Overcharging of Polyelectrolyte Complexes: An Entropic Phenomenon

Journal:	*Soft Matter*
Manuscript ID	SM-ART-08-2020-001466.R1
Article Type:	Paper
Date Submitted by the Author:	29-Sep-2020
Complete List of Authors:	Ghasemi, Mohsen; University of Michigan, Chemical Engineering Friedowitz, Sean; Stanford University, Chemical Engineering Larson, Ronald; University of Michigan, Chemical Engineering

Overcharging of Polyelectrolyte Complexes: An Entropic Phenomenon

Mohsen Ghasemi,[1] Sean Friedowitz,[2] and Ronald G. Larson[1]*

[1]*Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan 48109, USA*

[2]*Department of Chemical Engineering, Stanford University, Stanford, California 94305, USA*

Abstract

Overcharging in complex coacervation, in which a polyelectrolyte complex coacervate (PEC) initially containing equal moles of the cationic and anionic monomers absorbs a large excess of one type of polyelectrolyte species, is predicted using a recently developed thermodynamic model describing complexation through a combination of reversible ion binding on the chains and long-range electrostatic correlations. We show that overcharging is favored roughly equally by the translational entropy of released counterions and the binding entropy of polyelectrolytes in the polyelectrolyte complex, thus helping resolve competing explanations for overcharging in the literature. We find that the extent of overcharging is non-monotonic in the concentration of added salt and increases with both strength of ion-pairing between polyions and with chain hydrophobicity. The predicted extent of overcharging of the PEC is directly compared with that of multilayers made of poly(diallyldimethylammonium), PDADMA, and poly(styrene-sulfonate), PSS, overcompensated by the polycation in two different salts: KBr and NaCl. Accounting for the specificity of salt ions interactions with the polyelectrolytes, we find good qualitative agreement between theory and experiment.

Introduction

By mixing oppositely charged polyelectrolytes (PEs) in aqueous solutions or sequentially depositing them at interfaces, dense polymeric phases known as polyelectrolyte complex coacervates (PECs) are produced. PECs range from viscous fluids to solid-like precipitates and are useful for a variety of technological applications such as biomedicine and therapeutic protein delivery,[1, 2] water treatment,[3] and food processing.[4] In biological systems, an intriguing example of PEC formation is the spontaneous association of RNAs and proteins into membraneless compartments in cells.[5-8]

It is generally believed that PECs are held together by specific “ion-pairs” between charged groups on polyanion and polycation backbones, which impart viscoelastic properties to these materials.[9, 10] Association of units of opposite charge are influenced by contributions such as entropy gain due to release of hydration water,[11, 12] enhanced electrostatic attraction arising from a reduced dielectric constant near polyelectrolyte charged groups,[13] or other factors. Although charged groups on polyanions and polycations can associate and form ion-pairs during complexation, the driving force for formation of PECs is usually explained by the “counterion release” mechanism,[14-16] wherein the release of counterions upon ion-pairing of charged groups on the polyanions and polycations results in a favorable translational entropy gain. An additional entropic gain due to the multiplicity of the combination of binding pairs between polyanions and polycations has also been found by Lytle and Sing to drive PEC formation in 1:1 mixtures of polyanion and polycation.[17, 18]

In *stoichiometric* mixtures with equal numbers of charged groups on polycations and on polyanions, a neutral PEC is formed containing a 1:1 ratio of polyanion and polycation monomers.[19] On the other hand, if one type of polyelectrolyte is in excess, or if a neutral PEC is brought into contact with a large supernatant solution of one of the PEs, the PEC can adsorb more of the excess polyelectrolyte, leading to “overcharging” of the PEC.[19-21] We note that a bulk PEC phase remains *charge neutral* after adsorption of the excess PE by carrying counterions of the excess PE into the bulk phase. The presence of excess positive or negative polyelectrolyte charge on the PEC enables a myriad of novel applications, such as formation of polyelectrolyte multilayers (PEMUs),[22, 23] stabilization of nanomedicines,[24] and transfection of DNA into cells.[25-27]

Understanding how the degree of overcharging of a PEC depends on the physiochemical properties of the solution is key to the successful design of the aforementioned applications. Such design capability, however, has remained elusive due to the lack of a predictive approach for controlling the degree of overcharging, which is governed by several variables, such as concentration of salt,[20] ion-specifity,[28-31] solvent quality,[32] etc.[33-35]

The exact mechanisms controlling overcharging in PECs are still not entirely understood. With the advent of modern ultrasensitive titration calorimetric techniques, it has been found that association of polyelectrolytes in non-stoichiometric mixtures is often nearly athermal ($\Delta H \approx 0$),[36, 37] implying that PEC overcharging arises largely from some form of entropic force(s). That said, however, it remains unclear what entropic mechanisms drive overcharging. While a few prior studies have invoked the counterion release mechanism to explain overcharging,[38] no detailed quantitative analyses have been provided to substantiate these claims.

In the current study, we employ a polyelectrolyte solution theory to examine in detail the overcharging phenomenon, restricted in our study to bulk thermodynamic phases rather than small aggregates. The most relevant systems for application of our model are polyelectrolyte complex coacervates or multilayers in which the excess polyelectrolyte charge is distributed uniformly throughout the complex.[39] Next, we lay out our theory, and then in the Results section, we quantify the entropy change upon adsorption of excess polyelectrolyte by a stoichiometric PEC and identify key driving forces for adsorption that can unify different views on overcharging. Further, we explore ion-specific effects on the degree of overcharging. At the end, we provide comparisons of our model predictions with experimental overcharging data for multilayers made from poly(diallyldimethylammonium), PDADMA, and poly(styrene-sulfonate), PSS, in two different salts: KBr and NaCl, where we highlight the importance of ion-specific effects in phase behavior and overcharging of PECs.

