Macromolecules

A Molecular Thermodynamic Model of Complexation in Mixtures of Oppositely Charged Polyelectrolytes with Explicit Account of Charge Association/Dissociation

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S Supporting Information

ABSTRACT: Into an extended Voorn–Overbeek (EVO) free energy model of polyelectrolyte (PE) complexation and phase behavior, we incorporate three classes of short-ranged electrostatic effects, namely counterion association–dissociation, cross-chain ion pairing (IP), and charge regulation by treating each as a reversible chemical reaction leading to a corresponding law of mass action in a self-consistent fashion. The importance of each reaction is controlled by a corresponding chemistrydependent standard free energy input parameter. Our model also accounts for Born (or ion solvation) energy using a linear mixing rule for the effective dielectric constant. In monophasic systems, the proposed model can qualitatively explain the observed shifts in acidity and basicity observed in potentiometric titration of weak PEs in the presence of salt and oppositely charged PEs in accordance with Le Châtelier's principle. We



demonstrate how a competition between counterion condensation (CC) and IP alone can explain the complex coacervation of strongly charged PEs as well as the existence of a critical salt concentration. Binodal diagrams predicted in our model are also affected by long-ranged electrostatics and are most sensitive to IP strength both for weak and strong PEs. The extent of IP increases in the dense phase at the expense of reduced CC upon coacervation consistent with counter release view of complex coacervation. We compare binodal diagrams predicted by our model against experimental data for both weakly and strongly dissociating polyions pairs and find a plausible parameter set that leads to an acceptable and partial agreement with experiments in the two cases, respectively.

1. INTRODUCTION

Mixtures of oppositely charged macromolecules, i.e. polyelectrolytes (PEs), undergo associative phase separation, yielding inter-PE complexes at the meso scale or a dense liquid-like coacervate or solid-like precipitate at the macro scale, depending on the physiochemical conditions, e.g., monomer concentration, pH, ionic strength, and temperature. PE complexation (PEC) has proven to be a versatile means of assembling materials useful in numerous applications due to the relative ease of fabrication as well as wide variety of polymer chemistries of both biological and synthetic origin that can be employed. In fact, charge-driven assemblies have been historically invoked in origin of life theories. Promising biomedical applications of PEC have emerged over the past two decades in areas that include DNA condensation for gene delivery,^{1,2} nonviral gene therapy,^{3–6} delivery of various biotherapeutics,^{7–12} cell encapsulation^{13–15} and culture,¹⁶ and tissue engineering,¹⁷ as well as additives in the cosmetics and food industries.^{18,19} Sharing many features and properties with PE complexes, PE multilayer films provide another venue for practical applications in other areas.²⁰

Despite the rapidly growing applications, modeling of PEC has been slow to keep up. This is in large part due to highly correlated nature of unlike charges in the complex as well as a significance of chemistry specificity.²¹ In particular, functional groups along

oppositely charged PEs can bind together to form interchain ionic cross-links or "ion-pairs", the strength of which is strongly dependent on the chemistry of the chains involved. Cross-chain ion-pairs, referred to as simply "ion-pairs" hereafter, impart viscoelasticity to complex coacervates with their viscosity and storage modulus shown to decrease steadily as ion-pairs diminish with addition of salt.²²⁻²⁴ Counterion activity in single-PE solutions deviates strongly from ideal behavior²⁵ resulting from localization (condensation) of counterions along polymer chains,²⁶ lowering the effective chain charge density and mobility of counterions. "Counterion condensation" (CC) is chiefly controlled by a competition between loss of counterion translational entropy and monomer electrostatic repulsion²⁷ as well as gain in dipole polarization energy upon CC, the magnitude of which again depends on the physiochemistry of counterions and PE.²⁸ Throughout this work, the term "ionpairing" or "ion-pair" (IP) refers to binding of charges on oppositely charged PEs, while "counterion condensation" (CC) refers to binding of a small salt ion to an oppositely charged PE.

Received:July 7, 2016Revised:November 19, 2016Published:December 14, 2016

In the framework of the classical Voorn-Overbeek (VO) model proposed in 1957,²⁹ complex coacervation is driven primarily by the small translational entropy of polymer chains and the reduction in electrostatic energy as described by the Debye-Hückel (DH) theory of simple electrolytes. The VO model neglects finite ion sizes, chain connectivity, and the highly correlated nature of opposite charges at short length scales present in a complex coacervate, including CC and IP, and thus much of the chemistry specificity of the components involved cannot be accommodated. Furthermore, charge densities of either PE chain type remain constant in the VO model, thus neglecting the charge regulation effects induced by the local environment especially in weakly dissociating PEs. Despite its clear shortcomings, the predictions of the VO model can be brought into good qualitative^{30,31} and quantitative³² agreement with experiments by the liberal use of adjustable parameters and a possible fortuitous cancellation of competing neglected effects, the nature of which is poorly understood.³³

We refer the reader to a recent review for a detailed discussion of the extensions to VO model and modern developments in theory of complex coacervation.³³ Chain connectivity effects missing in the VO model have been addressed utilizing the random phase approximation (RPA) for ideal chains, the accuracy of which is limited to weak and long-ranged fluctuations and low charge densities. Chain connectivity has been recently evaluated explicitly at the Gaussian level in the high wavevector limit and shown to alter the long-ranged electrostatics dramatically at low salt³⁴ compared to DH expression derived for simple salts, but CC, IP, and charge regulation were not considered in this work. The short-range electrostatic correlations can be described by treating CC as a reversible chemical reaction. This has been done both for CC in single-PE solutions^{35,36} and for IP in oppositely charged PE mixtures.^{3'} We note that in their model Jha et al. accounted for the size mismatch between components as well as the salt-induced ionization of PEs.⁴⁰ They did so by using experimentally obtained acidity constants pK_A 's treated as explicit functions of salinity. They, however, did not consider CC or IP.

The VO model and subsequent extensions discussed above are based on the assumption that PE complexation is driven by longranged electrostatic field fluctuations of freely interacting charges. However, simulation and experimental studies^{41,4} explain PE complexation in terms of counterion release (CR) whereby PEs shed their bound counterions while forming IP's with the oppositely charged PE. Long-ranged electrostatic fluctuations are largely irrelevant in the CR mechanism since bound charges are practically free charges removed from the system. Following the work of Semenov and Rubinstein on associative polymers,⁴³ a molecular thermodynamic model has been proposed⁴⁴ for cross-associating polymers such as oppositely charged PEs undergoing IP, but this theory did not address long-ranged electrostatics, the effect of salt counterions, CC, and charge regulation. A unified theory of PE complex coacervation reconciling the two aforementioned views is still lacking. Moreover, both IP and CC as well as charge regulation have not been considered simultaneously in the context of PE complex coacervation, to the best of our knowledge.

Using liquid state (LS) integral theory, Perry and Sing recently demonstrated that coacervate formation is enhanced while CR intensifies as chains become more rigid, promoting strong correlations between oppositely charged groups.⁴⁵ Field-theoretic methods, LS integral equation methods, and their hybrid variants are sophisticated, yet they do not provide closed-

form expressions for thermodynamic function explicitly in terms of macroscopic variables for free energy. Closed-form thermodynamic functions that are mathematically tractable are desirable for the development of advanced transport models of PE multilayer buildup, where derivatives of free energy needed to be calculated sufficiently fast to obtain driving forces.⁴⁶

Inspired by a growing body of calorimetric and potentiometric titration studies on the complexation reaction in various systems,⁴⁷⁻⁴⁹ we propose a molecular model yielding closedform free energy expressions for a solvated mixture of PEs and ions, which incorporates short-ranged electrostatic interaction, namely CC, IP, and, in the case of weakly dissociating PEs, charge regulation effects on protonation. Not only do the preceding charge association phenomena need to be incorporated in a unified theory of complex coacervation to allow for consistency with thermodynamic measurements, we demonstrate in this work that they provide a picture of PE complex coacervation that can be driven entirely by CR, i.e., a competition between IP and CC. The paper is organized as follows: in the second section, we lay out the model. In the third, we first demonstrate that our model captures the charge regulation effects observed experimentally in potentiometric titration of single-phase systems. Later in the third section, we consider phase separation of both strongly and weakly dissociating oppositely charged PEs and compare our model predictions to experimental phase diagrams available for two such systems. Finally, in the fourth section, we summarize and draw conclusions.

