**LAB: Light Scattering Batch Experiment – Zimm Plots**

### Topics:

* Use of a MALS detector and flow cell for unfractionated samples (batch mode)
* Measure the ***weight average molar mass (Mw)***, the ***z-average root mean square radius (rms radius)***, and the ***second virial coefficient (A2)*** of a Dextran in aqueous solution.

### Goals:

* To become familiar with instrument and ASTRA software set-up
* Collection of batch data
* Optimization of fit parameters for generating a Zimm Plot for the analysis of unfractionated samples.

### What is a batch measurement?

* Measure weight average molar mass, z-average rms radius, and A2 of an ***unfractionated*** sample.

### When to use a batch measurement?

* No SEC method available (e.g. different column and/or different solvent needed)
* The column is suspected to alter the sample (*e.g.* high Mw polymers, adsorption or shearing of the sample on a column)
* To measure A2
* To determine equilibrium constant and kinetics
* To monitor a reaction

### How to perform a batch measurement?

* Measuring LS detector cells: **flow cell**, scintillation vial (HELEOS), microCuvette
* Need to know: dn/dc, concentration (can be also measured with RI or UV in series with LS detector)

### Experimental setup with a flow cell:

* Goal: Stable LS signal (plateau) with known corresponding concentrations
* Different setups:
  + **HPLC pump and a manual injector (500 – 1000 μL)** or
  + **Syringe pump and syringes**
  + Calypso system: two syringe pumps to deliver concentration gradients and online concentration measurement (more on that in a Scatter Session on Day 3)
  + On-line A2 determination (more on that in a Scatter Session on Day 3)
* Key point: Delivery of an air bubble-free and particle-free solution to the flow cell

### Specifics of this lab:

* Goal: Generation of a Zimm plot with at least 5 concentrations
* Use either syringe pump or HPLC pump with DNDC injection system (WISL), flow rate ~0.5 mL/min, exact flow rate not critical
* Collect baseline at start and end of the acquisition: aqueous solvent (100 mM NaNO3, 200 ppm NaN3 as preservative)
* Normalization standard (10 kDa Dextran at ~ 10 mg/mL)
* Five concentrations of sample (0.2– 2.0 mg/mL of high Mw Dextran sample)
* Plumbing of the tubing:
  + Reverse inlet and outlet plumbing to take advantage of the smaller diameter of the inlet tubing to pressurize the flow cell to keep air bubbles dissolved in solution.
* Filters:
  + 0.02 μm syringe tip filter for solvent
  + 0.2 μm for normalization standard
  + 0.45 μm for high molar mass dextran solutions
* Syringes:
  + For aqueous solvents – rubber tip syringes are okay as well as all polymer or glass
  + For organic solvents – do NOT use syringes containing plungers with a rubber tip as it will dissolve and contaminate your sample. Use all polymer or glass syringes, only!

### Topics:

* Setting up a syringe and a syringe pump (or Wyatt DNDC injector kit with an HPLC system) to pass solvent and samples through the DAWN or miniDAWN flow cell.
* Detecting air bubbles in the flow cell visually and by use of the forward laser monitor.
* Assessing the quality of flow cell cleanliness.
* Normalization using an isotropic scatterer (small molar mass polymer).
* Collection of data in ASTRA.
* Processing light scattering data and the generation of a Zimm Plot.
* Evaluation of the Zimm Plot.
* Selecting detector and concentration fit orders
* Deleting concentrations and/or detectors if needed
* Try different polynomial detector fit models (Debye, Zimm and Berry)

### Notes:

* A list with part numbers of supplies and equipment used in this session can be found in Section 14 of your LSU binder.
* An example ASTRA data file is located on your USB memory key in: **LSU Training Binder\04 - Flow Cell Batch Lab**

**ASTRA V Data Collection:**

***Open*** ASTRA V (current version) and ***Create*** a new experiment from a system template:

**File**

**New**

**Experiment from Template**

**System Templates**

**Light Scattering**

**Batch (Zimm plot)**

The default Light Scattering instrument is the HELEOS. To change to a miniDAWN TREOS (or EOS, miniDAWN Tristar, etc.):

Right-click on **Configuration**

**Replace Configuration**

**Example Configurations**

**Light Scattering**

**TREOS**

**LS Batch**

Click on the “**+**” sign next to your LS instrument (**HELEOS, TREOS, or other)** to expand the parameters, then double click to see the parameters in the dialog box that opens to the right of your workspace.

**HELEOS:**

Select **Physical Instrument** by clicking on “**…**”  
(Note: you may have to click on **Add** before you can **Select** your instrument, see Section 3 of this course binder)

**Sample Cell**: Fused Silica or K5 (for batch mode using the flow cell)

**Calibration Constant:** Enter correct value

(measured or from Certificate of Performance)

**OK**

**Solvent:**

**System Solvents:**  Water

**OK**

**Sample:**

**Dn/dc**: 0.138 mL/g

**OK**

***+* Procedures**

**Basic Collection**

**Duration:** 30 minutes

**Collection** **Interval:** 2 sec

**Apply**

**Run**

Start by collecting:

5 minutes of Baseline using filtered solvent

3-5 minutes 10 kDa Dextran

3-5 minutes of each concentration of the unknown Dextran in solvent (0.2 – 2.0 mg/mL)

Finish with original baseline solvent to complete the baseline.

The acquisition time cannot be extended during the run (ASTRA 5 cannot append data) However, you can increase the acquisition time by using the **Run Indefinitely** option if you find yourself running short of time (**Experiment \ Run Indefinitely**). Use **Stop Collection** to end the run when the data collection is complete.