Binding Equilibrium Free Energy Model

The thermodynamic theory used here, initially developed by Salehi and Larson, describes ion-pairing between polyelectrolytes (PEs) and binding of salt counterions to PEs as reversible reactions.[40] Recently, Friedowitz and

Qin,[41] extended the theory to incorporate the electrostatic correlations using a random phase approximation (RPA). We recently used this model to study the phase behavior of stoichiometric PECs through "doping" wherein salt is gradually added to the PEC, leading to breaking of ion pairs as salt ions bind to the PE's.[42] Here, we employ this model to explore overcharging and its driving forces.

The Helmholtz free energy density, f, normalized by the thermal energy $k_{\mathrm{B}}T$, the total solution volume V, and reference volume υ_{W}, can be written as

$$\frac{\upsilon_{\mathrm{W}}\,F}{k_{\mathrm{B}}TV} \equiv f = f^{\mathrm{T}} + f^{\chi} + f^{\mathrm{corr}} + f^{\mathrm{rxn}} + f^{\mathrm{comb}} \tag{1}$$

which has contributions from mixing (or translational) entropy f^{T}, dispersion interactions f^{χ}, long-ranged electrostatic correlations f^{corr}, local ion bindings f^{rxn}, and combinatorial entropy f^{comb}. We briefly introduce each free energy contribution below, while a more detailed definition of these terms can be found in our recent study.[42]

The mixing entropic free energy of each species f^{T} contributes,

$$f^{\mathrm{T}} = \sum_{i=\mathrm{A,C,\pm,W}} \frac{\phi_i \ln \phi_i}{N_i \omega_i}$$

$$= \frac{\phi_{\mathrm{A}}}{N_{\mathrm{A}}\omega_{\mathrm{A}}} \ln \phi_{\mathrm{A}} + \frac{\phi_{\mathrm{C}}}{N_{\mathrm{C}}\omega_{\mathrm{C}}} \ln \phi_{\mathrm{C}} + \frac{\phi_{+}^{\mathrm{f}}}{\omega_{+}} \ln \phi_{+}^{\mathrm{f}} + \frac{\phi_{-}^{\mathrm{f}}}{\omega_{-}} \ln \phi_{-}^{\mathrm{f}} + \phi_{\mathrm{W}} \ln \phi_{\mathrm{W}} \tag{2}$$

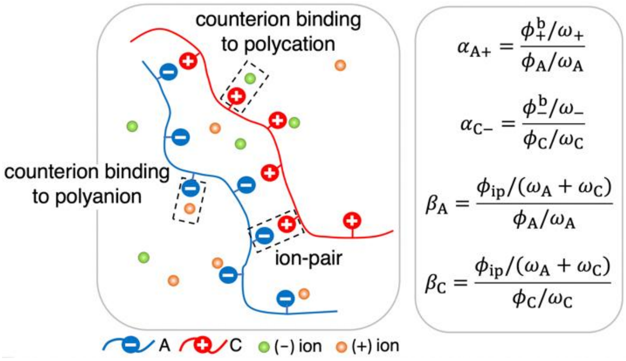
N_i represents the degree of polymerization of the species i. In this work, ϕ_{A} , ϕ_{C}, ϕ_{-} , and ϕ_{+} denote volume fractions of polyanion (A), polycation (C), anion ($-$), and cation ($+$), respectively. Salt ions are either free or bound to the polyelectrolytes with volume fractions of $\phi_{\mathrm{S}}^{\mathrm{f}}$ and $\phi_{\mathrm{S}}^{\mathrm{b}}$, respectively, and so $\phi_{\mathrm{S}} = \phi_{\mathrm{S}}^{\mathrm{f}} + \phi_{\mathrm{S}}^{\mathrm{b}}$ with $\mathrm{S} = +$ for cations or with $\mathrm{S} = -$ for anions. The charged groups are considered to be monovalent with (monomeric) molecular volumes υ_i for $i =$ A, C, and $\pm$. We normalize the molecular volumes of the charged species as $\omega_i = \upsilon_i/\upsilon_{\mathrm{W}}$, where $\upsilon_{\mathrm{W}} = 29.7\text{Å}^3$ denotes the molecular volume of water. The polyelectrolyte counterions and salt ions are taken to be identical; in either the PEC or supernatant phase, a portion of them bind to the polyelectrolytes while the rest remain free.

The dispersion interactions between polyelectrolytes and water are captured by $f^{\chi} = \chi_{\mathrm{PW}}(\phi_{\mathrm{A}}\phi_{\mathrm{W}} + \phi_{\mathrm{C}}\phi_{\mathrm{W}})$. Here, χ_{PW} is the Flory-Huggins interaction parameter, which reflects hydrophobicity of the polyelectrolytes (A and C). In this study, we take the polycation and polyanion to have the same interaction parameter with water (i.e. $\chi_{\mathrm{PW}} \equiv \chi_{\mathrm{AW}} = \chi_{\mathrm{CW}}$), and we ignore the other Flory-Huggins interactions parameters, including those involving salt ions.

Electrostatic correlations are treated with a modified version of the RPA applicable to systems with short-range ion binding,[41] in which a fixed Gaussian polyelectrolyte structure is used. Although it has been shown that a polyelectrolyte in the PEC takes on a Gaussian chain configuration,[43, 44] in the supernatant the configuration is likely more expanded. However, in the RPA theory used in this work, a Gaussian configuration is assumed for polyelectrolytes in both the PEC and the supernatant phase. The assumption of a Gaussian configuration in the supernatant likely leads to the failure of the model to accurately predict experimental overcharging at low salt. More sophisticated treatments of chain structure, such as that developed by Shen and Wang to treat adaptive chain flexibility, could be incorporated into the model in the future.[45]

