Extraction from Aqueous Komplexes (TALSPEAK) process, the harder lanthanide ions were extracted from an aqueous solution that selectively retained the somewhat softer actinides(III) in the form of complexes with polyaminopolyacetic acid, using the hard acidic extractant, di-(2-ethylhexyl)phosphoric acid (*HDEHP*) [4, 14]. In the reverse TALSPEAK the complexant solution was applied for selective stripping of actinides from the loaded organic phase [15].

An effective modification of the PUREX process was carried out later, which allowed the coextraction of the trivalent minor actinides and Np(V) together with U(VI) and Pu(IV) from the highly radioactive SNF solution. This was the result of introducing into the TBP organic phase an additional extractant, carbamoylmethylphosphine oxide (CMPO), selective for trivalent actinides. This led to the development of the TRansUranic EXtraction (TRUEX) process [16, 17].

In the 1970s a new idea appeared to separate all the actinides and some long-lived fission products from the SNF (partitioning) to transform (transmute) them into short-lived or stable nuclides [12, 18, 19]. The transmutation was expected to be carried out either in special accelerator-driven systems or in fast-neutron reactors of a new generation that would burn plutonium and the minor actinides [13, 19]. Further, it was concluded that the transmutation of long-lived fission products is not

technically relevant and was abandoned [7, 20]. The main aim of the partitioning and transmutation (P&T) strategy is to reduce the radiotoxicity of the remaining nuclear waste to the level of radiotoxicity of natural uranium (together with the decay products) in a relatively short period of several hundred years, incomparably less than over 10,000 years required for nuclear waste from the PUREX process, and the other aim is to improve the effectivity of the use of energy resources [18, 19]. This approach is essential to achieve the goal of a fully closed nuclear fuel cycle. Indispensable for the transmutation of minor actinides is their initial separation from much greater amounts of fission products, in particular from the lanthanides. The similarity of the chemical properties of these f-electron elements makes their mutual separation a difficult task. Therefore it was necessary to develop novel, efficient solvent extraction processes for the separation of minor actinides.

24.4 Americium(III) Recycling—Advanced Fuel Cycles

The need to improve the reprocessing of spent nuclear fuel, allowing effective separation of all actinides, including americium, has led to intensified research on the development of innovative extraction processes. Numerous actinide separation processes were developed, based on solvent extraction. They are described in several reviews and books [5, 7, 8, 10, 12, 18, 21–23].

Strong radiolytic degradation of the extractant and diluent during the reprocessing of the SNF requires frequent replacement and processing of the radioactively contaminated used solvent, completed by its incineration. To minimize the amount of secondary radioactive waste, generated as a result of destroying the used reagents, it was proposed to use completely incinerable reagents that contain only carbon, hydrogen, nitrogen, and oxygen atoms [24]. The adherence to this approach, called the CHON principle, leads to the formation of a relatively small amount of residual radioactive ash. From this point of view, the extractants containing P or S atoms should not be used for reprocessing [22].

Relatively soft poly-N-donor CHON ligands, the derivatives of bis-triazinylpyridine (BTP) or bis-triazinyl-bipyridine (BTBP), are considered the most promising selective actinide extractants for the separation of Am(III) from lanthanide fission products [23, 25]. The low basicity of these ligands allowed the successful extraction of Am(III) from $>$1-M HNO_3. The new 6,6′-bis(5,5,8,8-tetramethyl-5,6,7,8-tetrahydrobenzo-1,2,4-triazin-3-yl)-2,2′-bipyridine ligand ($CyMe_4$-BTBP, Fig. 24.2), resistant to hydrolysis and radiolysis, was selected as the European reference molecule for the development of the Selective ActiNide EXtraction (SANEX) process [22, 23, 25, 26]. The SANEX feed is the product solution from the DIAMide EXtraction (DIAMEX) process [7, 22], containing trivalent actinides and lanthanides coextracted from the PUREX raffinate.

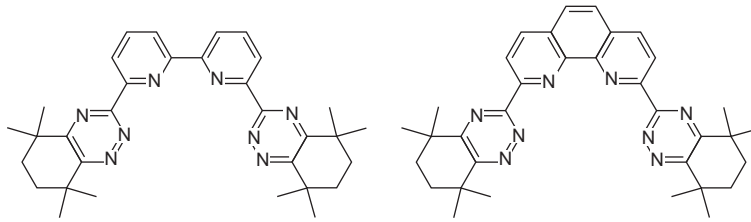

Fig. 24.2 Structures of the ligands $CyMe_4$-BTBP (1) and $CyMe_4$-BTPhen (2)

The M^{3+} ions (M = An or Ln) present in the aqueous HNO_3 solutions form strong cationic complexes, $[M(BTP)_3]^{3+}$ and $[M(BTBP)_2(NO_3)]^{2+}$, with the neutral BTP and BTBP ligands dissolved in an organic diluent, usually aliphatic hydrocarbons, 1-octanol, or their mixtures. These complexes are extracted to the organic phase as neutral salts with nitrate counterions. Also, neutral $[M(BTBP)(NO_3)_3]$ complexes can be extracted. Because of rather poor solubility of $CyMe_4$-BTBP in the kerosene/1-octanol diluents, the concentration of HNO_3 is practically the only adjustable parameter that enables effective separation ($D_{Am} > 1$ and $D_{Eu} < 1$, where Eu represents the Ln elements). High separation factors, $SF_{Am/Eu} = D_{Am}/D_{Eu} \approx 150$, were obtained in these extraction systems. To improve the slow kinetics of M^{3+} extraction, observed for the BTBPs [23], a phase-transfer agent (cf. Chapter 4), *N,N′*-dimethyl-*N,N′*-dioctyl-2-(2-hexyloxyethyl) malonamide (DMDOHEMA), was added to the organic phase [25]. A series of countercurrent tests carried out in multistage centrifugal contactors with model (spiked) and genuine (hot) fuel solutions in 2-M HNO_3 confirmed the usability of the $CyMe_4$-BTBP extractant for large-scale separation of Am(III) and Cm(III) from the lanthanide fission products in the regular SANEX (r-SANEX) process [27]. The flow sheet of the countercurrent process (Fig. 24.3) shows that a number of stages in the extraction, scrubbing, and stripping sections are needed to obtain the product with the required purity.

Another way to accelerate the slow kinetics of M^{3+} extraction by the BTBP ligands is the use of a novel lipophilic ligand, 2,9-bis(5,5,8,8-tetramethyl-5,6,7,8-tetrahydrobenzo-1,2,4-triazin-3-yl)-1,10-phenantroline ($CyMe_4$-BTPhen, Fig. 24.2), with a partially preorganized structure [28, 29]. The complex formation occurs with the *ccc* conformer of the BTBP ligand, and its formation requires rotation of a pyridine group around the C—C axis of bipyridine in the most stable *ttt* conformer of the free ligand, which is time-consuming and requires overcoming a significant energy barrier [29]. In contrast the required cis conformation of the two pyridine groups already exists in the free BTPhen ligand. Accordingly, not only the kinetics of Am^{3+} and Eu^{3+} extraction with $CyMe_4$-BTPhen is much faster than that with $CyMe_4$-BTBP, but also the respective distribution ratios, D_{Am} and D_{Eu}, and the

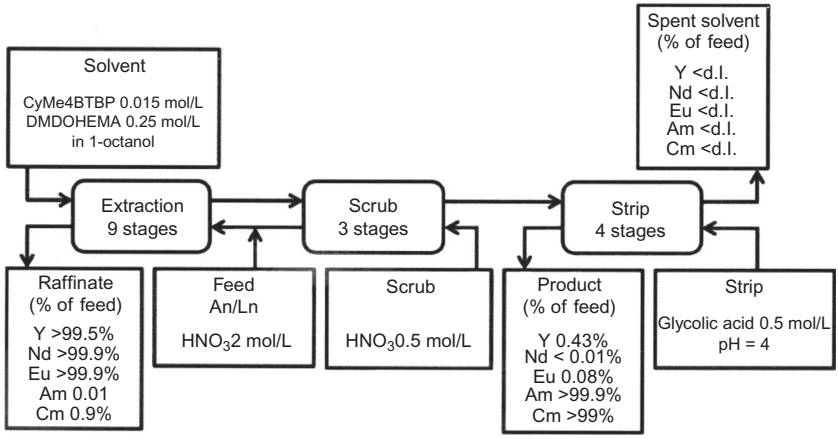

Fig. 24.3 Flow sheet of the hot test of the countercurrent r-SANEX process ($CyMe_4$-BTBP/DMDOHEMA). *(From Modolo G, Wilden A, Geist A, Magnusson D, Malmbeck R. A review of the demonstration of innovative solvent extraction processes for the recovery of minor actinides from PUREX raffinate. Radiochim Acta 2012;100:715–25 with permission from Walter de Gruyter GmbH.)*

separation factor, $SF_{Am/Eu}$, under comparable conditions are higher for $CyMe_4$-BTPhen [28] than for $CyMe_4$-BTBP [27].

The actinide selectivity of BTBP extractants is due to the formation of stronger complexes with An^{3+} (Am and Cm) than with Ln^{3+} ions [23]. This is commonly interpreted in terms of more favorable interactions of fairly soft BTBP ligands with the Am^{3+} cation, somewhat softer than Eu^{3+} [30]. Theoretical DFT studies on the formation and liquid-liquid distribution of Am(III) and Eu(III) complexes with a BTBP ligand, in particular the calculated energies of their formation in water, point to a greater stability of the Am complexes. A greater shift of electron density from the ligands onto the Am(III) than Eu(III) central atom in the complexes indicates a higher covalent contribution to the Am—N than Eu—N bonds [31].

Hydrophilic derivatives of the bis-triazinyl extractants can be used for back extraction of actinides from the loaded organic phase in the innovative SANEX process (i-SANEX). The water-soluble tetrasulfonated BTP derivative, 2,6-bis(1,2,4-triazin-3-yl)pyridine (SO_3-Ph-BTP, Fig. 24.4), selectively strips Am(III) from TODGA solutions in 1-octanol/kerosene diluent to 0.5-M HNO_3, leaving lanthanides in the organic phase [32].

N,N,N′,N′-tetraoctyl diglycolamide (TODGA, Fig. 24.5) [33] was widely studied as a nonselective extractant of lanthanides and actinides from the PUREX raffinate. This neutral tri-*O*-dentate ligand forms strong cationic complexes with numerous multivalent cations, especially lanthanides and actinides. They are eagerly extracted

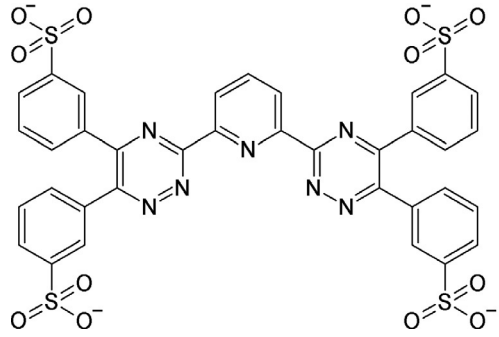

Fig. 24.4 Structural formula of the SO_3-Ph-BTP^{4-} anion

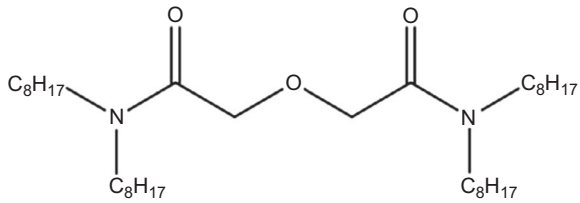

Fig. 24.5 Structural formula of the TODGA extractant

(as ion associates with nitrate anions) from aqueous HNO_3 solutions to inert organic solvents. The more stable Eu(III) complexes are somewhat better extracted than their Am(III) counterparts [34]. The stoichiometry of these complexes has not been precisely determined because of the aggregation of TODGA in aliphatic diluents. The applications of TODGA and its homologous diglycolamide extractants in SNF reprocessing and the coordination chemistry of the diglycolamides have been comprehensively reviewed [35]. Quantum mechanical DFT calculations performed for cationic 1:3 Am(III) and Eu(III) complexes with TEDGA—a hydrophilic homologue of the lipophilic TODGA ligand—have shown that due to the presence of two amide oxygen donor atoms, the TEDGA (TODGA) molecule is not a hard, as it was commonly considered, but a moderately soft ligand (HSAB concept). The covalent contribution to the essentially ionic Am—O and Eu—O bonds is slightly larger for the former, mainly because of a greater overlap of lone pair orbitals on the ligand oxygen atoms with the 6d(Am^{3+}) than 5d(Eu^{3+}) orbitals, due to the greater spatial range of the former [36]. This difference seems to be overcompensated by the stronger electrostatic attraction of the donor oxygen atoms of TODGA by the slightly smaller Eu^{3+} than by the slightly larger Am^{3+} ion.

An alternative concept for SNF reprocessing, the subject of investigation in Europe [37], is the Grouped ActiNide EXtraction (GANEX) process, aimed at the

homogeneous recycling of actinides by coextraction of transuranium elements (Np, Pu, Am, Cm, etc.). The process employs two extraction cycles. In the first, bulk uranium(VI) is selectively removed from strongly acidic (HNO_3) SNF solution, using di-2-ethylhexyl-isobutylamide extractant. In the second cycle, all the transuranic actinides are separated together. Hydrophilic complexants are used to prevent coextraction of certain fission and corrosion products present in the SFN solution. The approach is simpler and offers greater proliferation resistance than the PUREX-SANEX option. Various combinations of the actinide extractants were studied [38]. The recent variant of the second cycle—the EURO-GANEX—process is based on the combination of TODGA and DMDOHEMA extractants in the organic phase [39, 40]. The SO_3-Ph-BTP^{4-} ligand was evaluated as the stripping agent; it is very effective for the recovery of plutonium and americium in the alternative second cycle of GANEX [38–40]. Hydrophilic tetrasulfonated derivatives of 6,6′-bis(1,2,4-triazin-3-yl)-2,2′-bipyridine (BTBP) and 2,9-bis(1,2,4-triazin-3-yl)-1,10-phenantroline (BTPhen) proved to be even more effective in separating actinides(III) from lanthanides(III) by selectively forming water-soluble actinide complexes [41].