2. THEORY

We consider here a system comprising polycation and polyanion chains denoted by "C" and "A", respectively, counterions accompanying the chains, co-ions introduced by added salt, and the water molecules, represented by "W". For simplicity, we assume counterions and co-ions of like charge are of the same chemical identity so that we refer to them collectively as counterions hereafter. All ions are monovalent and the positive and negative simple ions are designated by "+" and "–" symbols. Further, we take both PEs to be weakly dissociating and monoprotic. We shall demonstrate that strong dissociation is a limiting case of weakly dissociating PEs, and we thus proceed assuming the latter for generality. We consider four additive contributions to the normalized Helmholtz free energy density f, each of which is described separately:

$$f \equiv \frac{l^3 F}{V k_{\rm B} T} = f^{\rm EVO} + f^{\rm C} + f^{\infty} + f^{\rm B}$$
(1)

Here *l* denotes the cubic root of the volume of water molecules ~0.31 nm taken as the reference length scale and *V* is the solution volume. The first contribution is here called the "extended VO" (EVO) free energy density $f^{\rm EVO}$, given by eq 2. It includes the translational entropy of components and long-ranged electrostatic field fluctuations (electrostatic screening) contained in the original VO theory,²⁹ although the electrostatic term is slightly improved from the lowest order expression of Voorn and Overbeek, as suggested by by Jha et al.⁴⁰ In addition, the EVO free energy includes the short-ranged van der Waals (VdW) interactions suggested by Veis⁵⁰ and Spruijt et al.³⁰ Following Jha et al.⁴⁰ the translational entropy of component "*i*" given in terms of volume fractions, ϕ_{ij} is inversely weighted by ω_{ij} the ratio of the corresponding molecular volume to that of solvent.

 N_i represents the polymerization index of *i* in eq 2 and is taken to be unity for all nonpolymeric components; ω_i for a polymeric species is thus the ratio of the volume of the monomer to the volume of a water molecule. Short-ranged VdW interactions f^e are quantified by Flory-type interaction parameter χ_{ii} between species. The third term in eq 2, f^{DH} , is the extended DH free energy expression employed by Jha et al.40 that captures electrostatic field fluctuations for a constellation of charges with a mean volume of $\overline{\omega}l^3$ where any explicit consideration of the connectivity effects is neglected. We simply retain the DH expression for long-ranged (screening) electrostatic free energy in the EVO model. (Connectivity can be encompassed implicitly in our model, however, through fits of standard state free energies or equilibrium constants, as will become apparent in what follows.) The nondimensional reciprocal Debye length denoted by $\overline{\kappa}$ is

$$\bar{\kappa}^2 \equiv \kappa^2 l^2 \bar{\omega}^{2/3} = \frac{4\pi l_B \bar{\omega}^{2/3}}{l} \sum_{i=A,C,\pm,OH,H_3O} \frac{\sigma_i \phi_i}{\omega_i}$$
(3)

The charge density σ_i of each ionic species is unity except for the PE monomers, where σ_i is the fraction of monomers that carry an unpaired charge and is controlled by the extent of ionization (which is $1 - \alpha_{AH}$ for polyanions or α_{CH} for polycations), and by CC, as well by as the fraction of bare charges paired with PE charges of opposite sign, given by eqs 4 and 5.

$$\sigma_{\rm A} = f_{\rm A} (1 - \beta_{\rm A}) = (1 - \alpha_{\rm AH})(1 - \alpha_{\rm A+})(1 - \beta_{\rm A})$$
(4)

$$\sigma_{\rm C} = f_{\rm C} (1 - \beta_{\rm C}) = \alpha_{\rm CH} (1 - \alpha_{\rm CH-}) (1 - \beta_{\rm C})$$
(5)

Here f_i denotes the overall fraction of repeat units of *i* available for IP. At equilibrium, a portion of the simple ions condense along PEs, losing their translational entropy in the process. Only free simple ions are active in electrostatic screening and thus contribute to the reciprocal Debye length. When chains become sufficiently charged, short-ranged electrostatic effects become important, and a portion of the simple ions as well as the PE charges become highly localized along chains of opposite charge to form electrostatic dipoles and multipoles. We assume that all the bare charges, condensed simple ions, and ion-pairs follow an annealed distribution along the chains such that they break up and re-form in a dynamic equilibrium (see Figure 1). Therefore, any given polyanion site could be protonated with probability α_{AH} , which is the fraction of polyanion sites that are protonated. A deprotonated PE group could be carrying a condensed counterion; the probability that a PE site is both deprotonated and carrying a counterion is thus $(1 - \alpha_{AH})\alpha_{A+}$. Also, there is a probability β_A that a bare deprotonated polyanion group forms an ion-pair with a polycation monomer. Similarly, there is a probability $\alpha_{\rm CH}$ that a given polycation site is protonated and thus charged while the probability of finding a site that is both protonated and carrying a condensed counterion is $\alpha_{CH}\alpha_{CH-}$. Note that in this subscript notation the "-" stands for the negatively charged condensed ion and does not indicate a charge on the polymer. The subscript "CH" means that a proton is bound to the neutral cationic monomer "C". In this notation, we



Figure 1. Definitions of extents of protonation of repeat units, CC (with counterions chosen to be potassium and chloride only for demonstration), and IP for two representative chains. Not shown are freely floating counterions, hydroxyl, hydronium ions, and water. The dashed ellipses represent ion-pairs. N_A and N_C denote the total number of repeat units.

truncate the "+" from the "H+", since we use the symbol "+" to designate the cationic salt ion. Lastly, there is a probability $\beta_{\rm C}$ that a bare protonated polycation group forms an ion-pair. Association and complexation are illustrated schematically in Figure 1.

In general, the charge densities σ_i 's, given by eqs 4 and 5 in our model, are functions of pH, salinity, and oppositely charged PE concentration. The charge densities of PEs in our model are not known a priori and need to be obtained self-consistently as explained later in this section. This stands in sharp contrast to the original VO model where the charge densities need to be prescribed. Jha et al.⁴⁰ treated PE charge densities as explicit functions of salinity and pH but ignored CC and role of oppositely charged PE (IP). Conservation of total ions yields two relations between the total volume fraction of the counterions, which are the parameters in eqs 6 and 7 superscripted with "o", and the free and bounds ions.

$$\phi_{+}^{o} = \phi_{+} + \phi_{A}(1 - \alpha_{AH})\alpha_{A+}\left(\frac{\omega_{+}}{\omega_{A}}\right)$$
(6)

$$\phi_{-}^{\circ} = \phi_{-} + \phi_{\rm C} \alpha_{\rm CH} \alpha_{\rm CH-} \left(\frac{\omega_{-}}{\omega_{\rm C}}\right) \tag{7}$$

Here we assume a 1:1 stoichiometry in IP, which is plausible for most systems, ⁴⁷ even though deviations from a 1:1 stoichiometry have been observed in calorimetric measurements.^{49,51} The 1:1 stoichiometry of IP between two oppositely charged PEs affords the following constraint, relating the extents of association/ dissociation.

$$\phi_{\rm C}\alpha_{\rm CH}(1-\alpha_{\rm CH-})\beta_{\rm C}\omega_{\rm A} = \phi_{\rm A}(1-\alpha_{\rm AH})(1-\alpha_{\rm A+})\beta_{\rm A}\omega_{\rm C}$$
(8)

The remaining contributions in eq 1, which we append to the EVO model, are respectively (I) the combinatorial entropy associated with the distribution of ion-pairs and condensed simple ions along the chains deriving from the short-ranged electrostatic correlations, (II) changes in free energy due to association/dissociation phenomena modeled here as reversible chemical reactions arising from strong short-ranged electrostatic correlations with the exception of water self-dissociation, and

(III) Born (solvation) free energy of ions. Note that we have also included the hydronium and hydroxyl ions volumes in eq 2, the sizes of which are taken to be equal to that of water. These species are included to allow for charge regulation effects. The chemical identities of functional groups and counterions change upon association/dissociation of ions, described by six linearly independent reversible reactions: protonation/deprotonation of each PE (eqs 9 and 10), CC on each PE (eqs 11 and 12), IP (eq 13), and water self-dissociation (eq 14), which is needed for completeness.

$$AH + H_2 O \stackrel{\Delta G_{AH}^{\infty}}{\longleftrightarrow} H_3 O + A \tag{9}$$

$$C + H_2 O \stackrel{\Delta G_{CH}^{\infty}}{\longleftrightarrow} OH + CH$$
(10)

$$(A+) \stackrel{\Delta G^{\infty}_{A+}}{\longleftrightarrow} A + (+) \tag{11}$$

$$(CH-) \stackrel{\Delta G^{\infty}_{CH-}}{\longleftrightarrow} CH + (-)$$
(12)

$$A + CH \stackrel{\Delta G_{p}^{\infty}}{\Longleftrightarrow} CHA \tag{13}$$

$$2H_2O \Leftrightarrow^{\Delta G_w} H_3O + OH$$
 (14)