Local ion binding effects, i.e. binding (or adsorption) of salt ions along polyelectrolytes and ion-pair formation, are described by reversible reactions with the contribution $f^{\mathrm{rxn}} = \frac{\phi_{\mathrm{A}}}{\omega_{\mathrm{A}}}\alpha_{\mathrm{A+}}\,\Delta G_{\mathrm{A+}} + \frac{\phi_{\mathrm{C}}}{\omega_{\mathrm{C}}}\alpha_{\mathrm{C-}}\,\Delta G_{\mathrm{C-}} + \frac{\phi_{\mathrm{C}}}{\omega_{\mathrm{C}}}\beta_{\mathrm{C}}\,\Delta G_{\mathrm{CA}}$ to the free energy. Here, ΔG_{ij}, with $ij = \mathrm{A+}$, $\mathrm{C-}$, and CA denote the standard free energies of binding between the (monomer) units of the species i and j.[40] ΔG_{ij} is defined as $\Delta G_{ij} = G_{ij}^{0} - G_{i}^{0} - G_{j}^{0}$ with G_{l}^{0} (l = i, j or ij) being the standard free energy of formation of the unit l in solvent (water) in the infinite dilution limit.[40] Each ΔG_{ij} is a phenomenological parameter that implicitly accounts for ion-specific effects such as changes of the solvent structure and dielectric permittivity upon binding, etc.[46, 47] The values of ΔG_{ij} are therefore expected to be sensitive to the specific ions and charged monomers, and would need to be obtained by fitting data or by simulation. The extents of binding reactions are described by $\alpha_{\mathrm{A}+}$ for cation binding along polyanions, by $\alpha_{\mathrm{C}-}$ for anion binding along polycations, and by β_{C} (or β_{A}) for ion-pair formation between charged groups of polyanions and polycations. Note that $\alpha_{\mathrm{A}+}$ and $\alpha_{\mathrm{C}-}$are identical to the "doping level" used by the Schlenoff group[48] and others.[49] Also, the ion-pairing fractions are related to each other through a stoichiometry constraint $\phi_{\mathrm{C}}\beta_{\mathrm{C}}/\omega_{\mathrm{C}} = \phi_{\mathrm{A}}\beta_{\mathrm{A}}/\omega_{\mathrm{A}}$. Using these definitions, one can find the effective charge density of polyanion and polycation as $\sigma_{\mathrm{A}} = 1 - \alpha_{\mathrm{A}+} - \beta_{\mathrm{A}}$ and $\sigma_{\mathrm{C}} = 1 - \alpha_{\mathrm{C}-} - \beta_{\mathrm{C}}$. See Scheme 1 for the definitions.

Scheme 1. Local ion binding reactions and definitions of their extent along each type of polyelectrolyte. For this particular instance, $\alpha_{\mathrm{A}+} = 3/7$, $\beta_{\mathrm{A}} = 3/7$, $\sigma_{\mathrm{A}} = 1/7$, and $\alpha_{\mathrm{C}-} = 2/6$, $\beta_{\mathrm{C}} = 3/6$, $\sigma_{\mathrm{C}} = 1/6$. (The drawing is adapted from ref 20, used with permission.) ϕ_{ip} represents the volume fraction of ion-pairs, and each ion-pair is assumed to occupy the normalized volume of (ω_{A} + ω_{C}). The ionic charges on the polymer can be either on the backbone or on short pendant groups off the backbone.



Counterion-monomer and monomer-monomer associations are not frozen ionic bonds,[14] but can permute positions along the polyelectrolyte backbones, giving rise to a (combinatoric) binding entropy. The corresponding free energy contribution due to the number of different combinations of distributing ion-pairs, bound counterions, and free (or unpaired) PE monomers along PE backbones, is given by,[13, 40, 50, 51]

$$f^{\,\mathrm{comb}} = -\frac{\upsilon_{\mathrm{W}}\, S^{\mathrm{comb}}}{k_{\mathrm{B}} V} = f_{\mathrm{A}}^{\mathrm{comb}} + f_{\mathrm{C}}^{\mathrm{comb}} + f_{\mathrm{CA-\Lambda}}^{\mathrm{comb}} \tag{6}$$

with,

$$f_{\mathrm{A}}^{\mathrm{comb}} = -\frac{\upsilon_{\mathrm{W}}\, S_{\mathrm{A}}^{\mathrm{comb}}}{k_{\mathrm{B}} V} = \frac{\phi_{\mathrm{A}}}{\omega_{\mathrm{A}}}[\alpha_{\mathrm{A+}} \ln \alpha_{\mathrm{A+}} + \beta_{\mathrm{A}} \ln \beta_{\mathrm{A}} + (1 - \alpha_{\mathrm{A+}} - \beta_{\mathrm{A}}) \ln(1 - \alpha_{\mathrm{A+}} - \beta_{\mathrm{A}})] \tag{7}$$

$$f_{\mathrm{C}}^{\mathrm{comb}} = -\frac{\upsilon_{\mathrm{W}}\, S_{\mathrm{C}}^{\mathrm{comb}}}{k_{\mathrm{B}} V} = \frac{\phi_{\mathrm{C}}}{\omega_{\mathrm{C}}}[\alpha_{\mathrm{C-}} \ln \alpha_{\mathrm{C-}} + \beta_{\mathrm{C}} \ln \beta_{\mathrm{C}} + (1 - \alpha_{\mathrm{C-}} - \beta_{\mathrm{C}}) \ln(1 - \alpha_{\mathrm{C-}} - \beta_{\mathrm{C}})] \tag{8}$$

and

$$f_{\mathrm{CA}}^{\mathrm{comb}} = -\frac{\upsilon_{\mathrm{W}}\, S_{\mathrm{CA}}^{\mathrm{comb}}}{k_{\mathrm{B}} V} = -\frac{\phi_{\mathrm{C}}}{\omega_{\mathrm{C}}}\beta_{\mathrm{C}} \ln\left[\frac{\phi_{\mathrm{C}}}{\omega_{\mathrm{C}}}\beta_{\mathrm{C}}(\omega_{\mathrm{A}} + \omega_{\mathrm{C}})\right] \tag{9}$$

The equilibrium values of the extents of binding reactions, i.e. $\alpha_{\mathrm{A+}}$, $\alpha_{\mathrm{C-}}$, and β_{C}, in each phase are determined by minimization of the free energy, given in eq 1, with respect to these extents, under the constraints of electroneutrality, incompressibility, and ion-pairing stoichiometry. This leads to an equilibrium constant for each binding reaction, referred to as laws of mass action (LMA's). See our recent study for the details.[40, 42]