To further improve the system, also CHON compounds were tested as Am(III)-selective stripping agents. Promising results were obtained for tri-*O,N*-dentate hydrophilic derivatives of dipicolinic acid (PDCA) and a deca-*O,N*-dentate *N,N,N′N′*-tetrakis[(6-carboxypyridin-2-yl)methyl]ethylenediamine (H_4TPAEN, Fig. 24.6) in the system with the TODGA extractant. However, the moderate basicity of these CHON ligands requires a pH of the aqueous phase to be greater than one [42, 43]. Another limitation of the H_4TPAEN ligand is its low solubility in acidic aqueous solutions. Nevertheless, it seems promising for the Am(III)/Cm(III) separation (see in the succeeding text), providing a separation factor $SF_{Cm/Am} \approx 3.5–4$ at pH 1.5 [42]. A novel CHON hydrophilic ligand, 1,10-phenanthroline-2,9-dicarboxamide, with a number of OH groups at the periphery (to ensure solubility in water) was synthesized and tested as an Am(III) stripping agent, but its low solubility makes the expected applications questionable [44]. Hydrophilic tri-*N*-dentate CHON ligands of low

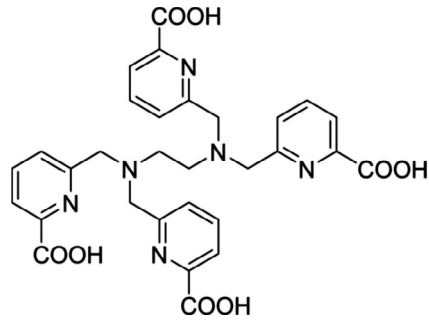

Fig. 24.6 Structural formula of the H_4TPAEN stripping agent

basicity, the derivatives of the pyridine-2,6-bis(1*H*-1,2,3-triazol-4-yl) with –OH groups at their peripheries, were synthesized as well. These PyTri ligands strip Am(III) from 0.2-M TODGA in octanol-kerosene solvent to 0.44-M HNO_3, leaving the lanthanides in the organic phase [45].

24.5 Separation of Americium(III) From Curium(III)

There are convincing arguments for not recycling curium and the heavier actinides, but routing these rather short-lived nuclides to the high-level waste, along with fission products. This is mainly caused by the neutron radiation of some of their isotopes, in particular ^{244}Cm, the most abundant curium isotope in SNF, which undergoes spontaneous fission. Also, californium-252, a very strong neutron emitter produced by prolonged neutron irradiation of curium, americium, and plutonium, would pollute the back end of the fuel cycle. The neutron radiation increases the exposure to process operators creating serious shielding problems [19]. Also the heat generated by the decay of ^{244}Cm results in technical problems, if curium accompanies the separated americium. France, one of the world's leaders in nuclear energy production and fuel reprocessing, intends to recycle americium alone, leaving curium and the heavier actinides in the nuclear waste [46].

Unfortunately the similar chemical properties of Am^{3+} and Cm^{3+} make these ions extremely difficult to separate. Attempts to separate americium from curium after oxidation of Am(III) to Am(VI) [47–49] proved it to be difficult to implement on a large scale. The separation of trivalent Am from Cm makes use of the inverse selectivity of the extractant (e.g., TODGA) and strippant (e.g., SO_3-Ph-BTBP^{4-}) for Am(III) than for Cm(III). This is the basis for the selective stripping of Am(III) in the Americium-Selective Extraction (AmSel) process, when the lanthanides and Cm(III) remain in the loaded TODGA phase [50]. A better separation of these elements, with a separation factor $SF_{Cm/Am} \approx 3.6$, was achieved using a hydrophilic tetrasulfonated derivative of BTPhen in 0.65-M HNO_3 [51, 52]. Also the use of the lipophilic $CyMe_4$-BTPhen ligand under nonequilibrium conditions of the extraction process allows the separation of Am from Cm(III) [52].

Attempts have also been made to use CHON stripping agents for the separation. The process of solvent extraction of americium (EXAm) was developed for the separation of americium alone from SNF. A mixture of two extractants DMDOHEMA and HDEHP (not CHON ligand) in an aliphatic diluent transferred Am(III) together with light lanthanide fission products from highly acidic (4–6-M HNO_3) PUREX raffinate, leaving Cm(III), the heavier actinides, and the remaining fission products in the aqueous phase. The Am/Cm selectivity was improved by a selective complexing agent, *N*,*N*,*N*′,*N*′-tetraethyl diglycolamide (TEDGA)—a hydrophilic homologue of TODGA, the presence of which raised the Am/Cm separation factor from 1.6 to 2.5

allowing a significant reduction in the number of stages needed for their effective separation in a continuous countercurrent process [53, 54].

Novel hydrophilic CHON ligands, derivatives of 2,9-bis-triazolyl-1,10-phenanthroline with hydroxylated-1,2,3-triazolyl moieties, were evaluated as Am(III) stripping agents, but their Am selectivity was not significantly improved [55]. The search for the most suitable Am(III)-selective stripping ligand of CHON composition is still ongoing.

24.6 Specific Problems of SNF Reprocessing by Solvent Extraction

In the solvent extraction of radionuclides present in spent nuclear fuels, we encounter issues also occurring in similar processes with nonradioactive metal ions. These are problems resulting from the limited solubility of extractants and/or stripping agents in a given liquid phase and from the limited metal loading capacity of the extraction systems. The limited solubility of the extracted complexes in hydrocarbon diluents is usually considered to be the reason for the sometimes observed adverse effect of the organic phase separation on two liquids, called third-phase formation. Organic modifiers are often introduced into solvent extraction systems to mitigate these adverse phenomena and to improve slow extraction kinetics. These and other typical issues will not be discussed in detail here, and the present section will deal with some specific problems arising from the presence of highly radioactive species in solvent extraction systems.

24.6.1 High-intensity ionizing radiation

All experiments with radionuclides, even if used in tracer amounts, require effective shielding from high-intensity ionizing radiation and prevention of radioactive contamination of personnel. Hundreds of radioactive isotopes present in SNF emit particle radiation, such as high-energy alpha radiation, beta radiation with a broad energy spectrum, and electromagnetic gamma radiation with various energies specific for a given isotope. Some isotopes of the heaviest actinides (curium and californium) emit also neutron radiation. The ionizing radiation makes a significant health hazard. Radiation protection relies on reducing exposure to radiation, which decreases with the increasing distance from the radiation source and with shielding through barriers of various materials depending on the kind of radiation.

Highly penetrating gamma radiation is best absorbed by heavy nuclei, lead barriers being most common. In contrast, alpha radiation is the least penetrating and presents no problems with shielding. More penetrating beta radiation can be absorbed even by thin metal barriers. However, high-energy beta particles can

interact with some shielding materials and produce secondary radiation, strongly penetrating bremsstrahlung X-rays. In such cases, shielding must be carried out with low atomic-weight materials, for example, plastics and water. Similar shielding is also required in the case of highly penetrating neutron radiation. Hydrogen-rich material is more effective than materials containing heavy nuclei. Fast neutrons are slowed down by the light nuclei as a result of elastic scattering and then react with atoms having high cross sections for thermal neutron absorption. It is often necessary to provide an additional shield that absorbs gamma radiation accompanying such reactions.

Equally or even more important is prevention of radioactive contamination of personnel. Operations with open sources of radioactive materials need effective ventilation. Manipulations with alpha and soft beta emitters should be carried out in sealed glove boxes equipped with HEPA filters in the exhaust system, and under a slightly reduced pressure to prevent dangerous outflows. Working with high activities of high-energy beta emitters and gamma-ray emitters requires additional shielding with thick layers of steel and/or lead, which is ensured in hot cells equipped with viewing windows made of sandwiched glass panes containing PbO, and with manual manipulators. Because of very high levels of radioactivity of SNF, all work in the reprocessing plants is conducted by remote control [56].

24.6.2 Radiolysis of solvent extraction systems

Ionizing radiation emitted by fission products and actinides present in SNF degrades the components of solvent extraction systems under reprocessing conditions. This is due to the interactions of gamma rays and high-energy beta particles with solvents when the organic phase comes into contact with the highly radioactive SNF solution in nitric acid, accompanied by short-range interactions of alpha particles from the actinides already extracted into the organic phase. The irradiated solvent molecules become ionized or excited, which results in the formation of transient reactive species. The most important of these in the biphasic, aerated, and nitric acid-containing systems are $^{\bullet}OH$, $^{\bullet}NO_3$, $^{\bullet}NO_2$, carbon-centered radicals, radical cations, $^{\bullet}H$ atoms, and solvated electrons. These short-lived species diffuse into the bulk solution and react with the solutes. Their reactions with extractants or stripping agents cause deleterious effects such as a decrease in the ligand concentration and the formation of degradation products that can also complex the separated metal ions, interfering with the desired separations. Also, longer-lived molecular species, in particular H_2O_2 and HNO_2, are produced in these systems and act as redox reagents affecting the oxidation state of metal ions in the irradiated solutions. All these phenomena, in particular the degradation of ligands, accumulation of irradiation products, the changes in the oxidation states of extracted metal ions, and changes in the physical properties of

solvents, significantly affect the separation processes by decreasing the extraction efficiency, loading capacity, separation factors, etc. [57, 58].

Solvent extraction systems used for SNF reprocessing, which have accumulated numerous degradation products acting as complexing and redox reagents, are so complex that it is difficult to predict the behavior of the system. As an example, we can discuss the hardly predictable changes in the oxidation state of some actinides in the system. In predominantly oxidative aerated nitric acid solutions, low absorbed radiation doses result in the oxidation of Np(V) to Np(VI), presumably by reaction with $^{\bullet}OH$ radicals [57, 58]. In contrast, HNO_2 reduces Np(VI) to Np(V). On the other hand, at low HNO_2 concentrations in highly concentrated HNO_3 solutions, HNO_2 accelerates the oxidation of Np(V) to Np(VI) [37]. Also, plutonium has multiple oxidation states coexisting in aqueous HNO_3, and their equilibrium is affected by the acid concentration and by ionizing radiation. The radiolysis products, H_2O_2 and HNO_2, reduce Pu(VI) to Pu(IV) and possibly even to Pu(III), upon accumulation of millimolar amounts of HNO_2 [57].

It is generally accepted that aromatic hydrocarbons are more stable to ionizing radiation than aliphatic hydrocarbons. The stabilization effect of the aromatic ring can extend to the alkyl groups in the same molecule and even to aliphatic compounds present in a mixture with the aromatic compounds [58]. However, an unexpected result was observed when studying the effect of different aromatic solvents on the radiolytic stability of the tetraethyl BTP extractant in 1-hexanol diluent. The addition of nitrobenzene to the organic phase protected the BTP molecule against radiolytic degradation, whereas *tert*-butyl benzene did not exert similar effects. This has been interpreted as a small protective effect of the aromatic ring alone and explained in terms of the ability to remove solvated electrons and α-hydroxyalkyl radicals by nitrobenzene, but not by aromatic hydrocarbons [59].

Tributyl phosphate (TBP) is the most commonly used ligand in SNF reprocessing—as the extractant in the large-scale PUREX process and as a phase modifier in some other solvent extraction processes. The main product of TBP radiolysis in the presence of nitric acid is dibutyl phosphoric acid (HDBP). Various possible mechanisms for its formation have been discussed [57]. The other products of radiolysis include nitrated, methylated, and/or hydroxylated phosphates, often of higher molecular weight. Some of these degradation products are hydrophilic complexing agents that retain uranium and plutonium in the aqueous phase. Therefore these compounds (particularly the organophosphorus species) must be removed from the degraded solvent prior to its reuse. The degraded TBP solvent is usually purified by washing with sodium carbonate or sodium hydroxide solutions, which eliminates retained uranium and plutonium, organophosphorus species, a portion of the low-molecular-weight neutral molecules (e.g., butanol and nitrobutane), and most ruthenium and zirconium fission products. However, the alkaline wash is insufficient to

completely restore the properties of the TBP solvent, so that additional cleanup procedures have been proposed [5].

Another class of extractants potentially useful for SNF reprocessing are some derivatives of (1,2,4-triazin-3-yl)pyridine (BTP) and (1,2,4-triazin-3-yl)bipyridine (BTBP, Fig. 24.2). The BTPs of the first generation, 2,6-bis(5,6-dialkyl-1,2,4-triazin-3-yl)pyridines, appeared susceptible to oxidative and radiolytic degradation; therefore several studies have been carried out to improve their stability. Since the degradation begins with the removal of the H-atom at the α-CH_2 position of the triazine alkyl substituent, efforts were made to replace the alkyl chains with groups less susceptible to hydrogen elimination. The cyclohexyl moieties substituted with methyl groups at the benzylic positions ($CyMe_4$) have been selected. Furthermore, to solve the problems associated with the subsequent back extraction of metal ions, the improved structure based on the bipyridine skeleton was designed as $CyMe_4$-BTBP (Fig. 24.2) [23], successfully used in the hot test of the SANEX process [27]. A protective effect of nitric acid against radiolytic degradation of $CyMe_4$-BTBP in the solvent extraction system with 1-octanol diluent was also observed [60].

Another promising extractant of lanthanides and actinides from the PUREX raffinate is TODGA (Fig. 24.5). The irradiation of its hydrocarbon solution in the presence of aqueous nitric acid leads to a highly efficient cleavage of the N—$C_{side\text{-}chain}$, N—$C_{carbonyl}$, and C—$O_{etheral}$ bonds, with the formation of degradation products such as *n*-octane and *N,N,N′*-trioctyldiglycolamide, *N,N*-dioctylamine and 5-(*N,N*-dioctyl)-amido-3-oxopentanoic acid, or *N,N*-dioctylglycolamide and *N,N*-dioctylacetamide, respectively. The presence of these products decreases the distribution ratios of Am(III) and lanthanides(III), deteriorating their extraction efficiency in the irradiated system [58]. The acidic product of TODGA degradation has an adverse effects on the Am(III) stripping from the loaded organic phase. The formation of these degradation products necessitates additional washing of the irradiated solvent prior to its reuse, similar to the alkaline washing of the irradiated TBP solutions, aimed at HDBP removal [57].