Note that the symbols used for the charged reactants and products in eqs 9-14 do not indicate the charge that they may possess; for instance, a bare deprotonated PE repeat unit, denoted by "A", carries a negative charge while a protonated polycation monomer, denoted "CH", is positively charged, despite the lack of any explicit indication of their charges on these symbols. As remarked earlier, the simple salt cations and anions are represented simply by "+" and "-", respectively. Oppositely charged counterions or PE groups can neutralize the bare charged groups along the chains, thus forming monomer-simple ion dipoles, (A+) and (CH-) (which are neutral), or neutral monomer-monomer ion-pairs, CHA. The quantities appearing over the double-sided arrows in eqs 9-14 denote the standard free energy change in $k_{\rm B}T$ units associated with the corresponding reactions. The standard free energies of all reactions are specified at a temperature T and in the infinite dilution limit, i.e., in the limit of $\phi_{\rm w} \rightarrow 1$, and hence are denoted by the superscript " ∞ " hereafter. In the latter limit, ions are solvated by pure water, and the dielectric constant and Bjerrum length tend to ϵ_r^w and l_B^w , respectively. The contribution to free energy due to chemical reactions, f^{∞} , is the sum of the standard free energies of the association/dissociation equilibria each weighted by the appropriate reaction extents and the normalized concentrations:

$$f^{\infty} = \left(\phi_{\rm H_{3}O} + \frac{\alpha_{\rm AH}\phi_{\rm A}}{\omega_{\rm A}}\right) \Delta G_{\rm w}^{\infty} + \frac{\alpha_{\rm CH}\phi_{\rm C}}{\omega_{\rm C}} \Delta G_{\rm CH}^{\infty}$$
$$- \frac{\alpha_{\rm AH}\phi_{\rm A}}{\omega_{\rm A}} \Delta G_{\rm AH}^{\infty} - \frac{\alpha_{\rm CH}\alpha_{\rm CH-}\phi_{\rm C}}{\omega_{\rm C}} \Delta G_{\rm CH-}^{\infty}$$
$$- \frac{(1 - \alpha_{\rm AH})\alpha_{\rm A+}\phi_{\rm A}}{\omega_{\rm A}} \Delta G_{\rm A+}^{\infty} + \frac{\alpha_{\rm CH}(1 - \alpha_{\rm CH-})\beta_{\rm C}\phi_{\rm C}}{\omega_{\rm C}} \Delta G_{\rm ip}^{\infty}$$
(15)

Note that the standard free energies do not include the translational entropy of reactants and products as they have already been accounted for in $f^{\rm EVO}$. The standard free energies in the infinite dilution limit used above are conceptually equivalent

to bond energy and dielectric mismatch parameters introduced by Semenov and Rubinstein⁴³ and Muthukumar,⁵² respectively. They should be in principle independent of composition, provided that the model captures all other contributions to the total free energy adequately. However, they can very well depend on the extents of association/dissociation equilibria due to the mean-field treatment employed here wherein all monomers are treated as if disconnected. (The effect of the connectivity could be captured phenomenologically in the standard free energies by allowing them to depend on polymer molecular weight, for example.) In the Supporting Information, we demonstrate that the front factor in the first term of eq 15 may be written equivalently in terms of hydroxyl volume fraction and α_{CH} , using electroneutrality and incompressibility conditions.

The combinatorial entropy associated with the short-ranged correlations along PE chain are treated in a mean-field fashion wherein the connectivity of the chains is neglected, following the earlier works on self-associating⁴³ and cross-associating polymers.⁴⁴ In the Supporting Information, we demonstrate that combinatorial free energy $f^{\rm C}$ is obtained as the sum of five contributions each associated with one of the five association/ dissociation equilibria represented by eqs 9–13, given by eq 16.

$$f^{\rm C} = -\frac{l^3 \ln Z}{V} = f_{\rm AH} + f_{\rm CH} + f_{\rm A+} + f_{\rm CH-} + f_{\rm ip}$$
(16)

The first two terms on the right side of eq 16 are entropic contributions of protonation/deprotonation equilibria, eqs 9 and 10, given by eqs 17 and 18.

$$f_{\rm AH} = \frac{\phi_{\rm A}}{\omega_{\rm A}} [\alpha_{\rm AH} \ln \alpha_{\rm AH} + (1 - \alpha_{\rm AH}) \ln(1 - \alpha_{\rm AH})]$$
(17)

$$f_{\rm CH} = \frac{\phi_{\rm C}}{\omega_{\rm C}} [\alpha_{\rm CH} \ln \alpha_{\rm CH} + (1 - \alpha_{\rm CH}) \ln(1 - \alpha_{\rm CH})]$$
(18)

The third and fourth terms on the right-hand side of eq 16 arise from entropic free energy associated with the CC equilibria along polyanions and polycation chains, respectively, represented by eqs 19 and 20.

$$f_{A+} = \frac{\phi_{A}}{\omega_{A}} (1 - \alpha_{AH}) [\alpha_{A+} \ln \alpha_{A+} + (1 - \alpha_{A+})]$$
$$\ln(1 - \alpha_{A+})]$$
(19)

$$f_{\rm CH-} = \frac{\phi_{\rm C} \alpha_{\rm CH}}{\omega_{\rm C}} [\alpha_{\rm CH-} \ln \alpha_{\rm CH-} + (1 - \alpha_{\rm CH-}) \\ \ln(1 - \alpha_{\rm CH-})]$$
(20)

The last contribution in eq 16 derives from the distribution of ion-pairs along chains of both types, given by eq 21, where the last of the three terms, the one with a negative sign, derives from the IP bond probability (see Supporting Information) favoring phase separation. (Note that the stoichiometric constraint, eq 8, would also allow eq 21 to be written in terms of the concentration of polyanion ϕ_A rather than polycation ϕ_C .)

$$\begin{split} f_{\rm ip} &= (1 - \alpha_{\rm AH})(1 - \alpha_{\rm A+})[\beta_{\rm A} \ln \beta_{\rm A} + (1 - \beta_{\rm A}) \\ &\ln(1 - \beta_{\rm A})] + \frac{\phi_{\rm C}\alpha_{\rm CH}}{\omega_{\rm C}}(1 - \alpha_{\rm CH-})[\beta_{\rm C} \ln \beta_{\rm C} + (1 - \beta_{\rm C}) \\ &\ln(1 - \beta_{\rm C})] - \frac{\phi_{\rm C}\alpha_{\rm CH}}{\omega_{\rm C}}(1 - \alpha_{\rm CH-})\beta_{\rm C} \\ &\ln\left[\phi_{\rm C}\alpha_{\rm CH}(1 - \alpha_{\rm CH-})\beta_{\rm C}\left(\frac{\omega_{\rm A} + \omega_{\rm C}}{\omega_{\rm C}}\right)\right] \end{split}$$
(21)

The Born (self- or solvation) energy⁵³ of all ions f^{B} is simply the sum of the individual contributions of each ionic species. However, only the deviations of Born energy from that in the infinite-dilution limit need to be considered here. Again, the condensed counterions do not contribute to f^{B} as given by eq 22.

$$f^{\rm B} = \frac{l_{\rm B} - l_{\rm B}^{w}}{2l} \sum_{i={\rm A,C,\pm,OH,H_3O}} \frac{\sigma_i \phi_i}{\omega_i^{4/3}}$$
(22)

The effective Bjerrum length, $l_{\rm B} \equiv (e^2/4\pi\epsilon_{\rm r}\epsilon_0 k_{\rm B}T)$, is lower in the polymer-rich phase, thus increasing the propensity of counterions to adsorb onto oppositely charged sites along the chains.^{52,54} Here *e* is the elementary charge, $k_{\rm B}$ is the Boltzmann constant, *T* is the absolute temperature, and ϵ_0 is the vacuum permittivity. Both PEs are assumed to have similar dielectric constants $\epsilon_{\rm r}^{\rm P} = 3$ and that the relative dielectric constant of all other components is equal to that of water $\epsilon_{\rm r}^{\rm w} = 78$, and we further use a linear volumetric average to evaluate the effective dielectric constant in each phase, eq 23 for simplicity.

$$\epsilon_{\rm r} = \epsilon_{\rm r}^{\rm w} + (\epsilon_{\rm r}^{\rm P} - \epsilon_{\rm r}^{\rm w})(\phi_{\rm A} + \phi_{\rm C})$$
⁽²³⁾

Note that eq 23 is only intended to capture the composition dependence of the dielectric constant qualitatively. The remaining six equations needed to close the model mathematically are obtained by setting to zero the variation of Helmholtz free energy at constant volume with respect to six independent degrees of freedom, namely $\phi_{H_{3}O}$, α_{AH} , α_{CH} , α_{A+} , α_{CH-} , and β_C , subject to the constraints introduced by eqs 6–8, and electroneutrality and incompressibility given respectively by eqs 24 and 25. The resulting six laws of mass action (LMA) are listed in the Appendix. Electroneutrality allows us to eliminate the hydroxyl volume fraction throughout.