**Basic collection window after the data acquisition:**



10 kDa dextran

unknown dextran

Baseline

**Important: Syringe-tip filters are required for batch mode light scattering operation!**

### ASTRA Experiment Data Processing

**Procedures**

**Baselines**

Define baseline for 90° detector

Click on **Autobaseline** to set baselines for all other LS detectors

Check the other detector baselines to verify correct baseline settings.

**OK**

**Define Peaks**

Click and drag on the 10 kDa Dextran plateau to select peak region for normalization

**OK**

**Configuration**  (top tool bar)

**Normalize**

Peak number: 1

Radius (rms): 6 nm

Click on **Normalize**

**OK**

Go back to **Peaks**

Delete peak 1 (only necessary for normalization, not for Zimm plot) by highlighting the peak on the graph or table and hit the “Delete” button on your keyboard.

Then define peaks for concentrations C1-C5 (see graph next page):

Ctrl + left mouse click and define a region to zoom in

Click and drag to define plateau

Ctrl + right mouse click to zoom back out

Enter the concentrations for each plateau, e.g.

C1 = 2.00 E-4 g/mL

C2 = 5.00 E-4

C3 = 1.00 E-3

C4 = 1.50 E-3

C5 = 2.00 E-3

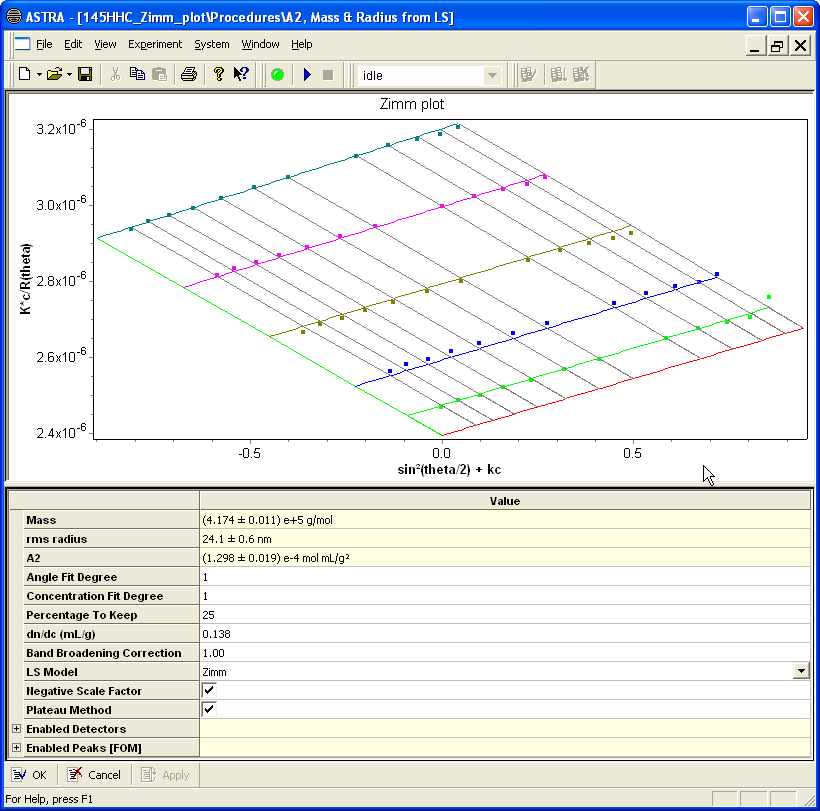
**OK**

**Select peak regions across the flat section of each individual plateau as shown below:**



***And then……***

Click on **Determine A2, Molar Mass and Radius from LS data** – voila!



**Molar mass:**

From extrapolation to zero angle and zero concentration

**rms radius:**

From slope of angular dependence

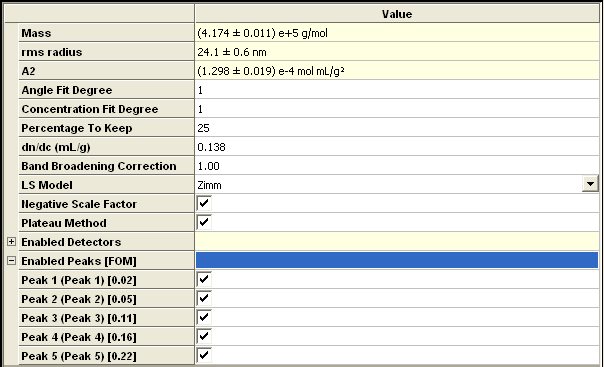
**A2:**

From slope of concentration dependence

**A few hints:**

* Make sure that **Plateau method** is checked and the value for *dn/dc* has been entered;
* Delete concentrations and detectors (by expanding the “+” sign of **Enabled Detectors** and **Enabled Peaks** to uncheck selected detectors and concentrations), if necessary;
* Generally, a ***Negative Scale Factor*** **k** (checked as shown above) makes the data easier to view.

**Figure of Merit (FOM)**



* The *Figure of Merit* indicates the fraction of the scattered light that originating from the *2A2Mc* term in the light scattering equation. A FOM of 0.05 means that 5% of the signal is due to the contribution of the *2A2Mc* term.
* If the FOM is very low (e.g. 0.05 or less) for the highest concentration measured, the determination of A2 will not be very precise. However, molar mass and rms radius (if greater than 10 nm) can still be determined precisely.
* If the FOM is very high (in the order of 1.0) higher order terms (the third virial coefficient) of the LS equation need to be included in the calculations.