Strong radiolytic degradation of the extractant and diluent during the reprocessing of SNF requires frequent replacement of the contaminated used solvent. The importance of using fully incinerable CHON reagents for the reprocessing was discussed in Section 24.4.

24.6.3 Prevention of criticality

In numerous variations of the SNF process, plutonium-rich homogenous solutions are formed and are later transformed into solid compounds. The accumulation of large amounts of fissile plutonium in small volumes poses a danger of accidental formation of a critical mass [4, 61]. Separation of the fissile materials from the fission products

(neutron poisons) and the presence of neutron moderators in the solvent extraction systems require provision for protection against an accidental nuclear chain reaction. Also the unintended precipitation of plutonium-containing solids or the formation of a third phase at high metal loading can be dangerous in this respect. The criticality safety for equipment containing fissile liquid is achieved by the geometry or shape of the containment. The design should provide for any potential leakage to a criticality safe containment. The evaluation of such designs should address the potential for such leaks to evaporate and crystallize or precipitate either at the leak site or on nearby hot vessels or lines [62]. Therefore the reprocessing plants are designed in criticality safe geometries and equipped with monitoring systems of ambient neutron fluxes [4]. Neutron poisons (like gadolinium) can also be added to the system to minimize this risk [61]. Nuclear *criticality* safety is concerned with mitigating the consequences of a nuclear *criticality* accident.

24.7 Summary

The open nuclear fuel cycle utilizes only a few percent of the energy contained in uranium. The partially closed fuel cycle, used on industrial scale in several countries for many years, consists in the separation of plutonium and uranium (in modified versions of PUREX process also neptunium and some fission products) from the spent nuclear fuel. Such an approach, which mainly uses solvent extraction processes, significantly saves uranium resources and to some extent reduces the potential risk associated with radiotoxic nuclear waste.

New, much more effective solvent extraction methods designed for reprocessing of spent nuclear fuel, comprising additional recycling of americium, are compared with the currently used technologies. This chapter discusses the use of novel americium-selective extractants and/or stripping agents aimed at supplementing the PUREX process and closing the nuclear fuel cycle. An alternative reprocessing concept based on homogenous recycling of all actinides by coextraction of transuranic elements (the EURO-GANEX process) is presented. Also discussed are specific problems of solvent extraction of highly radioactive actinides and fission products that result in the radiolytic degradation of solvents and interfere with the desired separations.

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Chapter 25

Continuous-Flow Extraction

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Chapter Outline

Liquid-Phase Extraction. https://doi.org/10.1016/B978-0-12-816911-7.00025-6

25.1 Introduction

The large of number of samples with which analysts can be confronted imposes the use of expeditious analytic methods (automatic methods). Despite the major conceptual and operational differences between partly and fully automated methods, these two terms are frequently confused. A fully automated method allows the whole analytic process to be completed with no intervention from the analyst; also, it can by itself make the decision as to whether the operating conditions should be altered in response to the analytic results. All too frequently, methods are deemed automated simply because one or several steps of the analytic process are performed in an automated manner. However, an automated method should be capable of completing all steps including sampling, sample preparation and dissolution, interference removal, aliquot withdrawal, analyte measurement, data processing, result evaluation and decision-making, and restarting the whole process in order to adapt it to the particular needs of a new sample, if needed.

Obviously, a fully automatic method is very difficult to develop (especially for solid samples, the first steps in the analysis of which can rarely be performed in an inexpensive manner). Usually the operations posing the greatest difficulties among those involved in such steps are those requiring some mechanical handling, automation of which is only possible in most cases by using a robot arm adapted to the chemical operations to be performed. Because this equipment is too expensive for most analytic applications, fully automated methods for the analysis of solid samples are very scant and largely restricted to the control of manufacturing processes in practice.

The automation of analyses involving fluid samples is facilitated by their usually adequate homogeneity and easy mechanical handling by the use of peristaltic or piston pumps or some other liquid management devices (e.g., a liquid driver).

Liquid-liquid extraction (LLE) is a classical and widely used technique for sample matrix separation and preconcentration prior analyte detection, having been applied to various analytic fields. Manual LLE requires large amounts of organic solvents and time-consuming multistage manipulations. Therefore, LLE was among

the earliest techniques implemented in flow assemblies in order to overcome these inherent drawbacks, that is, to reduce organic solvent consumption and to speed up extractions. As shown later, the way it is implemented varies from one flow technique to another. Flow-based LLE has been applied to various areas, such as environmental, pharmaceutical, clinical, and food analysis, among others. It has been mostly coupled to optical detectors, due to the fact that the influence of the organic phase is minimized in such systems.

25.2 Segmented Flow Analysis (SFA)

The need to seriously consider the development of automatic methods of analysis arose in the 1950s, where clinical tests started to be increasingly used for diagnostic purposes in medicine. This led to a rapid increase in the demand for laboratory tests that, for obvious economic reasons, could not be met simply by hiring additional laboratory staff. The solution to this problem was provided by segmented flow analysis (SFA), which afforded not only substantially increased analysis throughput but also substantial savings in samples and reagents. SFA laid the foundations for modern flow techniques.

Segmented flow analysis (SFA) is an automatic continuous methodology developed by Skeggs in 1957 [1]. Its associated equipment (Fig. 25.1) usually includes a peristaltic pump for continuous aspiration of the sample and reagents, a series of plastic tubes (the manifold) intended to carry liquid streams, and a detector. Once aspirated, samples are segmented by inserting air bubbles in the liquid streams that are subsequently removed before they can reach the detector.

The introduction of air bubbles has several purposes, namely:

(a) To avoid carryover between samples, which is facilitated by inserting a segment of flushing water (W) between individual samples (S) to remove any residues of the previous sample potentially remaining on the tubing walls

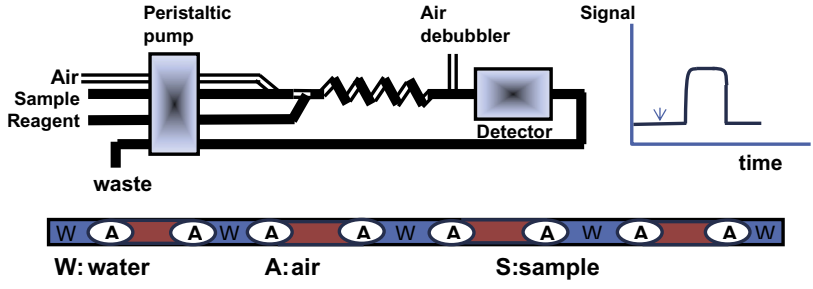

Fig. 25.1 Scheme of a segmented flow analysis system (SFA).

(b) To prevent dispersion of the sample plug
(c) To facilitate the formation of a turbulent flow homogenizing the sample-reagent mixture in the plug sandwiched between each pair of bubbles

The use of air bubbles also involves several limitations. The high compressibility of the air bubbles results in flow pulsation; their injection and subsequent removal complicate the operational design; and their presence reduces the efficiency of separation (dialysis, LLE) systems, hinders the implementation of stopped-flow methods, and precludes miniaturization in many cases.

Because each individual segment is isolated from the neighboring segments of flushing water, the recording provided by the detector is roughly a rectangle the height of which is proportional to the analyte concentration—if the reagents are permanently present in greater than stoichiometric amounts.

Since LLE is very often required in the development of analytic methods, the automation of this technique was carried out using the commercial Technicon SFA autoanalyzer. Using this analyzer, the solvent extraction of copper was semiautomated [2]. However, the implementation of LLE sample pretreatment in SFA analyzers was quite limited. The concept of flow segmentation was explored later for the development of aqueous-organic segmented continuous-flow solvent extraction, which will be introduced in the next section.

In its day, SFA provided an effective solution for laboratories engaged in large numbers of repetitive determinations daily. However, its high costs hindered its expansion to modest laboratories.

25.3 Flow Injection Analysis (FIA)

The name of this technique was coined by Ruzicka and Hansen [3] in Denmark in 1975. While it initially resembled SFA, FIA is rather different from it in both conceptual and practical terms. Thus the basic components of FIA are virtually the same as those of SFA and include a peristaltic pump to propel the sample and reagents, a series of plastic tubes (the manifold) carrying the liquids, and the detector (see Fig. 25.2). Unlike SFA, the sample is not inserted by continuous aspiration; rather, a constant volume of sample is inserted into a stream of liquid carrier via an injection (insertion) valve for merging with the reagents used by the analytic method applied. Tube lengths and the rotation speed of the peristaltic pump are dictated by the reaction time. Thus, if a long time is required for kinetic reasons, then a long piece of tubing is inserted—usually in coiled form—increasing the residence times of the sample and reagents in the reactor.

Unlike SFA, which operates under a turbulent flow regime, FIA uses laminar flow, which reduces the likelihood of carryover between successive samples.

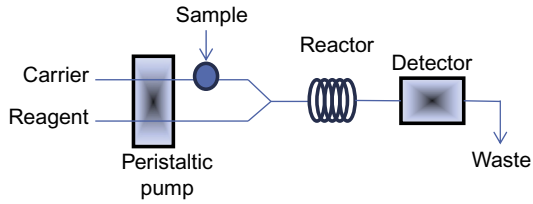

Fig. 25.2 Typical two-channel FIA manifold.

Also, FIA requires no separation of samples with intervening bubbles—it uses continuous flow.

The height and area of the peak are proportional to the concentration of the target species, which facilitates the construction of a calibration curve for its determination in unknown samples. To the left of Fig. 25.3 are shown the peaks obtained from quadruplicate injections of a series of standards of increasing concentration of analyte. While SFA usually requires that the analytic reaction reach chemical equilibrium, FIA does not. In fact, FIA only requires that the extent of reaction be constant and reproducible, which is facilitated by the high reproducibility in the hydrodynamic behavior of the system (this means that FIA may be considered as a fixed-time

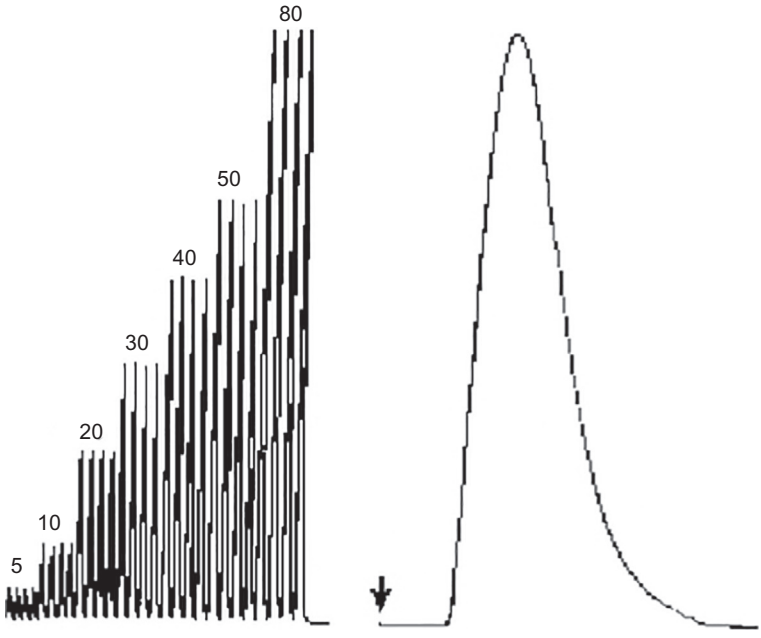

Fig. 25.3 Left: peaks used to construct a calibration curve. Right: typical FIA recording.

kinetic method). Because FIA uses much thinner tubing and much lower flow rates, it consumes samples and reagents more sparingly than does SFA.

In addition, FIA is much more flexible than SFA and allows the implementation of analytic methodologies unaffordable to the latter (e.g., kinetic methods and stopped-flow methods).

Another major advantage of FIA over SFA is its ease of implementation. In fact, a dedicated manifold can be readily assembled from inexpensive parts (viz., a peristaltic pump, injection valves, flow cells, and polytetrafluoroethylene [PTFE] tubing connectors) and available measuring instruments (e.g., spectrophotometers, potentiometers, ammeters, or atomic absorption spectrometry equipment). This has propitiated a vast expansion of FIA among research laboratories and led to the development of many applications relative to other more recent techniques within a few years after its inception.

25.3.1 Liquid-liquid extraction in FIA

Fig. 25.4 depicts a typical assembly for LLE in FIA. An organic extractant is merged with an aqueous carrier containing the sample, and after a long enough time for extraction to complete, the two phases are separated, and the organic one is driven to the detection cell for measurement.

These FIA systems have the disadvantage that the flexible tubing of peristaltic pump is vulnerable to the action of solvents and breaks easily lengthwise. This can be avoided by using the displacement technique, which involves passing water through the pump channel corresponding to the solvent. On entering a tightly closed vessel containing the solvent, the water displaces an equivalent amount for insertion into the manifold (see DB in Fig. 25.4). If the solvent is denser than water, then the latter is introduced at the top of the vessel; otherwise, it is introduced at the bottom.

The organic phase is inserted into the carrier stream via a T-shaped segmenter such as that of Fig. 25.4. The distance between the outlet tube and the solvent penetration point at the T-piece dictates the size of the aqueous and organic segments formed. If the outlet tube is made of glass, the aqueous drops and segments are convex and concave, respectively, in shape (see SG in Fig. 25.4); on the other hand, if the tube is made of a hydrophobic material such as PTFE, the two shapes are reversed. The key to a successful extraction by FIA lies in ensuring reproducibility of the organic segments that are dispersed in the carrier.