We numerically minimize the free energy, given in eq 1, with respect to the degrees of freedom of the system,[40] subjected to the aforementioned constraints applied to each of the co-existing phases, the LMA's, and an overall mass balance equation for each species,

$$C_i = C_i^{\mathrm{PEC}} \upsilon^{\mathrm{PEC}} + (1 - \upsilon^{\mathrm{PEC}}) C_i^{\mathrm{sup}} \tag{10}$$

Here C_i^{PEC} and C_i^{sup} denote, respectively, the concentrations of the species i in the PEC and in the supernatant, and υ^{PEC} is simply the volume fraction of the PEC phase ($\upsilon^{\mathrm{PEC}} = V^{\mathrm{PEC}}/V$ with V^{PEC} being the PEC volume). The concentrations can be converted into respective volume fractions using the general formula $\phi_i = C_i \omega_i / C_{\mathrm{W}}$, with $C_{\mathrm{W}} = 55.56$ M being the molar concentration of water.[40]

To investigate the thermodynamics driving the overcharging phenomenon, we first perform a qualitative analysis, where we mimic the equilibrium "overcompensation" experiments of PE multilayers by Schlenoff and co-workers.[20] They brought a stoichiometric complex with equal moles of monomers of each polyelectrolyte into contact with a salt solution containing a large excess of polycation, and measured the equilibrium uptake of additional polycation into the complex. We compare the equilibrium properties of the overcharged complexes with those of the stoichiometric ones to identify the forces driving transfer of excess polyelectrolyte into the PECs. For the stoichiometric solution, we start with equimolar bulk concentrations of $C_{\mathrm{A}} = C_{\mathrm{C}} = 0.1$ mM, and perform the free energy minimization, resulting in a dense phase containing virtually all polyelectrolytes and a dilute supernatant phase. To obtain a complex bearing excess polycation charge, we take a large excess of polycation $C_{\mathrm{C}} = 10$ mM in the solution, while keeping the concentration of the minor polyelectrolyte (polyanion here) at the same value as in the stoichiometric complex ($C_{\mathrm{A}} = 0.1$ mM). Minimization of the free energy then yields a dense phase containing nearly all of the polyanion and some larger amount of the polycation, coexisting with a supernatant consisting of the remaining excess polycation chains, where the volume of the supernatant phase is two orders of magnitude larger than that of the dense phase.

For the qualitative studies, we employ the same standard parameter values as in our recent study,[42] i.e. we set $\Delta G_{\mathrm{A+}} = \Delta G_{\mathrm{C-}} = -4\, k_{\mathrm{B}}T$, $\Delta G_{\mathrm{CA}} = -6\, k_{\mathrm{B}}T$, $\omega_{\mathrm{P}} = \omega_{\mathrm{A}} = \omega_{\mathrm{C}} = 6.5$, $N_{\mathrm{A}} = N_{\mathrm{C}} = 1000$, and $\chi_{\mathrm{PW}} = 0.5$. Additionally, since only a fixed PE conformation can be fed in the RPA approach, we take the statistical segment length of the polyelectrolytes to be $b_{\mathrm{A}} = b_{\mathrm{C}} = 2.5\, d_{\mathrm{P}}$, where d_{P} is the diameter of the polyelectrolyte monomers ($d_{\mathrm{P}} = (\omega_{\mathrm{P}} v_{\mathrm{W}})^{1/3} \approx 5.8$ Å). With this diameter, the polymer segment length is 14.5 Å, which is comparable to that of PSS in PDADMA/PSS complexes in simple salts ($b_{\mathrm{PSS}}^{\mathrm{experiment}} \approx 11$ Å).[43] Furthermore, for simplicity we assume that salt ions occupy the same molecular volume as water, i.e. $\omega_{\mathrm{S}} \equiv v_{\mathrm{S}}/v_{\mathrm{W}} = 1$. Note that, as highlighted in our recent study, this assumption should be relaxed when a quantitative comparison with real systems and experimental data is intended, due to the significant influence of the hydration shell of the salt ions on their translational entropy and hence, on the PEC phase behavior.

At the end of this paper, we compare quantitatively the predictions of the theory with the overcharging data for multilayers of PDADMA/PSS in KBr and NaCl solutions.

Results

The large 100-fold excess of polycation ensures that the composition of the PEC phase is insensitive to the concentrations C_{A} and C_{C}. The very small concentration of polyanion leads to a very small volume fraction of PEC over most salt concentrations; hence PEC formation has a negligible effect on the concentration of polycations in the supernatant. We define the degree of overcharging, Δ_{C}, as the relative excess of polycation with respect to polyanion in the complex,

$$\Delta_{\mathrm{C}}(\%) = (\frac{C_{\mathrm{C}}^{\mathrm{PEC,\,non-st}}}{C_{\mathrm{A}}^{\mathrm{PEC,\,non-st}}} - 1) \times 100 \ = (\frac{\phi_{\mathrm{C}}^{\mathrm{PEC,\,non-st}}}{\phi_{\mathrm{A}}^{\mathrm{PEC,\,non-st}}} - 1) \times 100 \tag{11}$$

where $\phi_i^{\mathrm{PEC,\,non-st}}$ ($C_i^{\mathrm{PEC,\,non-st}}$) is the volume fraction (concentration) of polyelectrolyte i in the overcharged PEC phase of the non-stoichiometric solution. In this work, variables superscripted with “st” and “non-st” are associated with the stoichiometric and non-stoichiometric cases, respectively.