$$0 = \phi_{\rm H_{3}O} - \phi_{\rm OH} + \frac{\alpha_{\rm CH}\phi_{\rm C}}{\omega_{\rm C}} - \frac{(1 - \alpha_{\rm AH})\phi_{\rm A}}{\omega_{\rm A}} + \frac{\phi_{\rm +}^{\rm o}}{\omega_{\rm +}} - \frac{\phi_{\rm -}^{\rm o}}{\omega_{\rm -}}$$
(24)

$$1 = \sum_{i} \phi_{i} = \phi_{W} + \phi_{+}^{o} \left(\frac{\omega_{+} + 1}{\omega_{+}} \right) + \phi_{-}^{o} \left(\frac{\omega_{-} - 1}{\omega_{-}} \right)$$
$$+ \phi_{A} \left(\frac{\omega_{A} + \alpha_{AH} - 1}{\omega_{A}} \right) + \phi_{C} \left(\frac{\omega_{C} + \alpha_{CH}}{\omega_{C}} \right) + 2\phi_{H_{3}O}$$
(25)

The composition of free counterions appears in the LMA equations corresponding to CC equilibria, given in the Appendix as eqs A-4 and A-5, and is related to the total counterion composition introduced through the conservation constraints, eqs 6 and 7. It can be readily verified that in the limiting case of a strongly dissociating polyanion, for sufficiently large K_A in eq A-2, α_{AH} tends to zero asymptotically such that f_{AH} vanishes and the term proportional to ΔG_{AH}^{∞} in eq 15 becomes linear in

composition and hence thermodynamically inconsequential. A similar argument can be made for a strongly dissociating polycation. Therefore, our model applies to both weakly and strongly dissociating PEs.

Since the main thrust of our model is the role of short-ranged electrostatics in driving complex coacervation, we have adopted the DH expression for long-ranged (screening) electrostatic free energy. Indeed, if CC and IP are proven to be the primary driving force for complex coacervation in some systems, a majority of charges could be neutralized by an opposite charge and thus do not contribute to long-ranged electrostatic fluctuations whether or not connectivity is considered. In our model, variations in conformational entropy of chains upon complex coacervation are not considered. This might be reasonable given that experimental SANS observations have confirmed that complex coacervation does not lead to a drastic stiffening or collapse of the chains, which continue to adopt nearly ideal Gaussian conformations over a range of molecular weight even in the coacervate phase.^{55,56} we have also neglected the dipole-dipole electrostatic interactions as they can be somewhat captured through VdW interactions by using appropriate FH parameters in f'. In fact, Muthukumar demonstrated that interactions of freely rotating dipoles mainly in single-PE solutions act to lower the effective short-ranged excluded volume interaction parameter.⁵² Lastly, our model does not accommodate inter-PE colloidal particles that can be formed in supernatant phases.

3. RESULTS AND DISCUSSION

As a direct consequence of the incompressibility assumption, eq 25, the partial molar volume of each species is a constant and is equal to the corresponding molecular volume. Therefore, the molar concentration C_i is related to volume fractions according to eq 26:

$$\phi_{i} = \frac{C_{i}\omega_{i}l^{3}N_{Avo}}{10^{24}}$$
(26)

where N_{Avo} is Avogadro's number and the characteristic length l = 0.31 nm. For convenience, we employ the $p[\cdot]$ function commonly used in analytical chemistry to quantify protonation and CC equilibrium constants of PEs expressed in (mol/L), as follows:

$$p[\cdot] \equiv -\log\left(\frac{10^{24}}{l^3 N_{\rm Avo}}[\cdot]\right)$$
(27)

The term $10^{24}/l^3 N_{Avo} \sim 55.56 \text{ mol/L}$ in the logarithm converts the nondimensional equilibrium constants in our model into ones expressed in mol/L. Here the factor 10^{24} is the conversion factor from nm³ into L. Instead of standard free energies, the equilibrium constants of protonation/deprotonation equilibria as well as CC reactions in the infinite dilution limit are chosen as the main parameters and expressed using the $p[\cdot]$ function in this section. This choice of parameter representation is intended to connect the model parameters to the conventional terminology for reporting the ionization equilibria of weak PEs. Conversion between free energies and equilibrium constants at the infinitedilution limit can be accomplished unambiguously using eqs A-1 to A-6 in the Appendix.

We first demonstrate that charge regulation, missing from all earlier models, follows naturally from our model by solving LMA equations even in single-phase PE mixtures such as multilayer films or vesicles. Moreover, the next section serves as an illustration of how equilibrium constants of weak PE employed in

Macromolecules

our model could be extracted from fairly straightforward potentiometric titration of weak PEs and subsequently used in phase behavior predictions.

3.1. Charge Regulation in Single-Phase Mixtures. Unlike the corresponding low molecular weight monomers, PE chains become progressively harder to ionize as the extent of ionization increases, 37-59 such that acidity/basicity of sufficiently long PEs cannot be characterized by a single ionization constant, K_A , for a polyanion chain, e.g., poly(acrylic acid), PAA, or K_C for polycations, e.g., poly(2-dimethylaminoethyl methacrylate), PDMAEMA. Experiments and theoretical studies have demonstrated that the apparent ionization constant for PAA and other weakly dissociating PEs in solution increase almost linearly as the extent of ionization of the chain increases, eq 28, implying that the chains become increasingly harder to ionize as charge fraction increases, relative to what is predicted by the Henderson-Haselbalch (HH) theory. The mean apparent ionization constant, denoted by the overbar in eq 28, can be simply taken as the pH at the halfway equivalence point, i.e., the point at which half the charges on the chain are neutralized.

$$pK_A^{\infty} = \overline{pK_A} + A(\gamma_A - 0.5)$$
(28)

The variable γ_A is here equal to the polyanion ionization fraction f_A . (For other cases discussed later, we take it to be the net fraction σ_A of unpaired charges on the polyanion.) The prefactor A in eq 28 is generally a function of salinity and decreases at higher salt. The preceding equation is valid if no other PE is present. However, it is plausible to expect a similar effect of ionization on the effective infinite dilution pK's in the presence of oppositely charged PEs. A crude and yet intuitive approach to capture the aforementioned effect involves setting $\gamma_A = \sigma_{A'}$ as discussed shortly.

Figure 2A compares our simulation results for a single-phase system to experimentally obtained titration results for a stoichiometric mixture of PAA/poly(diallyldimethylammonium chloride), PDADMAC. The experimental results, given by symbols in Figure 2A, measured the deprotonation degree of PAA in multilayer films⁶⁰ and in capsules as well as in inter-PE complexes⁶¹ exposed to a salt-free pH-adjusted bath. In the limit of zero IP constant, our result coincides with the experimental result for salt-free pure PAA films and complexes, given by open symbols in Figures 2A and 2C. These clearly do not conform to the dashed curve in Figure 2A, i.e., the prediction of the classical HH formula with a constant $pK_A^{\infty} = \overline{pK_A} = 6.5$ corresponding to the pH at the halfway equivalence point. Since the systems studied in Figure 2 are salt-free, we have neglected electrostatic screening effects. Also, we have absorbed the Born solvation contribution, i.e., the third term in the argument of the exponential function in eq A-2, with dissociation free energy of PAA, ΔG^{∞}_{AH} , amounting to using a constant p K^{∞}_{A} so that the Born free energy term is not explicitly considered, since the composition, and hence the dielectric constant, can be taken to be a constant in a single phase.

PDADMAC is a strong polycation with $\alpha_{CH} = 1$ irrespective of pH. The prefactor A = 2.5 and $\overline{pK_A} = 6.5$ in conjunction with eq 28 was found to provide the best fit in our predictions, given by the blue lines in Figure 2A and reproduced in Figure 2B, to the titration profile of salt-free PAA system with two molecular weights, given by the open symbols in Figures 2A and 2B. (Note that for pure PAA, there is no IP and hence there is no difference between f_A and σ_A .)



Figure 2. Comparison of model predictions at various ion-pairing constants for (A) deprotonation degree and (B) extents of ion-pairing of PAA (solid lines) and PDADMAC (dashed lines), in potentiometric titration of equimolar PAA/PDADMAC with experimental data reported by Choi and Rubner⁶⁰ (both open and filled circles) and Petrov et al.⁶¹ (both open and filled squares). Open symbols correspond to pure PAA and filled symbols to PAA/PDADMAC. The effective protonation constant of PAA was evaluated using eq 28 with $\gamma_A = \sigma_A$.

The presence of polycations shifts the apparent deprotonation constant of PAA by 1–3 units relative to pure PAA, depending on the chain's molecular weight and experimental conditions. A systematic variation of the IP constant weighted by polymer volume fraction in conjunction with eq 28 for PAA with $\gamma_A = \sigma_A$ demonstrates that IP between PAA and the oppositely charged PDAMDAC semiquantitatively predicts the observed experimental shifts, as shown by Figure 2A. Even though the prefactors used with pure PEs may not necessarily be the same as those for mixed polyanion/polycation systems and the crude approach taken to generalize eq 28 from pure PEs to mixed PEs may be inaccurate, our predictions should at least prompt more quantitative work to assess the protonation constants of weak PEs in the presence of oppositely charged chains.