Once extraction is completed, the aqueous and organic phase can be separated in various ways. One involves passing the mixture through a T-piece such as PS1 of Fig. 25.4. If the organic phase is denser than water, then the former leaves the tee through the bottom. This can be facilitated by inserting a small thin sheet of PTFE between the side and top branches to have the nonpolar organic solvent adhere to the

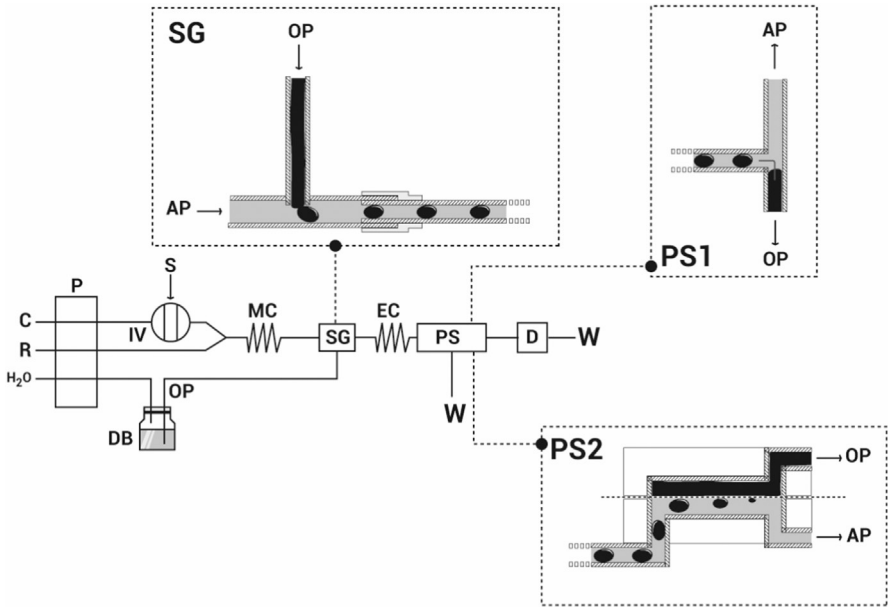

Fig. 25.4 LLE system in FIA and their main components: SG, T-shaped organic phase segmenter; PS1, phase separator for solvents denser then water; and PS2, phase separator for solvents less dense then water. Elements: C, carrier; R, reagents; S, sample, P, peristaltic pump; IV, injection valve; MC, mixing loop; EC, extraction loop; DB, displacement bottle of the organic phase by water; OP, organic phase; D, detector; W, waste.

PTFE walls, while the water leaves the T-piece via the top channel. It is advisable to aspirate the water through a piece of tubing leading to the peristaltic pump. The tube length used should ensure an adequate aspiration flow rate (viz., one coinciding with the combined flow rates of the carrier and reagent) so that the excess rate helps drive the organic solvent to the detector.

One other way of separating the organic and aqueous phase is by using a hydrophobic porous membrane sandwiched between two blocks having a carved circulation channel on one side each (PS2 of Fig. 25.4); a gas-diffusion or dialysis cell can be used for this purpose provided any plastic materials potentially attacked by the solvent are avoided. The mixture is forced through one channel the end of which is connected to a long and thin enough piece of tubing to obtain the load loss and pressure required to force part of the liquid through the membrane pores. Due to its hydrophobic nature, the membrane will only allow the organic solvent to pass through and be driven to the detector, excess liquid not crossing the membrane being sent to waste.

First examples reported for continuous LLE were based on segmentation of the organic phase in the aqueous phase [4, 5]. Classic approaches for LLE using FIA systems have been compiled in a review [6]. Recent developments toward the improvement of LLE using the FIA technique have been directed toward the automation of more efficient LLE techniques, such as the dispersive liquid-liquid microextraction technique (DLLME) [7]. In DLLME, the dispersion of a water immiscible extracting solvent is enhanced by the addition of a second solvent (disperser), which is miscible in both the aqueous and organic phases. By the action of the disperser, the extracting solvent will be dispersed as tiny droplets in the aqueous phase, enhancing the effective extraction area. This methodology was automated using the FIA technique for the determination of inorganic selenium species in water and garlic samples using electrothermal atomic absorption spectrometry (ETAAS), achieving automation of the DLLME method using an ionic liquid as extraction solvent for the Se-ammonium pyrrolidinedithiocarbamate complex, and using a Florisil-packed microcolumn as phase separator for the ionic liquid.

The continuous homogeneous microextraction of selenium and arsenic was achieved exploring the use of nonanoic acid as a switchable hydrophilicity solvent [8]. The procedure involved online mixing of the analytes with ammonium pyrrolidinedithiocarbamate and sodium nonanoate in a homogeneous aqueous phase. By acidifying the nonanoic acid is produced and dispersed into the acidic aqueous phase. Nonanoic acid droplets were separated by using a monolithic column packed with a block of porous PTFE. The retained analyte complexes were eluted with a basic solution and injected into a hydride generation atomic fluorescence spectrometry system for analyte quantification.

25.4 Sequential Injection Analysis (SIA)

Sequential injection analysis (SIA) was developed by Ruzicka and Marshall [9] as an alternative to FIA. With time, SIA has demonstrated that its scope departs markedly from that of the latter technique.

Fig. 25.5A shows a typical SIA system, whereas Fig. 25.5B is its schematic. The central port of a switching valve is connected to a two-way piston pump as are the side ports to the sample and reagent vessels and to the detector. The side ports can also be used for other purposes such as discharging waste or connecting to other devices (e.g., a microwave oven, photooxidation system, or mixing chamber).

One of the essential features of SIA is computerized control. The computer selects how the central port of the valve is connected to its side ports, starts and stops the pump to aspirate or dispense liquids, selects their volume and adjusts the flow rate. Also, it acquires and processes data.

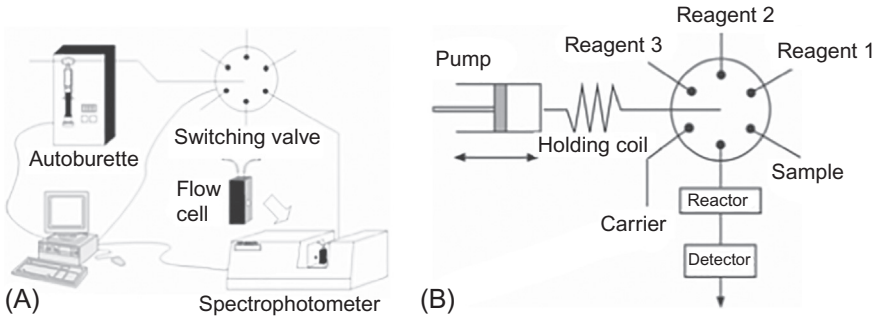

Fig. 25.5 (A) Sequential injection analysis system. (B) Schematic of the SIA of figure (A).

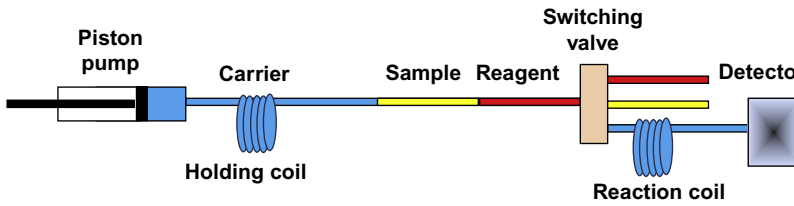

Fig. 25.6 Scheme of an SIA system following aspiration of the sample and reagent.

In an SIA system using a single reagent, the central port of the switching valve is connected to the sample channel, and the pump is set to aspirate a preset volume of sample at a low flow rate in order to avoid the formation of bubbles. Then the central port is connected to the appropriate side port to aspirate a preset volume of reagent (see Fig. 25.6). Next the valve is actuated to connect the central port with the channel leading to the detector, and an appropriate volume of carrier is dispensed to drive the sample and reagent to the detector.

All this occurs under a laminar flow regime that facilitates dispersion of the sample and reagent plugs; as a result, the detector profile is no longer rectangular, but rather exhibits the typical asymmetric shape of FIA peaks.

As is shown in Fig. 25.7, dispersion in an SIA system leads to the sample and reagent plugs overlapping and forming the reaction product to be detected. A typical SIA manifold includes two types of coil, namely, a holding coil inserted in the channel connecting the piston pump to the central port of the switching valve that is used to prevent the sample and reagents from reaching the piston pump—cleaning of which otherwise would be labor-intensive and time-consuming—and a reaction coil in the channel leading to the detector that is intended to ensure adequate overlap between sample and reagent plugs in order to allow a detectable amount of reaction product to form.

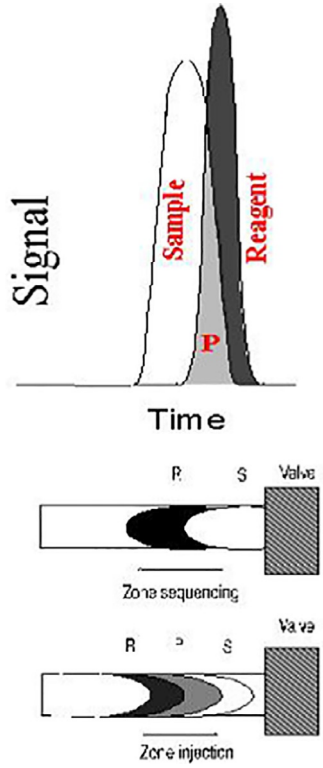

Fig. 25.7 Overlapping sample and reagent plugs in an SIA system.

Unlike FIA, SIA can be turned into a true multiparametric analysis system simply by using a switching valve with an appropriate number of channels to hold the different analytic reagents, delivery of which can be precisely programmed via the associated computer. Currently available switching valves can have more than 27 side ports. Also the number can be increased by connecting a side port of a valve to the central port of several others. Such a high degree of expandability is exclusive to SIA and multipumping flow systems, and no other flow techniques can match it in multiparameter determination capabilities.

On the other hand, in FIA operation, sample and reagent consumption are virtually independent of the analysis frequency as the peristaltic pump continuously propels the sample and reagents at a constant flow rate throughout. In SIA, however, the piston pump only works during the time strictly required to aspirate the amount of sample and reagent needed for a given determination. Aspirating an additional amount of sample is only required when the sample is replaced by the next sample, since the previous sample should be completely flushed out of the aspiration tube to

avoid carryover. By way of example, an SIA monitor for determining ammonium ion in wastewater uses 10 times less reagents than does a comparable FIA monitor; this is of economic and practical significance, especially for equipment that is to operate unattended over long periods (e.g., an automatic analytic monitor) [10].

Since it uses piston pumps, SIA is more robust than flow techniques using peristaltic pumps. In fact, peristaltic pumps use tubing of materials that are relatively easily damaged by some fluids (viz., acids, bases, and especially organic solvents); by contrast, SIA use glass piston pumps and rigid PTFE tubing, which are highly inert and ensure a long service life. Also, in SIA, the sample, reagents, and solvents seldom reach the propulsion system, which holds the carrier solution.

One difficulty of SIA operation arises from the way plugs are stacked; this hinders mixing of the sample and reagents (especially when more than two are needed, which require using a sandwich technique). One solution for determinations involving many reagents is the insertion of a mixing chamber in one of the side ports to homogenize the sample and reagent mixtures with the aid of a magnetic stirrer with withdrawal of small aliquots as required.

One of the greatest initial hindrances to SIA development—one that, in contrast to FIA, resulted in the development of barely a few tens of methods during its first year of existence—was the need to use a computer to govern the system. The scarcity of commercially available software and the lack of experience in interfacing computers to analytic instruments caused SIA to develop very slowly despite its proved advantages. Only during the past decade, with the inception of commercial software, SIA gained ground in the field of routine analyses [11].

On the other hand, the need to use a computer has been the origin of some advantages of SIA over FIA. Thus, residence times need no longer be controlled via the length of the manifold tubes and the flow rates of a peristaltic pump; rather, they are controlled in a highly reproducible manner by the computer. Also the ability to adjust the flow rate required in each step of the process and to change it at will at any time make SIA a highly flexible analytic tool. Thus, while using a different method in FIA very frequently entails altering the configuration of the manifold, switching to another method in SIA seldom requires more than using a different computer file containing the operational settings to be used with each procedure. Obviously, changing the reagents will also be necessary; however, a switching valve with an adequate number of ports can be used to hold the reagents needed for several determinations in different ports, so simply choosing the appropriate settings file will usually suffice to determine another analytic parameter.

The incorporation of computers into SIA systems has facilitated the implementation of stopped-flow methods. It suffices to calculate the volume of carrier to be delivered and stop the system when a peak is obtained at the detector in order to readily implement various analytic methodologies including classical kinetic, spectrophotometric, polarographic, voltammetric, and anodic stripping methods.

In addition, computers have increased the flexibility of analytic systems by allowing several operations mimicking those performed manually to be programmed for easy online implementation. Thus samples can be aspirated and supplied with a reagent; their mixture driven to a photoreactor; and an aliquot withdrawn, sent to a preconcentration unit and eluted from it with a view to sequentially detecting the analytes by several detectors arranged serially at the same port or radially at different ports—or even in a serial/radial mixed configuration.

25.4.1 Liquid-liquid extraction in SIA

LLE in SIA differ markedly from FIA in the way it is implemented. Thus SIA is subject to none of the limitations of FIA regarding the use of organic solvents as it employs no peristaltic pump tubes, or if they do (e.g., when a peristaltic pump is used instead of a piston pump in SIA), the solvent is never passed through them. While FIA affords substantial savings in samples, reagents, and solvents in extraction processes and minimizes the environmental impact of their waste, SIA provides even greater advantages over manual methods since, unlike FIA, the reagents are only used at the time a determination is performed.

SIA exploits the fact that the organic solvent adheres to the walls of hydrophobic tubing. The solvent, which can be a mixture, should be of an appropriate viscosity so that the film it forms is neither too thick (to avoid interfering with the back extraction [stripping]) nor too thin (so it will not break easily).