Driving force for overcharging – effect of added salt concentration

We first analyze how salt addition impacts the adsorption of excess polycations by a stoichiometric complex. Plotted in Figure 1a are the binodal diagrams for the stoichiometric and non-stoichiometric cases, which exhibit increases in the concentrations of polycation and its counterion in the PEC upon overcharging, over the stoichiometric PEC. Figure 1b shows the degree of overcharging of the PEC for the non-stoichiometric case as a function of the added salt concentration C_S, which is almost identical to the salt concentration in the supernatant, given the very small volume fraction of the PEC. The adsorption of excess polycation initially increases with salt concentration C_S, reaching in this case a maximum of 100%, and then decreases for $C_S > 500$ (mM), indicating loss of polycation from the overcharged PEC. This *erosion* of the overcharged complex can be attributed to extensive binding of salt ions to the polyelectrolytes as a result of reduced mixing entropy of salt ions, as will be analyzed shortly. The existence of a maximum in overcharging as a function of salt concentration has been observed in prior Layer-by-Layer (LbL) experimental studies and is discussed at the end of this section.[52-54]

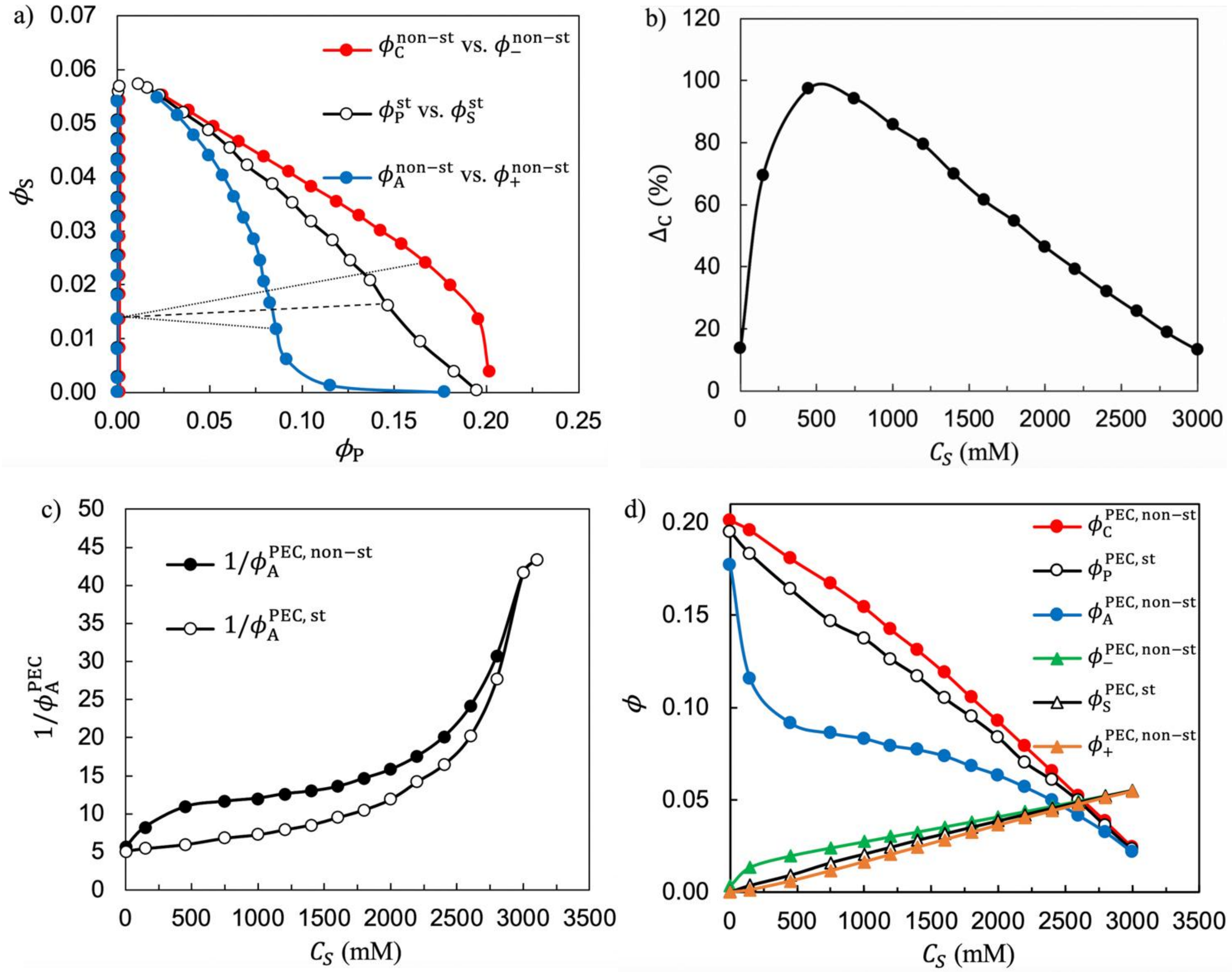


Figure 1. (a) Binodal phase envelopes, (b) the extent of polycation overcharging, (c) the swelling ratio of the PEC $1/\phi_A^{PEC}$, and (d) the volume fractions of polyelectrolytes and salt ions in the PEC for the stoichiometric and non-stoichiometric cases as functions of the added salt concentration, C_S. “P” in the subscript denotes either one of the two polymers in the stoichiometric case, while “S” denotes the salt. The color of the lines in (d) corresponds to the color of species (in Scheme 2), and open and filled symbols denote properties in stoichiometric and non-stoichiometric cases, respectively. In (a) and following figures the dashed lines on binodal diagrams show representative tie-lines. The parameters have the standard values, listed before the Results section.