As $K_{ip}\phi_p$ increases, the extent of ion-pairing of the deprotonated carboxylic groups increases accordingly, as shown by the solid lines in Figure 2B. For sufficiently high IP constant, β_A approaches unity, effectively removing bare carboxylate groups with which protons can interact and thus inducing more protonated groups to dissociate in order to maintain equilibrium, according to the Le Châtelier's principle. As pH increases, more PAA repeat units become deprotonated and thus available for ion-pair formation with fully ionized PDAMDAC groups resulting in an increase of β_C , as shown by the dashed lines in Figure 2B. Beyond pH ~ 6, extents of ion-pairing for both polymers reach a plateau which increases at higher values of the ion-pairing constant (Figure 2B). Application of Le Châtelier's principle to eqs 9, 10, and 13

suggests that ion-pairs remove the bare charged functional groups, thus inducing further chain ionization to maintain equilibrium. In fact, a similar concept based on IP has been used to explain pH shifts in PAA solutions in response to addition of another chain type capable of forming ionic or hydrogen bonds with PAA.^{62,63}

For PAA/PDADMAC at pH = 7, $K_{ip}\phi_p \sim 5$ can be inferred from experimental ITC measurements.⁵¹ Although this deviates from the values providing best fits to the experimental data in Figures 2A, namely $K_{ip}\phi_p = 10-100$, the proposed model captures the significant shifts in p K_A of PAA qualitatively well, given that the experimental titration data evidently depend on molecular weight as well as our assumption of a pH-independent ion-pairing equilibrium constant. The latter quantity has been shown to be sensitive to pH variations and even a 10-fold increase in response to pH variation can be expected.⁴⁹

Charge regulation in a weak polyanion such as PAA is also predicted in the presence of a weak polycation such as poly(allylammonium hydrochloride), PAH (see Supporting Information). The proposed model can also capture the main features of potentiometric titration of nonequimolar mixtures, as demonstrated for PSS/PAH system in the Supporting Information (see Figure S4). A similar observation has been reported for PDMAEMA in the presence of strongly charged poly(styrenesulfonate), PSS.⁵⁹ In the Supporting Information and consistent with experimental trends, salt counterions are also shown to induce further deprotonation (protonation) in PAA (PDMAEMA) and that the potentiometric titrations of singletype PE solutions can be used to obtain CC equilibrium constants, used in section 3.2.2.

Having demonstrated the capacity of the proposed model to capture the charge regulation effects in weakly dissociating PEs induced by simple salts and oppositely charged PE, we proceed to illustrate the effect of CC and IP on predicted binodal diagrams. We focus on monovalent PE mixtures prepared at stoichiometric conditions for simplicity, hereafter.

3.2. Binodal Diagrams. 3.2.1. Strong Polyelectrolytes. While the protonation degree of a strong PE is insensitive to pH, its effective charge density is determined by the PE, salt, and the oppositely charged PE concentrations through CC and IP, which lower the effective charge density. The charge density σ_i of a strong PE in VO-based models remains constant (usually unity) regardless. In our model, however, the charge density is evaluated self-consistently upon free energy minimization. Figure 3A depicts the binodal curves for an equimolar and symmetric system of strongly dissociating PEs such as the well-studied PSS/ PDADMAC strongly dissociating system ($\alpha_{AH} = 0$, $\alpha_{CH} = 1$), at a fixed IP equilibrium constant $K_{ip}^{\infty} = 120.8$ for various infinitedilution CC constants; note that the effective constants are controlled by eqs A-4 to A-6 in the Appendix. As both PEs are insensitive to pH, water self-dissociation has been neglected and the concentrations of hydronium and hydroxyl ions have been neglected, simplifying the model for this system. Note the definitions of molar concentrations are given in the caption to Figure 3, which we use throughout.

The binodal diagrams predicted for the system considered in Figure 3A exhibit richer features than those derived from the extended VO model that accounts for different components sizes. Note that in a symmetric system with identical CC parameters used for both PE–counterion pairs, the extents of CC and IP in a symmetric equimolar mixture are identical for both PEs. Also, the total concentration of negatively charged counterions in the single phase prior to coacervation is equal



Figure 3. Effect of CC equilibrium constant on (A) binodal diagrams for an equimolar symmetric mixture of two strongly charged PEs at a fixed IP constant of 120.8 in the infinite dilution limit, where the concentration C_s is the molar concentration of salt (moles/volume of either salt ion) and $C_{\rm p}$ is the molar concentration of monomers of either PE. Because of symmetry and equimolarity, concentrations of both PEs are identical and so are the total concentrations of either counterion. Open circles on either side of the diagrams mark the end-points of consecutive tie-lines corresponding to increasing salt concentrations. For clarity, only the dotted tie-lines for the black curve in (A) is presented here. (B) Extents of IP for polyanions (open squares) and extents of CC along polyanions chains (open circles), in the dense phase as a function of bulk salt concentration. The extents of IP and CC for the positively charged chain are identical to corresponding values of the polyanion chain due to symmetry. $\omega_+ = \omega_- = \overline{\omega} = 1$, $\omega_A = \omega_C = 2.4$, T =298 K, and f' = 0.

to the total concentration of positively charged counterions, and the concentration of either is denoted by C_S . Similarly, the total concentrations of oppositely charged PEs are equal to each other and the concentration of each is denoted by C_P . Our model clearly predicts an associative phase separation with a dense polymer-rich phase consisting of equal amounts of each PE and a supernatant phase almost devoid of polymers.

At sufficiently low salt concentrations, there is an abundance of charged PE repeat units and a dearth of counterions, resulting in much ion-pairing and little CC in the dense phase irrespective of the strength of counterion-PE local electrostatic interactions (see the circles in Figure 3B). Upon formation of each ion-pair for the system at hand in the infinite dilution limit, the total free energy (excluding the entropy of mixing) is lowered by $\sim 3.8k_{\rm B}T$. The free energy thus attains a minimum as more and more ionpairs are formed. PEs try to form as many ion-pairs as possible through phase separation into a dense phase where the probability of polyanion-polycation encounters is significantly higher. As evidenced by eq A-6, the extent of IP increases in denser systems such that f_{ip} tends to densify the coacervate as much as possible, expelling water. However, the substantial entropy of water opposes excessive densification of the gel phase. At the same molar concentration and IP constant, the PE with

the lower monomer volume occupies less volume in the dense phase and thus face less resistance from entropy of water. Therefore, the two-phase region of the phase diagram, expressed in terms of molarities, shrinks down for complex coacervation of oppositely charged PEs with bulky repeat units.

A comparison with predictions of the EVO model with the charge density of both PEs set to unity proves that the main driving force for complex coacervation in this system is the favorable IP which is increasingly counteracted as CC intensifies, i.e., at higher pK_{A+}^{∞} and pK_{CH-}^{∞} . This is a major departure from the classical VO model where complex coacervation is attributed solely to a gain in long-ranged electrostatic interactions. In fact, ~75% of the repeat units in the dense phase at low salt concentrations in our model (Figure 3B) are neutralized by an oppositely charged group increasing the Debye length, significantly diminishing the contribution of the long-ranged fluctuation energy to PEC.

As more salt is introduced, a competition between IP formation and CC controls the shape and curvature of the branch of binodal diagram corresponding to the coacervate phase. At high salt concentrations, CC is enhanced, causing some polymer-polymer ion-pairs to break up, which in turn translates into a lower mechanical gel modulus for the coacervate phase. The so-called salt-induced plasticization of PE complexes is well understood qualitatively.^{22,64} Consistent with molecular simulations and experimental observations on PSS/PDADMAC complexes and multilayers,^{65–67} the extent of IP (intrinsic charge compensation) decreases monotonically as salt concentration responsible for extrinsic compensation increases (see Figure 3B). As the strength of short-ranged counterion-PE interactions increases, represented by higher $pK_{A+}^{\infty} = pK_{CH-}^{\infty}$, more ion-pairs are broken up in the dense phase at any given salt concentration, leading to a lower critical salt concentration. Note in Figure 3B that for any value of $pK_{A+}^{\infty} = pK_{CH-}^{\infty}$, at the critical salt concentration, the extent of IP reaches a critical minimum of ~ 0.1 for the set of parameters employed in Figure 3B, while the CC extent reaches a maximum of ~0.65. In fact, all the curves in Figure 3B could be collapsed onto a single universal plot upon normalization of C_s by the corresponding critical salt concentration.