Fig. 25.8 depicts an SIA system for the determination of phenols in water [12] that uses acidity adjustment to effect extraction-back extraction.

A preset volume of solvent of appropriate viscosity is initially aspirated into the reaction coil, which is used as an extraction coil in the process. As the carrier (water) flows, a film of solvent is formed on the walls of the coil. Then, the system aspirates

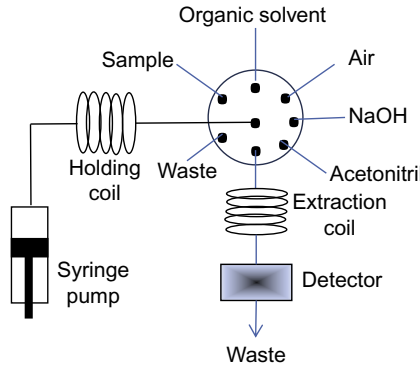

Fig. 25.8 SIA system for the determination of phenols by LLE.

acetonitrile, an air bubble, sodium hydroxide, and an appropriate volume of the sample to be extracted—which must previously be adjusted to the required pH—in this sequence. As these stacked liquids are propelled to the extraction coil, they enter it in the opposite sequence. Because phenols in the sample are undissociated at the pH of the medium, they are extracted by the organic solvent film together with other low-polarity substances present in the sample. When the next reagent in the sequence (sodium hydroxide) arrives, the phenols are converted into phenolates and back-extracted for delivery to the detector (a diode array instrument that provides the whole spectrum for the sample at the peak maximum). Any low-polarity compounds undergoing no change when the pH is adjusted are back-extracted to a negligible extent, so they remain in the organic phase. The air bubble inserted between sodium hydroxide and acetonitrile is intended to reduce dispersion in the plug of back-extracted phenols, and the acetonitrile to remove the solvent film formed and restore the initial conditions.

In fact the solvent film is not still during extraction and back extraction; rather, it moves as the aqueous phase flows, albeit at a slower rate. This requires using an appropriate length of extraction coil to avoid losses of organic phase through the detector before the analytes are determined in suitable forms.

By using standards to calibrate the system and spectra obtained at the maxima of the SIA peaks (see Fig. 25.9), one can simultaneously determine several phenols

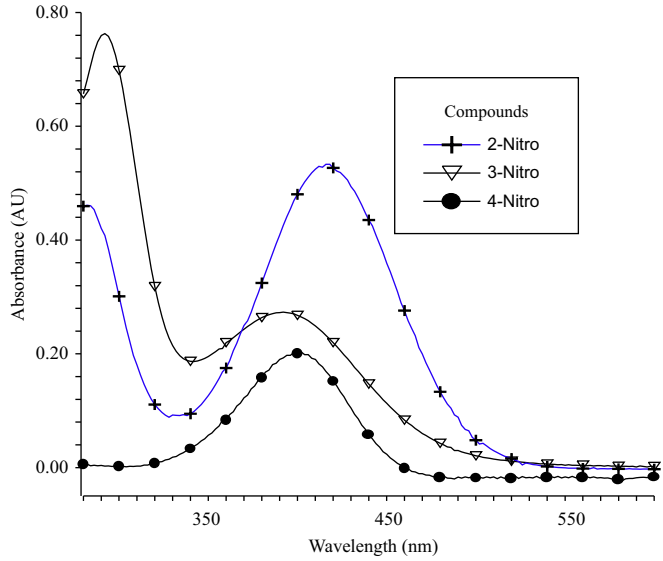

Fig. 25.9 Spectra recorded at peak maxima during the extraction of three nitrophenol. Standards.

without the need to separate them simply by using a multivariate chemometric approach.

Several advances in the automation of LLE using the SIA technique were developed in the earlier years. Anthemidis and Ioannou developed an automated method using the SIA technique for the extraction of copper and lead from water samples by online DLLME [13]. The SIA technique enabled online merging of the sample stream with a stream containing methanol, 2.0% (v/v) xylene, and 0.3% (m/v) ammonium diethyldithiophosphate. Copper and lead are complexed with the ammonium diethyldithiophosphate, and the resulting complex is extracted into xylene. The presence of methanol enabled dispersion of xylene in the aqueous sample stream, resulting in a cloudy mixture based on droplets of the dispersed xylene, enhancing the effective analyte extraction area. The dispersed xylene droplets were retained into a microcolumn packed with PTFE turnings, and 300-μL isobutylmethylketone was used for analyte elution and transportation to the nebulizer of a flame atomic absorption spectrometer (FAAS). The same approach was successfully implemented for the determination of lead and cadmium by ETAAS [14]. Xylene was replaced by more environmentally friendly solvents such as fatty alcohols for the DLLME of silver prior to FAAS determination [15]. Alternatively, the use of ionic liquids as extracting solvent was evaluated for SIA DLLME of thallium followed by FAAS analysis [16].

A dual-selection valve approach was developed for the automation of LLE using the SIA technique [17]. In this case the flow manifold is based on two SIA independent units, which are coordinated. The first SIA unit, the extraction unit, enabled the handling and mixing of the aqueous and organic phases, which are injected into a phase separator where the second SIA unit (detection unit) is connected. The detection unit is programmed to collect the separated organic phase from the phase separator and inject the extract into the appropriated detector. This method facilitated the extraction of picric acid in the form of an ion associate with 2-[2-(4-methoxy--fenylamino)vinyl]-1,3,3-trimethyl-3H-indolium reagent, with subsequent spectrophotometric detection. The double-valve SIA set up with spectrophotometric detection also enabled the extraction of thiocyanate in amyl acetate after the formation of an ion associate between the analyte and Astra Phloxine, followed by the detection of the ion associate at 550 nm [18]. An additional possibility afforded by the DV-SIA configuration is the programmed introduction of air bubbles in the extraction/separation chamber, enhancing the analyte mass transfer from the aqueous to the organic phase [19]. The introduction of a disperser solvent miscible with both aqueous and organic phases enabled the automation of the DLLME technique using the DV-SIA system [20]. The combination of DLLME with the DV-SIA system enabled the coupling of the DLLME sample preparation technique with spectrophotometric detection as exemplified by the determination of thiocyanate ions in the form of ion associate with dimethylindocarbocyanine reagent.

Automation of LLE by the SIA technique was automated by a single SIA manifold equipped with an additional syringe for the delivery of the organic phase into the extraction reservoir [21]. This manifold enabled the determination of thiamine in pharmaceutical preparations and dietary supplements using fluorescence detection. Alternatively, LLE was achieved using a simple SIA manifold without the addition of extra syringes/selection valves, by using an extraction/separation chamber connected to one of the peripheral ports of the selection valve to inject the aqueous and organic phases [22]. Another peripheral port of the selection valve is used to collect the separated organic phase based on an organic solvent lighter than water. This configuration enabled the spectrophotometric determination of Cr(VI) in water samples using the dimethylindocarbocyanine dye and air-assisted LLE with toluene. A similar approach was described for the extraction of polycyclic aromatic hydrocarbons, where the selection valve of the SIA manifold is coupled to the injection valve of an HPLC instrument, enabling the analysis of complex mixtures of organic pollutants [23].

Countercurrent extraction is a separation technique that involves two immiscible liquid phases flowing in opposite directions in a single or a multistage mode. Exploiting a lab-made microextraction chamber, the countercurrent flow of aqueous and organic phases was explored for the automated liquid-liquid microextraction of lead from environmental samples using the SIA technique [24].

Other applications of the SIA technique for automated LLE were developed for the determination of bisphenol A in tap water using spectrofluorimetric detection [25] and the spectrophotometric determination of acid number in biodiesel [26]. The versatility of the SIA technique was exploited for the automation of liquid-phase microextraction techniques, such as headspace single-drop microextraction for the spectrophotometric determination of ammonia in concrete [27]. The single-drop microextraction of caffeine was also successfully automated [28]. In this case the method was based on the extraction of caffeine by the solvent drop, followed by solvent evaporation and dissolution of the analyte in sulfuric acid prior to potentiometric detection. Different strategies have been adopted to improve the automation of the LLE process when executed in the SIA manifold. These include the automation of the salting-out effect to decrease the solubility of the analytes in the aqueous phase to enhance the extraction yield [29] and the exploration of less conventional solvents, such as solvents with switchable polarity [30], or deep eutectic solvents [31].

25.5 Multicommuted Flow Analysis (MCFIA)

The MCFIA technique, devised by Reis et al. [32], uses fast-switching three-way solenoid valves (see Fig. 25.10). The earliest MCFIA systems used a single-channel propulsion system to aspirate the liquids to be employed via individual valves.

Fig. 25.10 Three-way solenoid valves.

Because aspiration devices tend to insert air bubbles or degassed liquids into the system, it is preferable to use liquid propulsion devices such as peristaltic or piston pumps instead.

The system depicted in Fig. 25.11 can be used for several purposes by rapidly switching the valves. It uses a peristaltic pump and has solenoid valves V1, V2, V4, and V5 arranged in such a way that the propelled liquid is returned to the reagent reservoir while the valves are in the OFF position but inserted into the system when in the ON position. By alternately switching V1 and V2 ON, one can dilute the sample to a preset level by the carrier liquid. Because solenoid valves can be switched rapidly, one can alternately insert variable volume, thick slices of carrier, and sample that interpenetrate on their way through loop B1. Valve V3 allows the flow to be

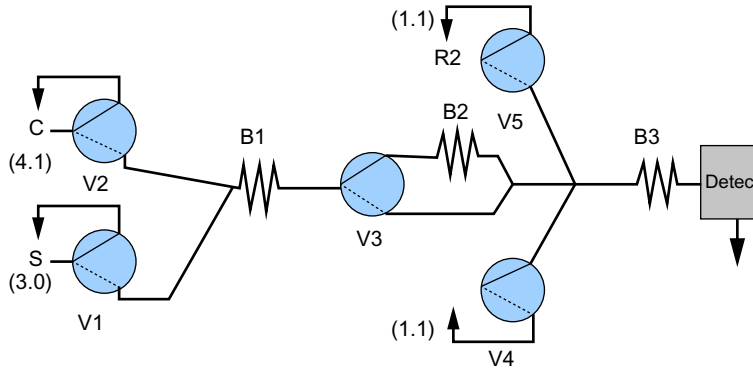

Fig. 25.11 Multicommuted flow analysis system.

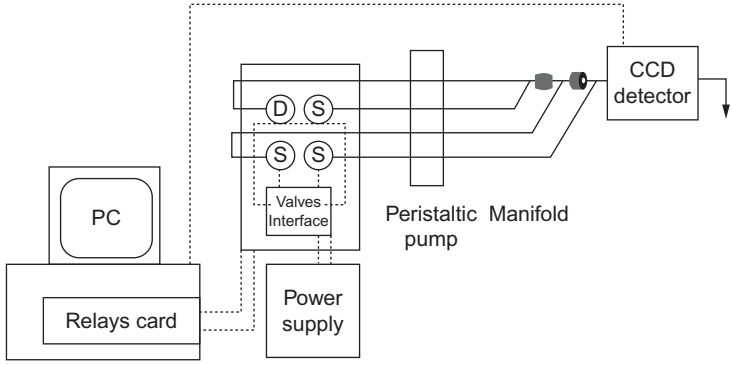

Fig. 25.12 Block diagram of an MCFIA system using a peristaltic pump to propel liquids.

directed to a copperized cadmium column to reduce nitrates to nitrites, while switching to the lower channel avoids this reduction reaction. Finally, valves V5 and V6 can be used to inject preset volumes by switching them ON over an appropriate interval. Loop B_3 is intended to facilitate homogenization of the diluted unknown sample with the reagents added in the last step.

Fig. 25.12 depicts an MCFIA system including a computer intended to switch between the solenoid valves via appropriate interfaces and to start and stop the peristaltic pump and acquire and process data.

One major shortcoming of solenoid valves is the unfavorable effect of the heat released by the solenoid coil when the valves are ON for a long time. The resulting increase in temperature can deform the PTFE inner membranes of the valves and render them unusable. Overheating can be avoided by using an effective electronic protection system. Fig. 25.13 shows a system where the valve is switched ON by

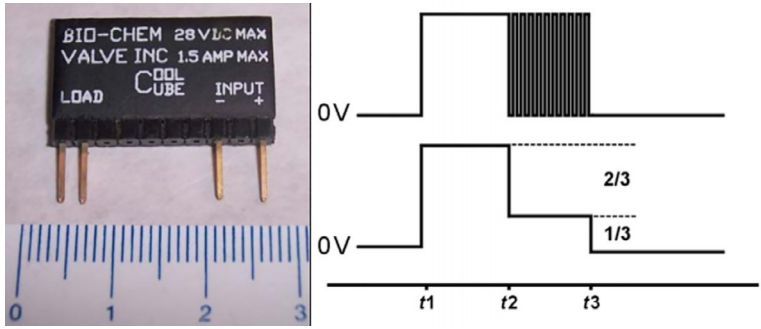

Fig. 25.13 Left: photograph of a valve protector. Right: voltages used to switch and keep the valve ON.

applying a dc voltage and then continues to operate under a pulse train of the same nominal voltage but a much lower voltage, which substantially reduces the amount of dissipated power.

25.5.1 Liquid-liquid extraction in MCFIA

Several papers report the use of MCFIA for LLE, for example, the determination of anionic surfactant in water [33, 34], molybdenum [35] and lead in plants [36], diltiazem in pharmaceuticals [37], and the iodine value for biodiesel [38].