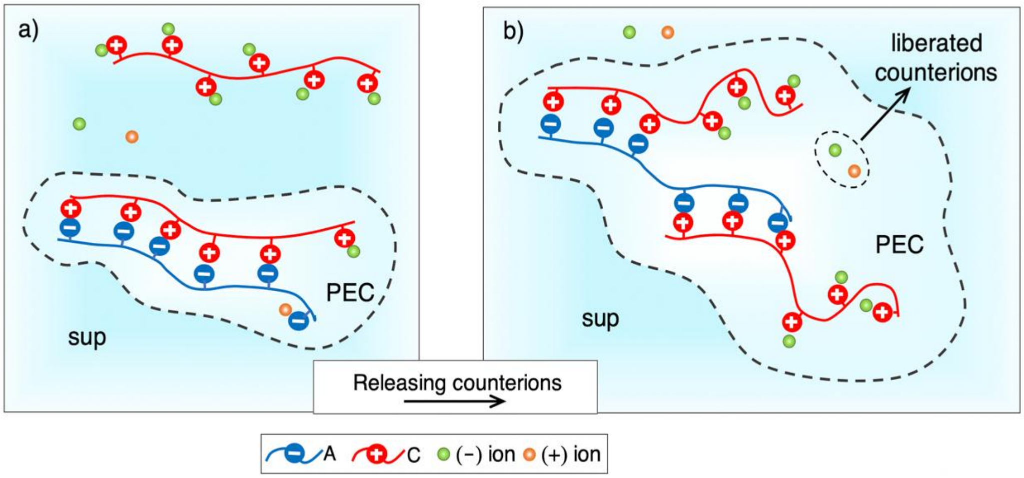
Figure 1c shows the swelling ratio of the PEC, $1/\phi_{\mathrm{A}}^{\mathrm{PEC}}$. We note that the minor polyelectrolyte, polyanion here, remains entirely in the PEC unless C_S is near the dissolution point ($C_S \approx$ 3150 mM). For the stoichiometric case, the dependence of the swelling ratio on salt concentration resembles that of experimental PECs doped with salt.[42, 48] As seen here, both the stoichiometric and non-stoichiometric complexes expand with added salt, and at any salt concentration, adding excess polycation to a stoichiometric mixture increases the complex volume ($1/\phi_{\mathrm{A}}^{\mathrm{PEC,\,non-st}} > 1/\phi_{\mathrm{A}}^{\mathrm{PEC,\,st}}$). The difference between the stoichiometric and overcharged swelling ratios ($1/\phi_{\mathrm{A}}^{\mathrm{PEC,\,non-st}} - 1/\phi_{\mathrm{A}}^{\mathrm{PEC,\,st}}$) can be interpreted as the volume expansion due to the adsorbed polycation mass. Near $C_S \approx$ 500 mM the difference is maximum, suggesting a high amount of the excess polycation mass adsorbed, which reasonably correlates with the overcharging trend in Figure 1b.

Both the adsorbed polymer mass and uptake of water and salt contribute to the increase in the volume of the complex, with the uptake of water and salt being dominant at high salt concentrations. This explains why, although the extent of overcharging is small at high salt (say, $C_S >$ 2500 mM), the PEC volume reaches a steep maximum due to the uptake of water and salt. Such information may help to engineer polyelectrolyte multilayers with desired polymer mass and thicknesses.

The volume fractions of ionic species in the stoichiometric and overcharged complexes are shown in Figure 1d. Interestingly, the dissimilarity between the stoichiometric and non-stoichiometric cases diminishes at high salt. A similar trend is observed in the fractions of counterion binding and ion-pairing for each type of polyelectrolyte (α's and β's) in the complex, illustrated in Figure S1 in the Supporting Information (SI). These trends reflect a ubiquitous feature of overcharging experiments, i.e. the overcharged PECs become more stoichiometric, and the overcharging capability decreases, at very high salt concentrations near the dissolution point ($C_S \approx$ 3150 mM).[55]

Figure S2 in the Supporting Information (SI) also reveals that, interestingly, the fraction of polyanion monomers bound to small cations in the overcharged complex, $\alpha_{\mathrm{A+}}^{\mathrm{PEC,\,non-st}}$, is lower than that in the stoichiometric one, $\alpha_{\mathrm{PS}}^{\mathrm{PEC,\,st}}$, at any fixed C_S, indicating that some polyanion counterions are liberated upon adsorption of excess polycation. ("P" denotes either the polycation or polyanion, since both polyions bind the same number of counterions in the stoichiometric case.) This points to the gain in translational entropy of counterions as a possible driving force for the adsorption of excess polycation (see Scheme 2).

Scheme 2. (a) Exposure of a stoichiometric PEC to polycation solution results in (b) complexation of some of the excess polycations with the PEC polyanions, and liberation of some counterions. "sup" denotes the supernatant phase.



To explore this, we calculate the concentration of each type of counterion released from (i.e., no longer bound to) each type of polyelectrolyte upon adsorption of excess polycation by the stoichiometric PEC at fixed (added) salt concentration. We do this by finding the difference between the stoichiometric and non-stoichiometric PECs in the total number of counterions bound to PEs. Plotted in Figure 2 are the concentrations of released cations, $C_{+}^{\mathrm{released}}$, and of released anions, $C_{-}^{\mathrm{released}}$, from the polyelectrolytes in the PEC during overcharging. The explicit expressions for the concentrations of released ions are provided in Appendix A.

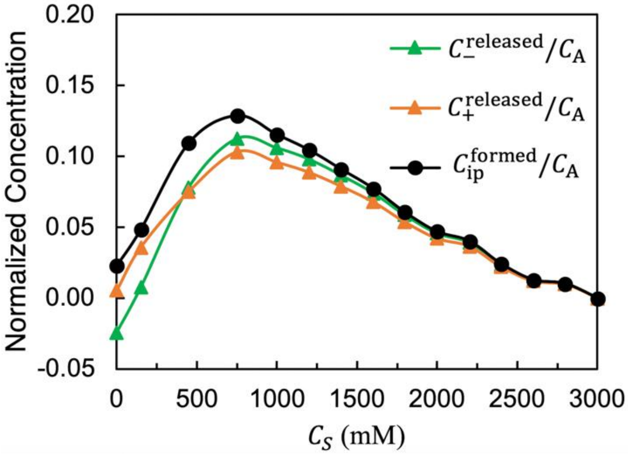


Figure 2. Normalized concentrations of released counterions and of newly formed ion-pairs released by overcharging as functions of added salt concentration. Note that concentrations are normalized by the overall concentration of polyanion ($C_\mathrm{A} = 0.1$ mM). The parameters have standard values.

As seen in this figure, the concentrations of released counterions, i.e. C_+^{released} and C_-^{released}, are almost the same over a wide range of salt concentration, implying a nearly one-to-one release of polyanion and polycation counterions upon overcharging, especially at high salt. It is interesting that these concentrations, especially at high salt, nearly match the molar concentration of newly formed ion-pairs, $C_{\mathrm{ip}}^{\mathrm{formed}}$, whose expression is also provided in Appendix A. This indicates that the counterions are liberated by the formation of ion-pairs between excess polycations and the PEC polyanions. We note that, at low salt, there are a small fraction of charge groups on polyanions that are neither ion paired nor bound to salt ions that can *also* form ion-pairs with the free charge groups on incoming polycation without releasing any counterions, giving rise to slightly more ion-pairs formed than counterions released (as can be seen at $C_S < 1000$ mM in Figure 2).