Perry and Sing⁴⁵ applied LS theory to strongly and oppositely charged PEs of segment size either similar to or smaller than that of counterions and demonstrated that LS predicts a significantly *lower* counterion concentration in the dense phase than in the supernatant, which at least for the case of PSS/PDADMA/KBr system does not seem to be valid.²⁴ In the limit where both IP and CC are weak, our model for strongly charged PEs reduces to the EVO model modified to account for Born energy effects. Our proposed model predicts the counterion release (CR) from a chemical standpoint. Upon adding eqs 11–13, we arrive at another form of complexation reaction generally envisaged in CR view of complex coacervation.

$$(A+) + (CH-) \xleftarrow{\Delta G_{ip}^{\infty} + \Delta G_{A+}^{\infty} + \Delta G_{CH-}^{\infty}} CHA + (+) + (-)$$

$$(29)$$

Equation 29 makes clear that increasing the salt concentration pushes the equilibrium in the backward direction causing the ion pairs (condensed counterions) to decrease (increase). At any given overall polymer and salt concentration greater than zero within the binodal envelope, the CC (IP) extent is lower (greater) in a dense phase than it would be if the system remained in a single phase. In other words, chains release their associated counterions to form ion-pairs with oppositely charged chains upon complex coacervation.

According to eq 29, a higher IP constant increases the extent of IP at fixed salt and PE concentrations. Figure 4 demonstrates the



Figure 4. Similar to Figure 3 except with constant CC parameters $pK_{A+}^{\infty} = pK_{CH-}^{\infty} = 0.73$ at three IP constants. The dotted tie-lines only for the intermediate IP strength are presented here, for clarity. Inset illustrates the extents of IP for polyanions (open squares) and extents of CC along polyanions chains (open circles), in the dense phase as a function of overall salt concentration. The extents of IP and CC for the positively charged chain are identical to the corresponding values of the polyanion chain.

role of the IP constant on binodal diagrams. Unlike the salt concentration that primarily sets the critical condition for phase separation, the IP constant alters the phase behavior in a more drastic way. At no-salt condition, the tendency of oppositely charged PEs to form ion pairs is in large part balanced by the entropy of water. As the extent of IP increases (see the squares in the inset of Figure 4), the extent of CC decreases at any given salt concentration.

It should be noted that there exists a maximum PE volume fraction in the dense phase, which is achieved in the no-salt conditions in equimolar mixtures (where $\alpha_{\rm P} = \alpha_{\rm A+} = \alpha_{\rm CH-} = 0$) and in the limit $K_{\rm ip}^{\infty} \to \infty$, i.e., $\beta \to 1$. Such a maximum is obtained by setting the osmotic pressure of the water to zero for infinitely long, strongly dissociating PEs in the aforementioned limit. For the parameter set in Figure 4, the maximum total PE concentration in the dense phase for a symmetric PE mixture predicted by our model—which is a function of $\omega_{\rm P} = \omega_{\rm A} = \omega_{\rm C}$ and FH parameter in water—is found to be ~3.75 M as $K_{\rm ip}^{\infty} \to \infty$ (see section SI.8 in Supporting Information for further details).

Figure 5 illustrates the comparison of our model predictions with and without DH free energy contribution to the binodal diagrams inferred from the phase diagram reported by Wang and Schlenoff for equimolar mixtures of PSS ($N_A \sim 2000$)/PDADMAC ($N_C \sim 1000$) in the presence of KBr.²⁴ Their reported polymer weight fractions in coexisting phases were converted to volume fractions using the mass densities of pure KBr (2.75 g/cm³) water and polymer (1.13 g/cm³). Molar concentrations and weight fractions of PEs and KBr in coacervate phases reported by Wang and Schlenoff,²⁴ $\omega_P = \omega_A = \omega_C = 6.5$ and $\omega_+ = \omega_- = 1$ can be inferred according to eq 26. Table 1 summarizes the parameters used in Figure 5. We have taken the CC constants and FH parameters for both PSS and PDADMAC repeat units to be identical to limit the parameter space.



Figure 5. Binodal diagram of PSS/PDADMAC/KBr system predicted by EVO (cyan lines) and by our model with (violet lines) and without (green lines) the Debye–Hückel (DH) free energy. The volume fraction of either repeat unit is denoted by ϕ_P while the total KBr volume fraction is given on the vertical axis. Black stars represent the experimental data demarcating the binodal boundary.²⁴ The open circles mark the KBr and PE volume fractions in coexisting phases connected by the tie-lines, which are dotted lines. The relevant parameters are listed in Table 1.

Table 1. Parameters Employed in Obtaining the BinodalDiagram in Figure 5

pK_{A+}^{∞}	pK_{CH-}^{∞}	$K^{\infty}_{ m ip}$	$\chi_{\rm AW} = \chi_{\rm CW}$	$\omega_{\rm A} = \omega_{\rm C}$	$\omega_+ = \omega$
2.58	2.58	347300	0.5	6.5	1.0

Even though neutral and sufficiently long chains for which the Flory-Huggins (FH) χ parameter in water exceeds ~0.5 are poorly soluble in water, a single-component PE can tolerate γ parameters up to unity and still remain soluble in water owing to the huge loss of translational entropy of the counterions that would occur if the polyions were to phase separate. However, if both chains of both charges are present at equimolarity, these chains phase separate at any salt concentration when both chains have γ values with respect to water that exceed 0.5. The critical γ in this case is not higher than this because the oppositely charged chains can achieve charge neutrality without carrying their counterions with them as they phase separate, and thus there is no entropic cost that might raise the critical value of χ above 0.5, as there is when only chains of a single charge phase separate. The same is true of the EVO model and we believe all models with a constant FH χ parameter between water and PEs. The effective χ parameter of PSS/PDADMA multilayers where the repeat units are expected to be highly ion-paired has been reported to be ~0.85.⁶⁸ For the maximum constant χ parameter that has a critical salt concentration above which there is no phase separation, namely $\chi = 0.5$, the maximum PE volume fraction predicted in our model is ~0.4, considerably underestimating the experimentally observed value of ~0.55 in Figure 5. Nonetheless, our model clearly outperforms the EVO model in Figure 5. Figure 5 also shows the prediction of our model without the DH term, thereby illustrating that IP and CC terms alone can predict complex coacervation and critical behavior at high salinity with the DH term influencing the predictions only at elevated salinity in our model.

Any higher IP constant beyond that reported in Table 1 could be used in conjunction with correspondingly higher pK_{A+}^{∞} and pK_{CH-}^{∞} to provide a similar fit to the experimental data in Figure 5. This suggests that the underlying equilibrium free energy in this system with strong IP is that of counterion release (CR), whose net free energy change is given by eq 29. This gives $\Delta G_{ip}^{\infty} + \Delta G_{A+}^{\infty} + \Delta G_{CH-}^{\infty} \sim 6.2k_{B}T$ for the IP and CC parameters used in Table 1, which agrees reasonably well with the experimental range of values $(4.7-7.9)k_{B}T$ reported for "doping equilibria" of PSS/PDADMA with simple salts.^{66,69} Upon measurement of two of the CC and/or IP equilibrium constants for this system (which is equivalent to measuring $\Delta G_{A+}^{\infty} \Delta G_{CH-}^{\infty}$ or ΔG_{ip}^{∞}), the third free energy contribution could be inferred unambiguously.

Near the critical salt concentration in Figure 5, the measured polymer concentration in the supernatant becomes appreciable, as can be observed in the asterisks near the top of the phase diagram. However, due to the high polymerization index used in the work of Wang and Schlenoff,²⁴ our theory as well as VObased models predict a vanishingly small PE concentration in the supernatant phase, since neither accounts for interpolyelectrolyte complexes that presumably exist as colloidal particles in the supernatant phase. In the Supporting Information, we demonstrate that allowing χ parameters to increase linearly beyond 0.5 as a function of IP extent, we can improve our model predictions, especially in the low salt limit. Additionally, the supernatant phase in this case can contain a sizable amount of both PEs at the critical point. Lastly, experimentally, it is evident in Figure 5 that at low salt concentration, addition of KBr raises the PE concentration in the dense phase, a behavior that has yet to be captured by any theory. Even though it is possible to suggest remedies such as the aforementioned compositiondependent χ parameters for such a shortcoming, we defer such refinements to future work until more systematic investigations become available.

3.2.2. Weak Polyelectrolytes. In a mixture of two weakly dissociating chains in the presence of added salt, protons, counterions, and oppositely charged repeat units compete to bind to PEs. This competition is determined by the free energies associated with eqs 9-13. Unlike in a mixture of strongly dissociating PEs, with weak PEs water self-dissociation eq 14 cannot be ignored. In controlled experiments, the pH and salinity are commonly adjusted prior to mixing the stock solutions containing either PE individually. Upon mixing, the pH is subject to change even if the system does not phase separate. Given that either buffer solutions or strong acid/bases are commonly used to adjust the pH of the stock solutions, here assumed to be the same for both starting PE solutions and designated hereafter by pH°, the concentrations of simple ions and PEs depend on the particular mixing and pH-adjustment protocol and thus cannot be varied independently. In previous models, the pH is typically specified for the final *mixed solution*, which ignores changes in pH resulting from charge regulation effect upon mixing of stock solutions.