Fig. 25.14 represents a liquid-liquid extraction flow analysis procedure for the spectrophotometric determination of molybdenum in plants at the microgram-per-liter level [35]. The flow network comprises a set of solenoid valves assembled to implement the multicommutation approach under computer control. An LED radiation source (475 nm), photodiode detector, and separation chamber are nested together with the flow cell as a compact unit. The consumption of reagents, potassium thiocyanate and stannous chloride, and extracting solvent (isoamyl alcohol) were optimized as 32 mg and 200 μL per determination, respectively. Accuracy was assessed by comparing results with those obtained with ICP-OES, and no significant difference at the 95% confidence level was observed. Other favorable

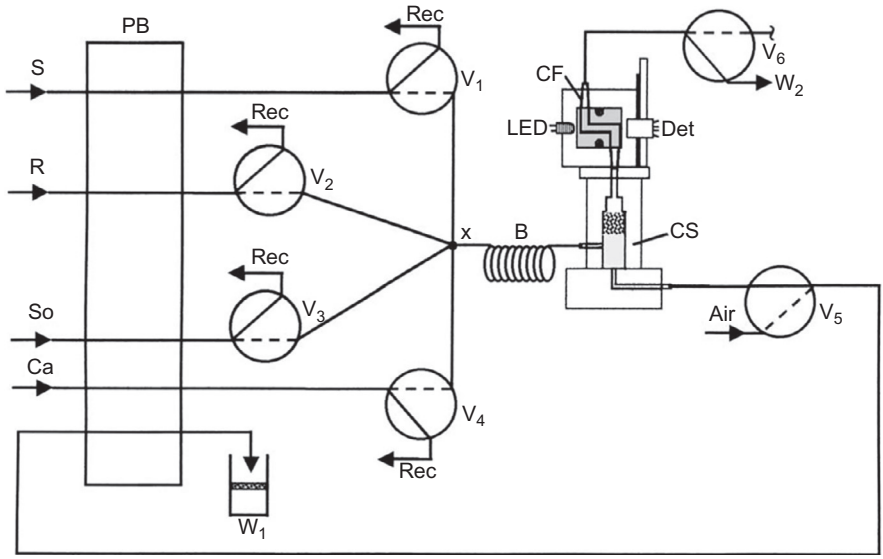

Fig. 25.14 Flow diagram of the MCFIA system for LLE. S: sample; R: reagent (KSCN +$SnCl_2$); So: isoamyl alcohol; Ca: carrier; PB: peristaltic pump; B: reaction coil; V1-V6: solenoid valves; CS: separation chamber; CF: flow cell; Det: photodiode detector; LED: blue LED 475 nm; Rec: solution store vessel; x: joint device; W1 and W2: wastes.

characteristics are a linear response range from 25- to 150-μg L^{-1} molybdenum ($r = 0.999$), detection limit of 4.6 μg L^{-1}, sample throughput of 25 determinations per hour, and relative standard deviation of 2.5% ($n = 10$).

This flow system for the solvent extraction based on multicommutation approach provided a significant reduction in reagent consumption. Since the sampling throughput was 25 determinations per hour, the waste volume of organic phase generated during a workday was 30 ml. This value is at least four times less than that observed for other procedures based on solvent extraction [39–41]. Nesting the aqueous-organic phase separation chamber to flow cell, radiation source, and photodetector resulted in a compact system, robust, safe, and easy to operate, contributing to savings in cost and time related to the analysis and further treatment of effluent.

25.6 Multisyringe Flow Injection Analysis (MSFIA)

This flow technique was developed in 1999 by Cerdà et al. [42]. The aim was to combine the advantages of the previous flow techniques while avoiding their disadvantages.

Fig. 25.15 shows a typical multisyringe burette for use in MSFIA. The device consists of a conventional automatic titration burette adapted in such a way that the motor can simultaneously move the pistons of four syringes in order to avoid the need to have four separate burettes operate in parallel.

This is accomplished by using a metal bar that is moved by the stepper motor of the burette, the bar accommodating the four syringes, and each syringe head containing a fast-switching solenoid valve. Obviously the motor moves the pistons of the

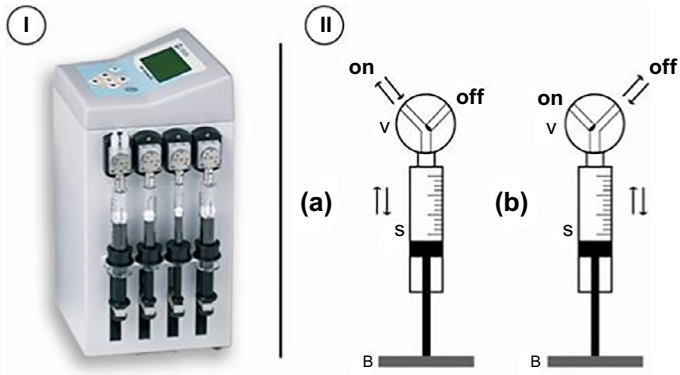

Fig. 25.15 Left: photograph of a multisyringe burette used in MSFIA. Right: schematic depiction of its operating modes.

four syringes simultaneously; this is equivalent to using a multichannel peristaltic pump in FIA but avoids the disadvantages of its fragile tubing. The ratio of flow rates between channels can be modified by using syringes of appropriate cross section similar to tubing diameters in FIA.

Injecting the desired volume of sample entails using a module including a typical FIA rotary injection valve that allows the delivered volume to be adjusted via the dimensions of its loop.

The process leading to the generation of an analyte peak with the earliest MSFIA prototype involved the two steps depicted in Figs. 25.16 and 25.17. In the first step (Fig. 25.16), the syringe pistons were lowered in order to aspirate the sample and reagents. While the solenoid valve for syringe 1, which delivered the sample, was ON (left), the others were OFF (right). Because the injection valve was in its load position, syringe 1—the one on the extreme left—aspirated the sample through the loop defining the injection volume. Simultaneously, the other syringes aspirated the carrier and reagents.

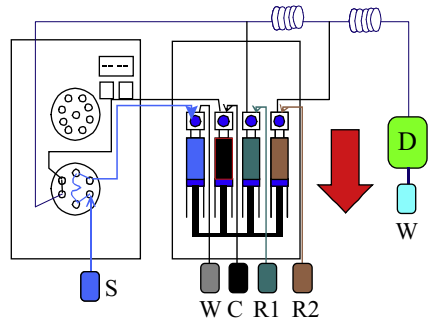

Fig. 25.16 Loading of the sample injection loop. S, sample; W, waste; C, carrier; R_1 and R_2; reagents; D, detector.

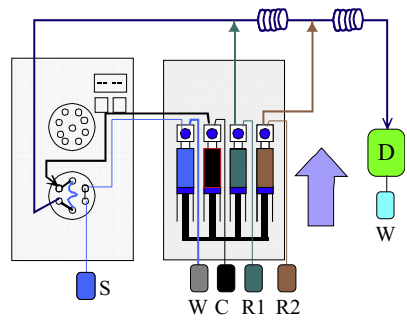

Fig. 25.17 Injection of sample and reagent in the original MSFIA prototype.

The second step (Fig. 25.17) was started by reversing the direction of the pistons. First the injection valve was switched to its inject position, the valve of syringe 1 was switched ON to drive excess sample to waste, and the valve of syringe 2 was switched ON to have the carrier flush the sample held in the loop into the manifold. The valves of syringes 3 and 4 were kept OFF in order to return reagents R_1 and R_2 to their respective containers. Immediately before the sample merged with reagent R_1, the valve of syringe 3 was switched ON to have the two liquids mix. Subsequently the valve was switched OFF after the sample passed by the merging point. This procedure was repeated for reagent R_2.

The MSFIA systems combine some of the advantages of the earlier described flow techniques, namely:

(a) The high throughput of FIA, which is a result of the sample and reagents being incorporated in parallel. This in turn leads to an improved mixing efficiency in relation to SIA.

(b) The robustness of SIA in terms of solvents and aggressive reagents. In fact, the liquids only come into contact with the walls of the glass syringes and PTFE tubing as no peristaltic pump tubes are used.

(c) The low sample and reagent consumption of SIA by the reaction because they are used in the amounts strictly required to perform a given determination. Also the reagents are only inserted when the sample passes by their points of merging.

(d) The high flexibility of SIA manifolds. Thus, residence times are not determined by tubing dimensions, but rather by commands of the computer used to govern the whole system, which sets the times and flow rates to be employed. Usually, switching to a different analytic method simply requires loading the file containing the appropriate settings for the new method and changing the reagent vessels.

(e) The ability to use MCFIA solenoid valves, which can be actuated without the need to stop the syringe pistons. Switching between valves is so rapid that no overpressure arises in the operation.

Unlike FIA, MSFIA requires the use of a computer to control the system. This, however, poses no special problem as a variety of affordable software for implementing any flow technique is now available [2].

The original MSFIA prototype was later modified for economy and simplicity. The alterations included the incorporation of two fast-switching valves independent of the syringes but controlled by the same burette. This allowed the injection valve module to be eliminated while still allowing accurately known liquid volumes to be injected as shown in Fig. 25.18.

The syringes are filled by switching solenoid valves V_5 and V_6 (see dashed lines in Fig. 25.18); in this way, syringe 2 aspirates a volume of sample, and syringes 1, 3, and 4 aspirate the carrier and reagents, respectively.

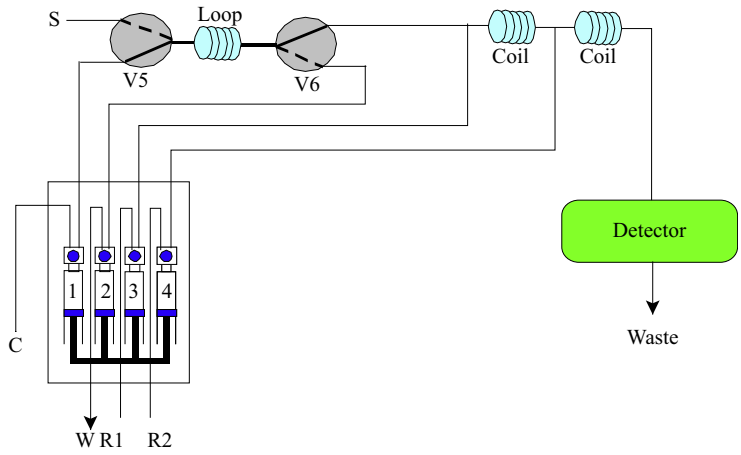

Fig. 25.18 Schematic depiction of the multisyringe burette using two additional independent solenoid valves.

Measurements are made by reversing the flow direction, which is accomplished by switching valves V_5 and V_6 OFF (solid line in Fig. 25.18). In response, syringe 1, with its valve ON, propels the sample volume held in the loop into the manifold. Simultaneously, the valve of burette 2 is switched OFF to have excess aspirated sample sent to waste. The remainder of the process is similar to the original prototype.

The latest-generation multisyringe burettes, marketed by SCIWARE, no longer include independent valves, which have been replaced by a four-outlet connecting strip at the rear of the panel supplying 12 V at up to 300 mA each to facilitate the control of single, double, and triple solenoid valves via the burette itself (Fig. 25.19). The strip can also be used to govern other devices (e.g., relays, pumps, fans, and LEDs). Provided the maximum nominal current is not exceeded, each outlet can be used to connect several devices for synchronous operation (e.g., the pair of single solenoid valves needed for injection).

25.6.1 Liquid-liquid extraction in MSFIA

The LLE methodology developed with MSFIA [43] is implemented in the three steps depicted in Figs. 25.20–25.22.

If necessary, the throughput of the LLE process can be increased by using a dual system such as that of Fig. 25.23 [44].

Recent LLE applications based on the MSFIA technique rely on the implementation of sample pretreatment in the dispersive mode, by adding an additional solvent (disperser) miscible in both water and the extracting solvent [45]. The versatility of the MSFIA technique was exploited combining two syringes moved simultaneously by the same stepper motor, enabling the automated DLLME of sixteen polycyclic

Fig. 25.19 Rear panel of the multisyringe burette.

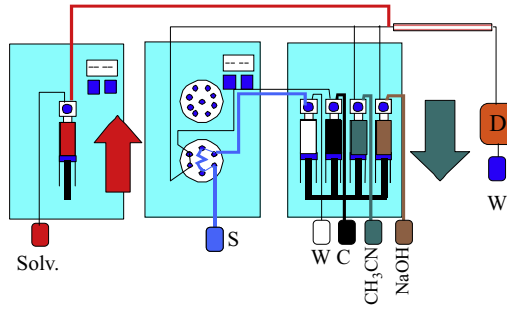

Fig. 25.20 First step in an MSFIA LLE. The extraction coil is loaded injecting the organic solvent with one of the single-syringe burette, the loop of the injection valve is loaded with sample aspirated by the first syringe, and the other syringes are loaded with carrier, NaOH and acetonitrile, all at once.

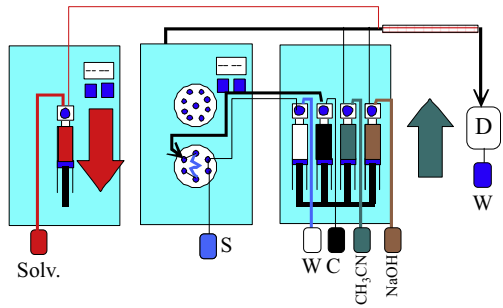

Fig. 25.21 Second step of the MSFIA LLE. Both burettes are actuated to change the flow direction, while the single-syringe burette aspirates solvent and the multisyringe burette starts to force the sample to the extraction coil. Previously inserted carrier forms a solvent film on the coil walls. As the acidified sample is passed through the coil, phenols are extracted.

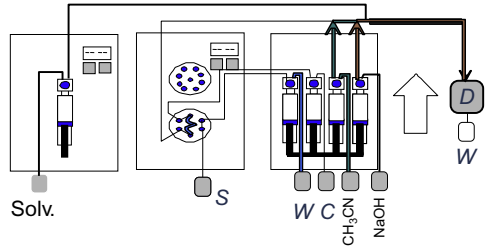

Fig. 25.22 In the third step of the MSFIA liquid extraction process, NaOH is passed through the coil to back-extract phenols and followed by acetonitrile to remove the solvent film previously formed and make the system ready for a new determination.