To gain further insight into the forces responsible for the adsorption of excess polycations, we now quantify the entropy change associated with overcharging. To do so, we develop an expression for the entropy change of the solution, ΔS, as excess polyelectrolyte is adsorbed onto the overcharged complexes. The full expression is developed in Appendix B, and accounts for changes in the translational entropy of salt ions ΔS^{T} and the combinatorial entropy of ion-binding sites along the chains ΔS^{comb}. To calculate ΔS^{T}, we note that $-k_\mathrm{B} \ln \phi_\mathrm{S}^{\mathrm{f},\alpha}$

denotes the translational entropy of each single (unbound) salt ion of type S residing in the phase α. Using this and the concentration of released counterions, we can easily calculate the gain in ΔS^{T}. In addition, the binding entropy of oppositely charged species to the polyelectrolytes in the complex can change upon overcharging. This entropy accounts for the number of microstates (or configurations) of a polyelectrolyte, each corresponding to a polyelectrolyte configuration with a specific arrangement of ion-pairs, bound counterions and free (or unpaired) monomers along the backbone. We find the combinatorial binding entropy ΔS^{comb} by using equations 7 and 8 to find the combinatorial entropy for each single polyanion and polycation chain before and after polycation adsorption (i.e. using the stoichiometric and non-stoichiometric cases). We note that entropic considerations from the excess components of our free energy model (i.e. the RPA electrostatic term) are neglected from this analysis. In addition, we keep in mind that although the salt concentration in the PEC phase varies upon adsorption of excess polycation, the (added) salt concentration averaged over both phases C_S remains almost identical to the supernatant salt concentration due to the very small volume fraction of the PEC. However, in our calculations of the entropy changes and also of released counterions, we use the exact composition of the phases, obtained from the free energy minimization.

The net entropy change, ΔS, which is the sum of the contributions ΔS^{T} and ΔS^{comb}, upon overcharging as a function of salt concentration C_S is plotted in Figure 3. As expected, the dependence of the gain in the translational entropy of counterions, ΔS^{T}, on the salt concentration is similar to that of the concentrations of released counterions ($C_{\pm}^{\mathrm{released}}$ in Figure 2).

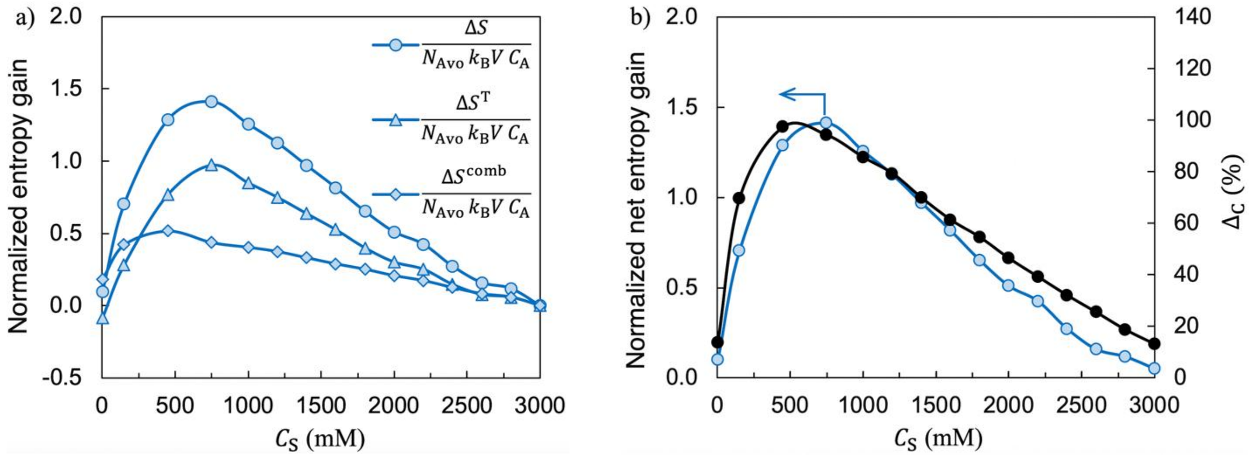


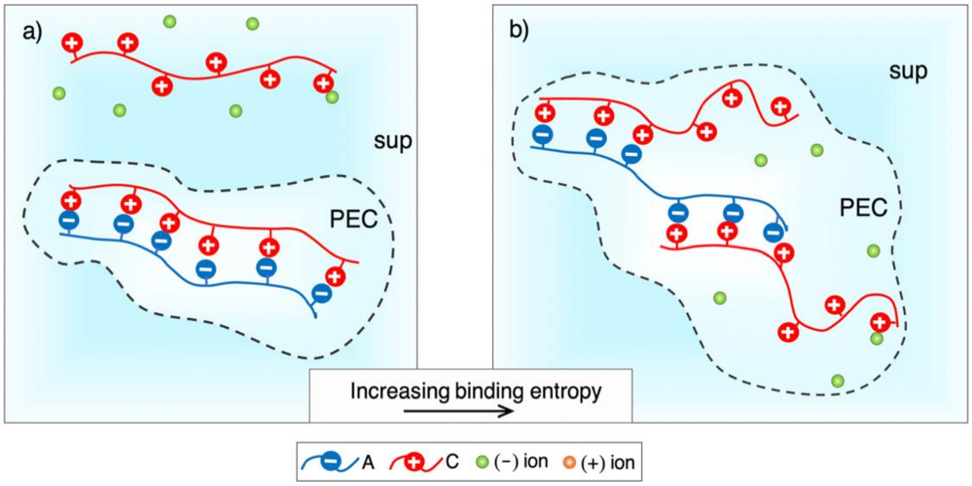
Figure 3. a) Normalized net entropy change (ΔS, blue lines with circles), and its translational (ΔS^{T}, blue line with triangles) and combinatorial (ΔS^{comb}, blue line with diamonds) contributions upon adsorption of excess polycation onto the stoichiometric complex. Note that $\Delta S = \Delta S^{\text{T}} + \Delta S^{\text{comb}}$ and that entropies are normalized by the overall concentration of polyanion ($C_{\text{A}} = 0.1$ mM). b) Normalized net entropy change (ΔS) and degree of overcharging from Figure 1b. The parameters have standard values.