In the remainder of this section, we denote the molar concentration of added salt (moles of either ion per unit volume of added salt, beyond any ions introduced through the acid or base used for pH regulation) in each of the stock PE solutions by C_S^B and follow a computational sequence mimicking the typical experimental procedure whereby the pH of each PE stock solution is adjusted by a strong inorganic acid or base sharing the counterions corresponding to the salt used. For instance, if the added salt is chosen to be KCl, as is the case in the remainder of this section, enough HCl or KOH is added to the stock solutions to adjust the pH to the desired pH^o at a given bulk salt concentration. As a result, the concentrations of the counterion from the acid or base (Cl⁻ or K⁺) can be significantly different depending upon the prescribed pH^o. The red and blue dashed



Figure 6. Binodal diagram illustrating the concentrations of two weakly dissociating PEs in coexisting phases versus their corresponding counterion concentrations for an aqueous equimolar system at (A) $pH^{\circ} = 7$ and (B) $pH^{\circ} = 5$. The dashed blue and red lines (which nearly superimpose in (A)) mark the salt–polymer concentrations below which the pH of either stock solution cannot be adjusted to the specified value, pH° . (C) and (D) correspond to the binodal diagrams in (A) and (B), respectively, where the bulk salt concentration in the stock solutions (assumed to be the same in each) is plotted against the PE concentrations in coexisting phases. Note that circles and squares in (C) are almost indistinguishable. The PE concentration in both stock solutions is fixed at 0.11 M. Experimental binodal data³⁰ for PAA/PDMAEMA system at pH = 6.5 are represented by stars in (C). (This is slightly different from the value $pH^{\circ} = 7$ used in our calculations.) The insets show the dependence of the fraction of PE units in the dense phase that are charged and therefore available for IP.

lines in Figures 6A and 6B depict the minimum counterion concentration introduced at an initially no-salt condition through the addition of enough KOH or HCl to achieve the designated pH values in each stock solution of PAA and PDMAEMA for a given polymer concentration. To adjust the pH to $pH^{\circ} = 7$, almost equal amounts of KOH and HCl are required, while to achieve $pH^{\circ} = 5$, considerably more HCl should be added to the PDMAEMA solution than the amount of KOH that needs to be added to PAA.

A survey of experimental studies indicates a dearth of systematic studies on binodal compositions of weak polyelectrolyte coacervates. We here use the experimentally obtained binodal diagrams³⁰ of the PAA/PDMAEMA/KCl system (both chains with a polymerization index \sim 500) at pH = 6.5 ± 0.2, as a reference in the following calculations. Spruijt et al.³⁰ assumed that the KCl concentration was the same in both phases and that within each phase the PAA and PDMAEMA concentrations were equal to each other. We explain in section SI.6 in the Supporting Information how the protonation and CC constants for both PEs in the infinite dilution limit can be extracted from the potentiometric titration data for PAA and PDMAEMA measured at various salinities (see Figure S5). Note that we do not consider any dependence of protonation constants on protonation extents here since the titration data for these polymers conform reasonably well to the Henderson-Haselbalch (HH) model at

the no-salt condition (see Figure S5). Given the scatter in the experimental data, the ion-pairing equilibrium constant was adjusted to provide a reasonable fit to the experimental coacervate phase concentrations, shown in Figure 6C. The value so obtained along with other parameters tabulated in Table 2 were consistently employed to predict the binodal diagram at

Table 2. Association/Dissociation Equilibrium Constants atthe Infinite Dilution Limit Employed in Figure 6

pK_A^∞	pK_C^∞	pK_{A+}^{∞}	pK_{CH-}^{∞}	$K^\infty_{ m ip}$	pK_w^∞
6.65	8	1.89	2.29	43800	14

pH^o = 5 (see Figures 6B and 6D). Unlike the PSS/PDADMA/ KBr system where the IP and CC could not be uniquely determined, the corresponding values in PAA/PDMAEMA/KCl system are uniquely determined as the extents of CC were independently obtained using the potentiometric titration data discussed in the Supporting Information.

The sizes of the PAA and PDMAEMA repeat units are taken to be equal to each other and determined from the reported water content of the coacervate at no salt-conditions, i.e., ~72 wt %,³⁰ using eq 28 ($\omega_A = \omega_C = 2.4$), while the effective sizes of potassium and chloride ions are both assumed to be equal to that of the water molecule. Molecular simulations have demonstrated that the VdW interactions of protonated and deprotonated PAA repeat units with water are different enough to justify neglecting the χ parameter for the deprotonated monomer against water and thus $f' = \chi_{AHW} \alpha_{AH} \phi_A \phi_W$. Thus, we take $f' = 0.45 \alpha_{AH} \phi_A \phi_W$ where $\chi_{AHW} = 0.45$ is the FH χ parameter of protonated PAA repeat units in water, which was taken from the literature,⁷² consistent with the calculations in Figure S5 used to obtain the protonation and CC parameters. For PDMAEMA, repeat units were shown not to differ significantly upon protonation.⁴⁰ Here, for simplicity, we neglect any FH χ parameter for PDMAEMA, or any other pairs of species, keeping only the χ parameter for protonated PAA in water.

IP between PEs upon mixing perturbs the pH. As a result, PAA chains at $pH^{\circ} = 5$ in the stock solution undergo further deprotonation upon mixing at low salt conditions, leading to a pH of ~2.55 and 3.3 in the supernatant and coacervate phases, respectively. For $pH^{\circ} = 7$, the pH in both phases after mixing is ~7.85. This result cautions against the use of the pH measured in the supernatant as the local pH inside the coacervate phase, as is often assumed in the VO model and other models.⁷⁰ We reiterate here that none of the existing models of complex coacervation account for charge regulation effects.

At pH° = 5, there is an excess of chloride ions introduced by HCl, as evidenced by the minimum chloride and potassium concentration required to adjust pH in stock solutions, represented by the red and blue dashed lines in Figure 6B. The high protonation degree of the PAA chain at low salt at pH° = 5 cannot be completely overcome by the propensity of the repeat units to form IPs; this leads to a higher concentration of PAA units in the coacervate phase to provide sufficient PAA units to ion-pair with PDMAEMA groups. Given that PDMAEMA chains are predicted to be 35% ionized at the no-salt condition while PAA groups are merely 10% ionized (see Figure S5) the resultant coacervate is nearly three and a half times more concentrated in PAA monomers than it is in PDMAEMA repeat units.

Unlike the PSS/PDADMAC system discussed in Figure 5, in the experiments, the supernatant phase in PAA/PDMAEMA system remains almost fully depleted of either PE at neutral pH (see Figure 6C), indicating the absence of inter-polyelectrolyte complex particles in the supernatant for this system. In the Supporting Information, we have compared our predictions to those of the EVO model with an empirical expression to account for charge regulation by Jha et al.⁴⁰ for weak PEs for this system using identical parameters as those used in Table 2. These parameters, along with $\chi_{AHW} = 0.45$, are much more realistic than the fitting parameters used by Jha et al.; the model of Jha et al. significantly underestimates the experimental binodal diagrams at pH^o = 7.

As demonstrated in section 3.2.1, the predicted binodal diagrams are most sensitive to the IP standard free energy (see Figure 4). Similarly, the phase behavior of weak PEs is highly sensitive to IP strength. In Figure S6, the critical KCl concentration of PAA/PDMAEMA system at pH° = 3 is shown to increase over 30-fold (from ~ 0.12 to 4 M) when the pK_{ip}^{∞} is decreased by 1 unit—equivalent to a 10-fold increase in K_{in}^{∞} . Note that PAA/PDMAEMA at pH^o = 3 and 5 with the bulk concentrations used in Figure 6 undergoes phase separation at salinities as high as 3 M KCl,²¹ suggesting the necessity for considering the a pH dependence of K_{ip}^{∞} to capture the critical salt concentration at different pH values. Our treatment of IP and CC in eqs 11-13 is a simplified picture wherein the water structure around individual participants is neglected. However, the release of hydration water molecules has been shown to play a significant role in PE complexation in multilayers.⁷¹ The number

of hydrating water molecules forming H-bonds to deprotonated PAA units was shown to decrease at low pH, causing a reduction of around ~1 $k_{\rm B}T$ in the complexation enthalpy of PAA/PDADMAC as pH was lowered from 10 to 3.⁷³ Similarly, the IP equilibrium constant for poly(L-ornithine hydrobromide)/poly-(L-glutamic acid) was found to increase over 13-fold as the pH was raised form 7 to 8.8.⁴⁹ Once relevant experimental and simulation data become available, the IP and CC free energies and even FH χ parameters can be made functions of composition, pH, IP and CC extents, etc., to reflect the chemistry-specific details missing in VO-based models and LS theories.