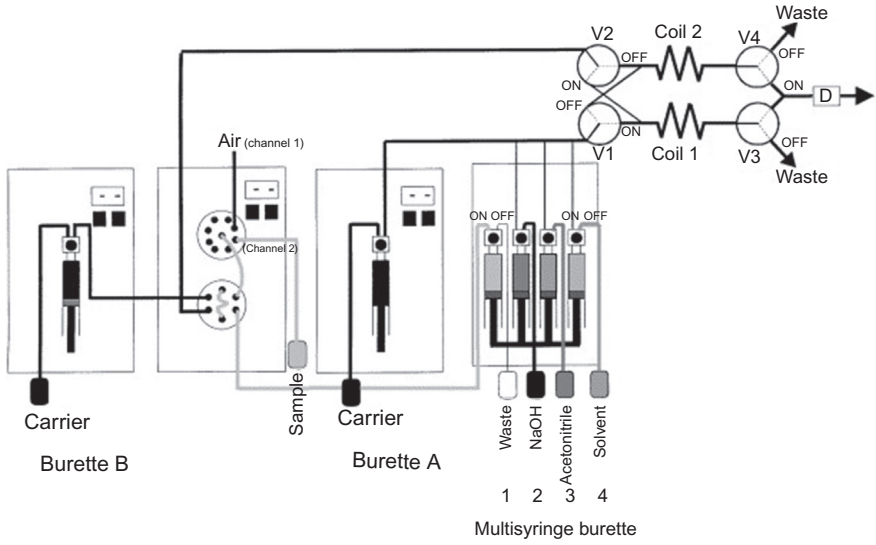

Fig. 25.23 Dual system for the determination of phenols by use of LLE in combination with multivariate analysis.

aromatic hydrocarbons from water samples. This method was fully automated by the hyphenation of the MSFIA technique with gas chromatography-mass spectrometry (GC-MS). The versatility of the MSFIA systems was also exploited for the development of an effervescence-assisted DLLME method [46], where the sample and all required aqueous reagents are loaded in a mixing chamber, followed by simultaneous counterflow injection of the extraction solvent (dichloromethane), the mixture of the effervescence agent (Na_2CO_3) and a proton donor solution (CH_3COOH). Carbon dioxide microbubbles are generated in situ enhancing the dispersion of the extraction solvent in the aqueous sample, facilitating the extraction of the analyte by the organic

phase. The method was applied to the determination of antipyrine in saliva using spectrophotometric detection.

25.7 Multipumping Flow Systems (MPFS)

Multipumping flow systems, simultaneously developed in 2002 by two research groups [47], are based on the use of solenoid piston pumps (Fig. 25.24) where each stroke propels a preset volume of liquid (3, 8, 20, 25, or 50 μL), the flow rate of which is determined by the stroke frequency.

Fig. 25.25 represent the behavior of a solenoid micropump controlled with voltage pulses.

The main advantage of these systems is their high flexibility, easy configuration, robustness, and low cost—as a result of the pump operating as both a liquid propeller and a valve. Like the previous flow systems, MPFS uses samples and reagents sparingly. Usually, it employs a combination of solenoid pumps and valves.

The analyte peaks provided by multipumping flow systems are higher than those obtained with other flow techniques. This can be attributed to turbulence generated by the pump piston strokes facilitating mixing of the sample and reagents.

Fig. 25.24 Solenoid piston micropump.

As can be seen from Fig. 25.26, a typical multipumping system is similar to an MCFIA system (Fig. 25.12). In fact, the former can also be used to implement MCFIA as it allows control of any combination of valves. The primary differences between the two techniques are that multipumping systems only require control of switching valves and the stroke frequency of pumps, in order to ensure reproducible flow rates. The simplicity and economy of MPFS should facilitate the development of portable equipment for field measurements.

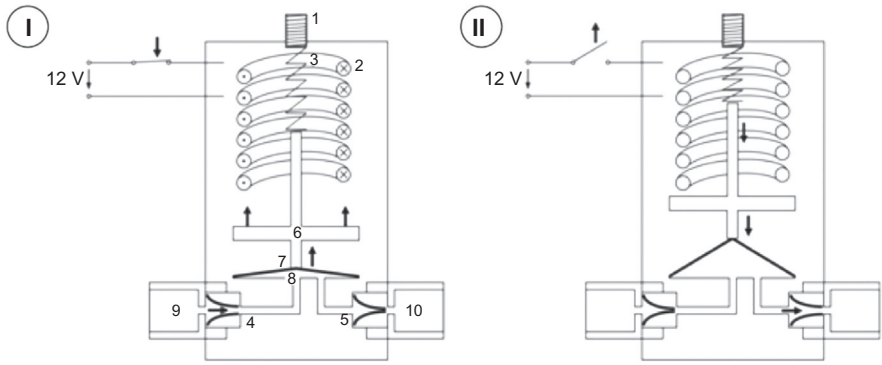

Fig. 25.25 Schematic behavior of a solenoid micropump (I) activated (loading) and (II) deactivated (dispensing). Elements: 1, spring tensor; 2, solenoid; 3, spring; 4, input valve; 5, output valve; 6, inductor; 7, membrane; 8 inner volume of the pump; 9, input; 10, output.

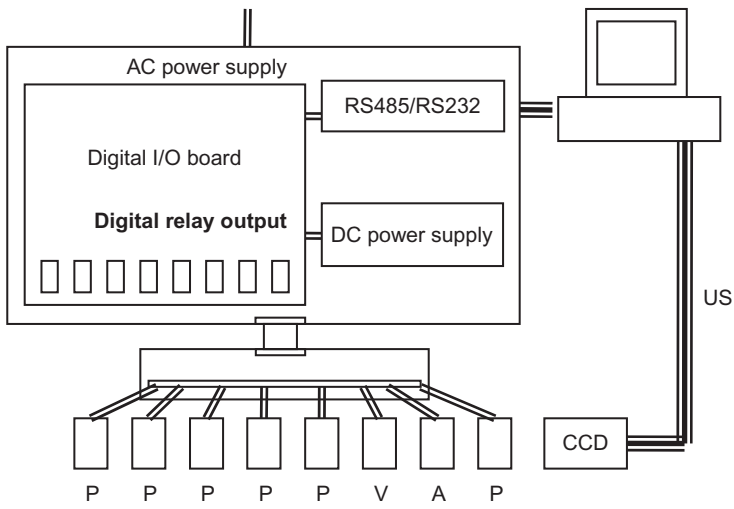

Fig. 25.26 Multipumping flow system (MPFS).

The MPFS technique was used for the flow-batch LLE of formaldehyde in milk. The pulsed flow of the MPFS pumps was exploited to enhance the dispersion of the extractant in the aqueous phase in the extraction chamber, quantifying the analyte by spectrophotometric measurements directly carried out in the organic phase using an optical fiber spectrophotometer [48].

25.8 Lab-in-Syringe: Dispersive Liquid-Liquid Microextraction

The DLLME technique [49] has attracted much attention due to its simplicity and the high enrichment factors that can be achieved. DLLME is a microextraction technique based on the use of a ternary mixture consisting of an aqueous phase, an organic phase (extractant), and an additional organic solvent (disperser solvent), which is miscible in both phases. The disperser is initially mixed with the extractant and then rapidly injected into the sample. By the fast dissolution of the disperser into the aqueous phase, the extractant is disrupted into small droplets enhancing the effective surface area for the extraction. The separated extractant droplets are then settled at the bottom of the vial or float upon the aqueous sample-disperser phase, depending on the density of the extractant used.

Required extraction times are usually short in DLLME, since equilibrium is quickly reached due to the large transfer area for the extraction procedure. The efficient recovery of the settled or floating extractant and its further injection into an analytic instrument for the quantification of target compounds is the most troublesome part of the procedure, mainly when the extractant is floating on top of the sample. Automation is one of the challenges of the DLLME technique.

Some advances have been made by exploiting SIA and an additional peristaltic pump, in order to mix, by confluence, the sample and the disperser/extractant mixture. However, additional steps are required, such as the need for a solid support for the retention/collection of the dispersed organic microdroplets and the reelution of the retained microdroplets from the PTFE support prior to detection. Other SIA systems have been proposed using a conical tube and adding an auxiliary solvent to adjust the density of the extraction solvent when this is lighter than water [20, 50, 51]. Other authors use microcolumns packed with hydrophobic sorbent materials after extraction as phase separators requiring a second extraction [13].

The complete automation of DLLME was achieved using the MSFIA technique [52]. DLLME was performed inside the syringe and was successfully combined with a liquid chromatography procedure utilizing the same syringe pump, which is known as multisyringe chromatography (MSC) [53].

Later, DLLME with spectrophotometric detection was integrated and fully automated inside a glass syringe [54], which acted as the container for the sample

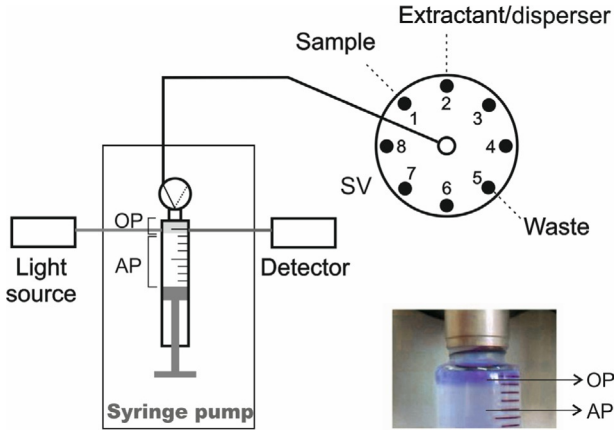

Fig. 25.27 Lab-in-a-syringe system. AP: aqueous phase; OP: organic phase; SV: selection valve.

treatment and as the detection cell, enabling the whole procedure (microextraction + detection) inside the syringe. This technique has been called lab-in-a-syringe. This system is shown in Fig. 25.27.

The initial applications of the in-syringe DLLME techniques were followed by the development of novel analytic methods for the spectrophotometric determination of copper [55] and the phenol index [56] in environmental water samples. The fluorimetric determination of aluminum in seawater was also achieved by incorporating a lab-made detector into the automated manifold [57]. In-syringe DLLME was also applied for the LLE of ^{99}Tc from biological samples and hospital residues prior to off-line liquid scintillation counting [58]. In order to facilitate the development of complex multianalyte determinations, in-syringe DLLME was interfaced with GC-MS for the determination of six phthalates in water samples [59]. In-syringe liquid-liquid extraction can be carried out using a miscible solvent, such as acetonitrile, followed by phase separation using glucose as a sugaring-out reagent [60]. This approach was implemented for the in-syringe extraction of pesticides in fruit and berries, followed by HPLC-MS/MS quantification.

25.9 Lab-in-Syringe: Magnetically Assisted Dispersive Liquid-Liquid Microextraction

In-syringe magnetically assisted DLLME (in-syringe MSA-DLLME) consists of a liquid-liquid microextraction step inside the syringe of an SIA system containing a magnetic stirrer to produce dispersion of the organic phase in the aqueous sample

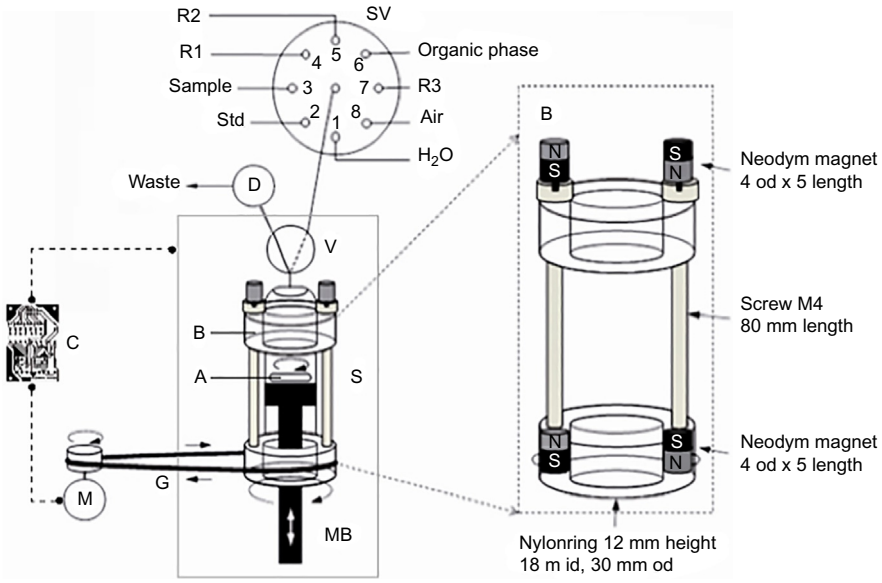

Fig. 25.28 Schematic representation of the in-syringe MSA-DLLME system: S, 5-mL syringe installed in a MB module; SV, selection valve; V, three-way solenoid valve; D, detection cell; A, magnetic stirrer; B, conductor of the magnetic stirrer placed around the syringe; M, motor that transmits the rotation through a rubber band (G); C, electronic circuit controlling the motor connected to one of the auxiliary outputs of the MB. An enlarged view of the magnetic stirring device B and its components is shown to the right.

[61]. The movement of the stirrer is controlled by a computer through the assisted magnetic stirring system (MSA). Fig. 25.28 shows an outline of MSA and its coupling to an SIA system. The SIA system comprises a multiburette MB with a single syringe (S) installed and a switching valve SV for the sequential loading of the reagents and the sample.

As shown in Fig. 25.28, the MSA system has four main parts: first, a small magnetic stir bar placed inside the syringe (A). Second, an external magnetic stirring device (B), which is placed around the syringe. Third is a motor (M) that forces the rotation of the external device. Finally, a circuit (C) that controls the on/off and the speed of the motor through one of the auxiliary outputs of the syringe pump.

The magnetic stirrer (10 mm long, 3 mm in diameter) is placed inside the syringe (S) on the plunger head. The plunger is adjusted in such a way that there is a space of at least 4 mm, to avoid damage when completely emptying the syringe. This space also allows the free rotation of the stirring bar when the piston is in the upper position.

The external magnetic device (B) [52] is specially designed to create a rotating magnetic field around the body of the syringe. In the enlarged view of Fig. 25.28, the components of this device are shown. It consists of two nylon rings, which can be easily placed around the glass tube of the syringe. These rings are connected by means of two screws with a space of 60 mm between them. Six neodymium magnets are inserted into the ends of the screws, one above and two below. These small magnets are located to ensure that the screws are fully magnetized and with opposite polarities. The magnetized screws provide a field of sufficient force to exert an effective attraction on the magnetic stir bar within the syringe, regardless of the height of the piston. A DC motor is used to rotate the magnetic stirrer and is controlled by software. Fig. 25.29 shows a photograph and circuit diagram of the control circuit.

The assisted magnetic agitation (MSA) system inside the syringe allows rapid and homogeneous dispersion of reagents and sample without the need for an additional mixing chamber or dispersing reagent. In addition, this system offers the possibility of performing other operations, such as standard addition or automatic preparation of standards, and pH change by the addition of a reagent prior to extraction and after the formation reaction of the complex.

In the first application of MSA-DLLME reported by Horstkotte et al., a novel methodology for the determination of aluminum in seawater and freshwater samples using fluorescence detection was described [61]. The scope of the MSA-DLLME technique was rapidly extended to include the automation of analytic methods for the spectrophotometric determination of Cr(VI) [62], methylene blue active substances [63], cationic surfactants [64], uranium [65], and lead [66], in water samples. In addition, it was utilized for the efficient extraction of caffeine in coffee beverages [67]. The MSA-DLLME also enabled the automation of in-syringe single-drop

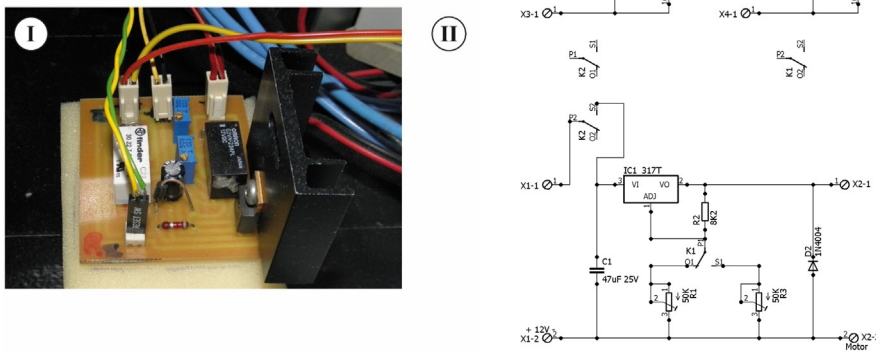

Fig. 25.29 (I) Photograph and (II) scheme of the circuit (from Eagle) to control the in-syringe stirring.

headspace microextraction for the determination of ethanol in wine [68] and the on-drop sensing of ammonia in water [69].

In-syringe MSA-DLLME was combined with chromatographic techniques, for example, for the automated extraction of estrogens [70], ultraviolet filters [71], and herbicides [72] in water followed by silylation and off-line GC-MS. It was also interfaced with high-performance liquid chromatography for the fully automated determination of UV filters in waters [71, 72]. The DLLME technique based on the solidification of the floating drop was automated by coupling MSA-DLLME to an automated Peltier cell handled by a programmer multiaxis robotic arm [72]. The in-syringe MSA-DLLME has also been combined with ETAAS [73] and inductively coupled plasma-optical emission spectrometry [74, 75] and combined with ETAAS in the headspace single-drop microextraction mode [76].

25.10 Software

Instrument control for the automatic flow techniques and the acquisition and processing of data is possible using the AutoAnalysis 5.0 program package, which is designed to offer the user a great versatility in the automation of analytic methods. AutoAnalysis was also developed on the Microsoft Windows platform (Win32) with three layers, namely, hardware (layer 1), instruments and communication channels (layer 2), and application (layer 3).

The application of the program AutoAnalysis involves the following stages:

25.10.1 Configuring the connected hardware

The user chooses the equipment and connections to be used and selects the most appropriate settings for the channels and instruments to be utilized. In the menu the configuration with the communication channels and instruments contained in the system is specified, as shown in Fig. 25.30.

The windows on the right contain the list of installable DLLs of the connection channels (earlier) and the installable equipment (later). In the left window appears the configuration for an MPFS system with conductometric detection, which also includes an automatic sampler in another serial channel of the computer.

25.10.2 Designing the analytic method to be used with the connected hardware

The programming tools are used to develop the most appropriate method via the sequence of operations and decisions to reach the expected results. In Fig. 25.31 the menu for editing the methods is shown. In addition to operations with

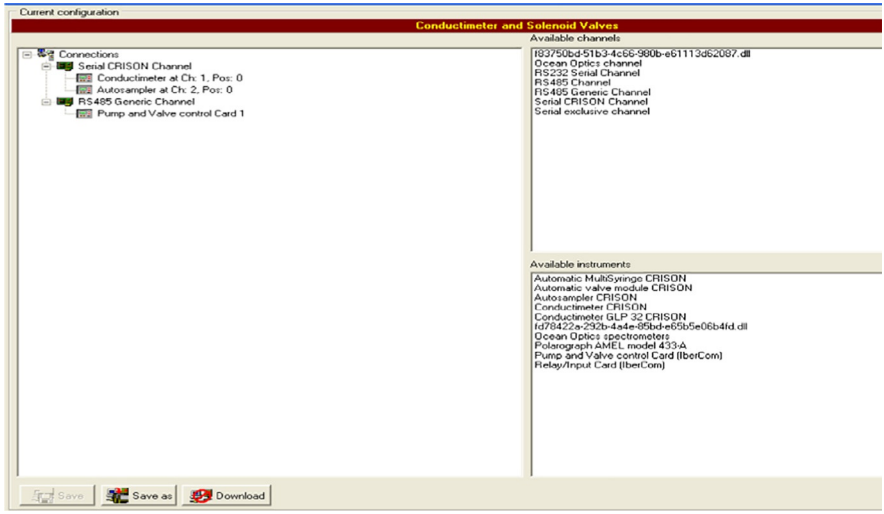

Fig. 25.30 AutoAnalysis menu for the configuration of the system hardware.

	Instrument	Command
52:	Empty instruction	
53:	Conditional	LavarBomba5: IF [CO2_NH3.Clean_Pump_A_CO2 = 1.0000] THEN
54:	Forms	Show form: «CO2_NH3»
55:	Conductimeter at Ch: 1, Pos: 0	Measure every 0.25 seconds.
56:	Pump and Valve control Card 1	P1[Stop] - P2[Stop] - P4[Stop] - P5[Stop] - P6[8.000mL,4.000mL/min,E] - V7[OFF]
57:	Conductimeter at Ch: 1, Pos: 0	Stop measure
58:	Change variable	CO2_NH3.Clean_Pump_A_CO2 = 0
59:	Forms	Hide form: «CO2_NH3»
60:	Conditional	LavarBomba5: ENDIF
61:	Empty instruction	
62:	Conditional	Medir NH4: IF [CO2_NH3.Measure_NH3 = 1.0000] THEN
63:	Forms	Show form: «CO2_NH3»
64:	Change variable	Sample = 5
65:	Change variable	Wait = 8
66:	Call procedure	Call "Medir NH3"
67:	Change variable	CO2_NH3.Measure_NH3 = 0
68:	Conditional	Medir NH4: ENDIF
69:	Empty instruction	
70:	Conditional	Medir CO2: IF [CO2_NH3.Measure_CO2 = 1.0000] THEN
71:	Forms	Show form: «CO2_NH3»
72:	Change variable	Sample = 5
73:	Change variable	Wait = 8
74:	Call procedure	Call "Medir CO2"
75:	Change variable	CO2_NH3.Measure_CO2 = 0
76:	Conditional	Medir CO2: ENDIF
77:	Forms	Hide form: «CO2_NH3»
78:	Loop	END: Repeat while CO2_NH3.End_Method = 0 repeat from Method
79:	Comment	

ods | Procedures | Editor | Execute

Fig. 25.31 Menu for editing a method with the AutoAnalysis 5.0 program.

instruments, the software offers its own programming operations such as the use of variables, conditional flow control and iterations, instructions for waiting times, comments on the method, or marks on the charts. These functions enhance the creation of new automatic methods, more versatile and complex and capable of making decisions. These advantages have allowed a considerable saving of time and a greater autonomy of the method by not requiring the presence of the analyst to change the conditions of one experiment to another. In AutoAnalysis, it is also possible to create methods that, once saved, can be loaded and executed from within another method. These "insertable" methods are known as "procedures." The procedures are very useful in long methods in which, from time to time, operations must be repeated. In addition, the same procedure can be used in different methods. The most commonly used procedures are system cleaning and measurement protocols. In any case the procedures simplify methods and facilitate their understanding and follow-up.

Variables may be programmed saving the value of the measured signal at the corresponding time with maximum of the signal. This use of variables is applicable when programming methods capable of making decisions. For example, you can program the method so that you decide to do another injection of the same sample when one of the three previous replicas is significantly different from the previous one or execute one procedure or another depending on the value of the height of the peak.

25.10.3 Executing method to realize expected commands and data capture

It executes commands previously programmed with related instrumentation. When measurement instruments are started, the corresponding data are acquired. During the execution of the AutoAnalysis methods, it records and presents the data in the execution window (Fig. 25.32). For optical measurements, AutoAnalysis also allows the simultaneous visualization of the evolution of the absorption spectrum of the solution analyzed. During a measurement, you can use tools, such as scale adjustment, magnified view, and insert a mark. You can also access a window that allows you to track the execution of the method's operations and see the values of the variables at that moment.

25.10.4 Data processing

After executing the analytic method, it is possible to perform a mathematical treatment of the acquired data, such as derivation, calibration, or spectral refining. In addition, data can be exported to be processed by other software (e.g., Excel).

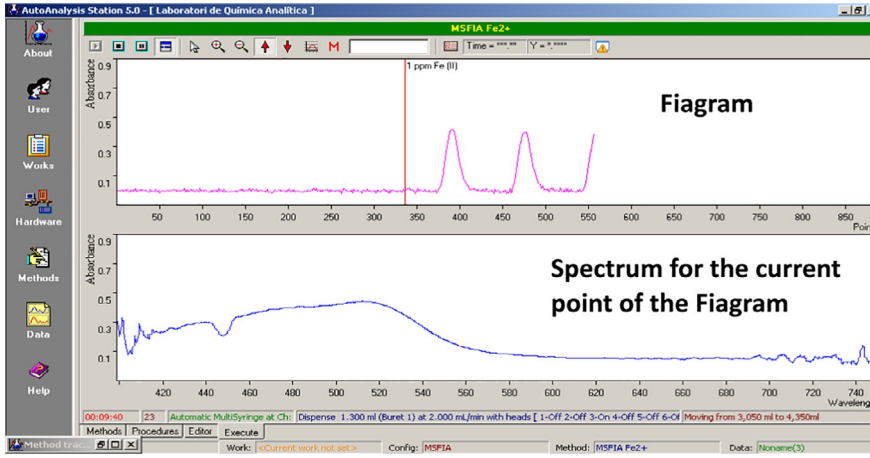

Fig. 25.32 Execution of a developed AutoAnalysis method using a CCD spectrophotometric detector. The upper figure is displaying the signal versus time. In the lower part, the spectrum obtained for the measurement of absorbance at a given moment is represented.

The program calculates the height and area of the peaks using the Savitzky-Golay method. Through a menu, you can choose the degree of polynomials and other parameters related to peak smoothing. The data processing panel is shown in Fig. 25.33. The height and peak area data are tabulated below the signal graph. Along with these values also appear the values of absolute height and the times of beginning, end, and maximum of the peak.

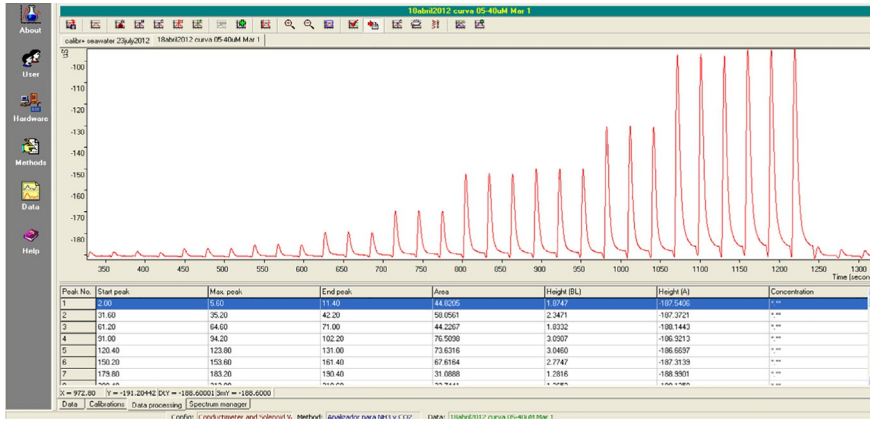

Fig. 25.33 Data processing panel of the AutoAnalysis program.

25.11 Conclusions

After the pioneering work in the automation of liquid-liquid extraction using FIA systems, improvements made exploring the different generations of flow techniques were described. These include the incorporation of multicommutation (MCFIA) or pulsed flows (MPFS) and the use of syringe-based flow techniques (SIA, MSFIA, and lab-in-a-syringe). With syringe-based flow techniques, a higher degree of automation has been achieved, including the full automation of complex liquid-phase microextraction techniques, such as dispersive liquid-liquid microextraction and headspace single-drop microextraction. In addition, the use of syringe-based flow techniques enabled the direct coupling of automated liquid-liquid extraction techniques to more sophisticated instruments, such as high-performance liquid chromatography, gas chromatography, or inductively coupled plasma-optical emission spectrometry increasing the selectivity and sensitivity of these methods. The automation of liquid-liquid extraction using flow analysis techniques enabled a reduction in the consumption of organic solvents, samples, and reagents, leading to the development of cost-effective and more environmentally friendly analytic methods.

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Further Reading

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