4. CONCLUSIONS AND FUTURE WORK

Our experimentally informed closed form free energy proposed here is shown to explain complex coacervation as a competition between three categories of association/dissociation equilibria, namely counterion condensation (CC), ion-pair formation (IP), and, for weakly dissociating polyelectrolytes (PEs), protonation or deprotonation. The strength of each of these phenomena is set by a corresponding standard free energy or equivalently an equilibrium constant defined at a reference state, taken to be the infinite dilution in water. The model presented here provides a tractable way to extend the Voorn–Overbeek (VO) model⁴⁰ of coacervation by allowing for the effects of IP, CC, and charge regulation as well as van der Waals (VdW) interactions, longrange electrostatic interactions, captured by the Debye-Hückel (DH) free energy, and translational entropy of mixing. Our new theory incorporates the specificity of PE physicochemistry and salt chemical identity, which are captured in parameters such as IP, CC, and ionization equilibrium constants as well as the volumes of ions and monomers, Flory-Huggins interaction parameters, and composition-dependent dielectric constants. While we found a reasonable set of reference free energies to use in our model to capture the experimentally obtained binodal compositions of weak PAA/PDMAEMA systems, our model predictions deviated from the experimental binodal diagram of the strong PSS/PDADMA system at low salt regime presumably due to the high hydrophobicity of this system which cannot be captured simply by using a constant FH χ parameter greater than 0.5. The main features and advantages of the proposed model are summarized as follows:

- Charge regulation of weak polyelectrolytes due to salt and oppositely charged PE as well as the pH drifts upon mixing of two oppositely charged PE solutions follow selfconsistently from Le Châtelier's principle embedded in laws of mass action (LMA) describing protonation, CC and IP equilibria, both in mono- and biphasic systems.
- The proposed model formalizes the counterion release (CR) picture of complex coacervation. While the critical salt concentration in the VO model is set solely by a competition between the translational entropy of simple counterions and long-ranged electrostatic fluctuation energy, the competition between IP and CC alone can predict complex coacervation and the presence of critical salt concentration in the present model, consistent with the CR picture. Nonetheless, long-ranged electrostatic interactions affect the critical salt concentration beyond which no coacervation occurs.
- As the propensity of counterions to condense increases at a fixed IP strength, or as IP strength is lowered for fixed CC equilibrium constants, the salt–polymer concentration

binodal envelope shrinks. The strength of IP between the two ionized PE groups is found to have the most drastic effect on the binodal diagrams both for weak and strong PEs.

Thus, the inclusion of CC and IP in our model drastically changes the picture of coacervation relative to the mechanism considered by the VO-based model, in which the driving force is long-ranged electric field fluctuations. The ability of VO-based models to fit experimental phase behavior data reasonably well through adjustments in sizes of species and other parameters disguise its omission of ion pairing, which can be an equally important driver for coacervation, at least for some systems. More careful measurements of IP and CC energies through calorimetry or indirectly through charge regulation effects measured through IR spectroscopy would help confirm this point. Accurate measurements of effective ionization extents in the coacervate can establish the exact contributions of longranged electrostatic versus IP and CC to driving force of complex coacervation. Our model can be improved and extended by allowing for composition dependencies of the above parameters and their measurement through experiments and molecular simulations. Future comparison with experiments of the binodal diagrams predicted by the proposed model in various systems using the thus-obtained parameters will allow for a systematic evaluation of the model, which may warrant the use of composition-dependent free energies and FH χ parameters to capture subtle effects observed experimentally.

APPENDIX

Here we present six law-of-mass-action (LMA) equations that follow from minimization of total Helmholtz free energy with respect to six degrees of freedom.

$$\begin{split} \frac{\phi_{\mathrm{H}_{30}}\phi_{\mathrm{OH}}}{\phi_{\mathrm{w}}^{2}} &= K_{\mathrm{w}} \equiv \exp\left[-\Delta G_{\mathrm{w}}^{\infty} + \frac{\kappa l_{\mathrm{B}}}{1+\overline{\kappa}} + \frac{l_{\mathrm{B}} - l_{\mathrm{B}}^{\infty}}{l} - \frac{\partial f^{\chi}}{\partial \phi_{\mathrm{H}_{30}}}\right|_{T,V} \right] \\ (A-1) \\ \frac{(1-\beta_{\mathrm{A}})(1-\alpha_{\mathrm{A}+})(1-\alpha_{\mathrm{AH}})\phi_{\mathrm{H}_{30}}}{\alpha_{\mathrm{AH}}\phi_{\mathrm{w}}} &= K_{\mathrm{A}} \\ &\equiv \exp\left[-\Delta G_{\mathrm{AH}}^{\infty} + \frac{\kappa l_{\mathrm{B}}}{1+\overline{\kappa}} + \frac{l_{\mathrm{B}} - l_{\mathrm{B}}^{\infty}}{2l}(\omega_{\mathrm{A}}^{-1/3} + 1) - \frac{\partial f^{\chi}}{\partial \alpha_{\mathrm{AH}}}\right|_{T,V} \right] \end{split}$$

$$\frac{(1-\beta_{\rm C})(1-\alpha_{\rm CH-})\alpha_{\rm CH}\phi_{\rm OH}}{(1-\alpha_{\rm CH})\phi_{\rm w}} = K_{\rm C}$$

$$\equiv \exp\left[-\Delta G_{\rm CH}^{\infty} + \frac{\kappa l_{\rm B}}{1+\bar{\kappa}} + \frac{l_{\rm B}-l_{\rm B}^{\rm w}}{2l}(\omega_{\rm C}^{-1/3}+1) - \frac{\partial f^{\chi}}{\partial \alpha_{\rm CH}}\Big|_{T,V}\right]$$
(A-3)

$$\frac{(1-\beta_{\rm A})(1-\alpha_{\rm A+})\phi_{\rm +}}{\alpha_{\rm A+}} = K_{\rm A+} \equiv \exp\left[-\Delta G_{\rm A+}^{\infty} + \frac{\kappa I_{\rm B}}{1+\kappa} + \frac{I_{\rm B}-I_{\rm B}^{\rm w}}{2l}(\omega_{\rm A}^{-1/3} + \omega_{\rm +}^{-1/3}) - \frac{\partial f^{\chi}}{\partial \alpha_{\rm A+}}\Big|_{T,V} - 1\right]$$
(A-4)

г

$$\begin{aligned} \frac{(1-\beta_{\rm C})(1-\alpha_{\rm CH-})\phi_{\rm -}}{\alpha_{\rm CH-}} &= K_{\rm CH-} \\ &\equiv \exp\left[-\Delta G_{\rm CH-}^{\infty} + \frac{\kappa l_{\rm B}}{1+\overline{\kappa}} + \frac{l_{\rm B}-l_{\rm B}^{\rm w}}{2l} \\ (\omega_{\rm C}^{-1/3} + \omega_{\rm -}^{-1/3}) - \frac{\partial f^{\chi}}{\partial \alpha_{\rm CH-}}\Big|_{T,V} - 1\right] \\ &\frac{\beta_{\rm A}\omega_{\rm C}}{(1-\beta_{\rm C})(1-\beta_{\rm A})\phi_{\rm C}\alpha_{\rm CH}(1-\alpha_{\rm CH-})(\omega_{\rm C}+\omega_{\rm A})} = K_{\rm ip} \\ &\equiv \exp\left[-\Delta G_{\rm ip}^{\infty} - \frac{\kappa l_{\rm B}}{1+\overline{\kappa}} - \frac{l_{\rm B}-l_{\rm B}^{\rm w}}{2l}(\omega_{\rm C}^{-1/3} + \omega_{\rm A}^{-1/3}) \\ - \frac{\partial f^{\chi}}{\partial \beta_{\rm C}}\Big|_{T,V} + 1\right] \end{aligned}$$
(A-6)

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macro-mol.6b01464.

Additional derivations and predictions of the potentiometric titration of PAH, PDMAEMA, and PAA in salt, the numerical algorithm for solving the nonlinear and coupled set of laws of mass action, and the procedure used to perform the flash calculations for obtaining binodal diagrams; comparisons of the predictions of other models to those of ours in PAA/PDMAEMA system as well as the role of the IP equilibrium constant on the binodal diagram for PAA/PDMAEMA/KCl at pH° = 3 and that of FH χ parameter on the binodal diagram of PSS/PDADMA/ KBr; a MATLAB script to perform flash calculations is available upon request (PDF)

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Notes

(A-2)

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ACKNOWLEDGMENTS

We acknowledge the support of the National Science Foundation (NSF) under Grant DMR 1403335. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF.

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