A surprising observation in Figure 3a is that the combinatorial binding entropy ΔS^{comb} is almost as significant as the counterion release entropy ΔS^{T}. To understand the role of ΔS^{comb}, we examine the adsorption behavior at zero (added) salt, whereby the contribution from counterion release is negligible because in the stoichiometric PEC the polyelectrolytes are almost fully ion-paired and harbor essentially no counterions (notice $\alpha_{\text{PS}}^{\text{PEC, st}} \approx 0$ at zero salt in Figure S2) to be released. Thus, the overcompensation of $\Delta_{\text{C}} \sim 15\%$ seen at zero salt in Figure 1b is caused by the change in the binding entropy alone. In the salt-free condition, since the polyelectrolytes are almost fully ion-paired in the stoichiometric PEC (see Scheme 3(a)), fewer microstates are available for these polyelectrolytes, resulting in a less favorable (combinatorial) binding entropy. Migration of excess polycations from the solution into the PEC allows polyanion sites to transfer some of their ion pairing to the incoming polycations, enabling the polycations already present in the complex to move off some of the polyanion sites. This increases the number of microstates for the polycations in the PEC and so the binding entropy (see Scheme 3(b) and the SI). The increase in the binding entropy thus explains the non-zero overcharging value at zero salt.

In support of this result, molecular dynamics (MD) simulations as well as prior experimental works of multilayer formation have reported overcharging in *salt-free* solutions,[56, 57] where no counterion release effects are expected. Furthermore, a host of experimental and simulation studies have also suggested the existence of a large number of tails and loops of excess PE protruding out of PECs.[56, 58-60] These observations can be rationalized by the binding entropy effects. In parallel, recent studies by Sing and coworkers have revealed the key role of a similar entropic contribution originating from an increase in the number of polyanion-polycation chain interactions in polyelectrolyte complexation.[17, 18]

Counterion release has been suggested as a driving forces for overcharging and the adsorption of excess polyelectrolyte.[38, 61, 62] Our findings quantitatively substantiate this, and expose combinatorial binding entropy as a second, equally important, mechanism. The entropic forces driving adsorption of excess polyelectrolyte obviously play a key role in the complexation of PEs in *stoichiometric* mixtures as well; interestingly, contributions from both translational and combinatorial entropies constitute the main part of the doping equilibrium constant of the PEC, derived in our recent study,[42] which confirms the intuition of Schlenoff and coworkers.[48]

Scheme 3. (a) Exposure of a stoichiometric PEC to a supernatant solution of excess polycation at zero added salt, where polyelectrolytes are almost fully ($\sim$ 90%) ion-paired in the PEC. (b) Complexation of some of the excess polycations with the PEC polyanions, driven by increase in combinatorial entropy ΔS^{comb}. Note that in our model the unpaired monomers of (polycation) chains are randomly dispersed along the chains, rather than forming non-random runs along the contours of the chains.



The net entropy gain upon adsorption of excess polycation roughly mirrors the non-monotonic overcharging trend (see Figure 3b), further demonstrating that overcharging is an entropic process, consistent with experimental observations in the literature.[19, 37, 63] At intermediate salt levels, the transfer of polycations to the complex is favored by (1) a higher extent of counterion release (see $C_{\pm}^{\mathrm{released}}$ in Figure 2 and ΔS^{T} in Figure 3a), and (2) an increase in the combinatorial entropy of the polyelectrolytes (see ΔS^{comb} in Figure 3a). At high salt concentrations, however, most polyelectrolyte sites are increasingly bound with the salt ions due to a substantial decrease in translational (or mixing) entropy of the salt ions (compared to low-intermediate salt concentrations). As a result, at high salt the gains in translational and combinatorial entropy are less due to the release of fewer bound ions (see Figure 2), leading to less adsorption of excess polycation. The result is a non-monotonic dependence of polycation adsorption on salt concentration.

In light of this prediction, in buildup of Layer-by-Layer (LbL) films one can expect the highest mass of polyelectrolyte to be deposited at intermediate salt concentrations. In fact, as mentioned earlier, in LbL experiments a maximum in film growth rate is observed at intermediate salt concentration, corresponding to a maximum in overcharging upon addition of each new layer.[52-54] For instance, this behavior is exhibited in Figure 4a, which shows the total amount of adsorbed polymers in the buildup of a nine-layer film made of quaternized poly(N-ethyl-4-vinylpyridinium bromide), QPVP, and poly(methacrylic acid), PMAA in NaCl solution from ref. 54.

To scrutinize the origin of the maximum in our overcharging predictions, we redo the overcharging calculations, but this time with a simplified free energy model without ion binding effects which reads $f = f^{\mathrm{T}} + f^{\chi} + f^{\mathrm{corr}}$. Since $\chi_{\mathrm{PW}} = 0.5$, any overcharging and complexation would be attributed in this case to the electrostatic correlations, f^{corr}. Figure 4b exhibits an almost *constant* polycation overcharging prediction of $\approx 45\%$ with the salt concentration for this case, which does not follow qualitatively the experimental trend of Figure 4a. Interestingly, doing the reverse, i.e. turning off the electrostatic correlations which leads to $f = f^{\mathrm{T}} + f^{\chi} + f^{\mathrm{rxn}} + f^{\mathrm{comb}}$, does not affect the existence nor the height of the maximum in the adsorbed amount of excess PE. This implies that at least for the multilayers of QPVP/PMAA, the translational and combinatorial entropies that drive overcharging owe their origin mostly to the ion binding effects (possibly as a result of strong ion binding effects), and not to the electrostatic correlations.

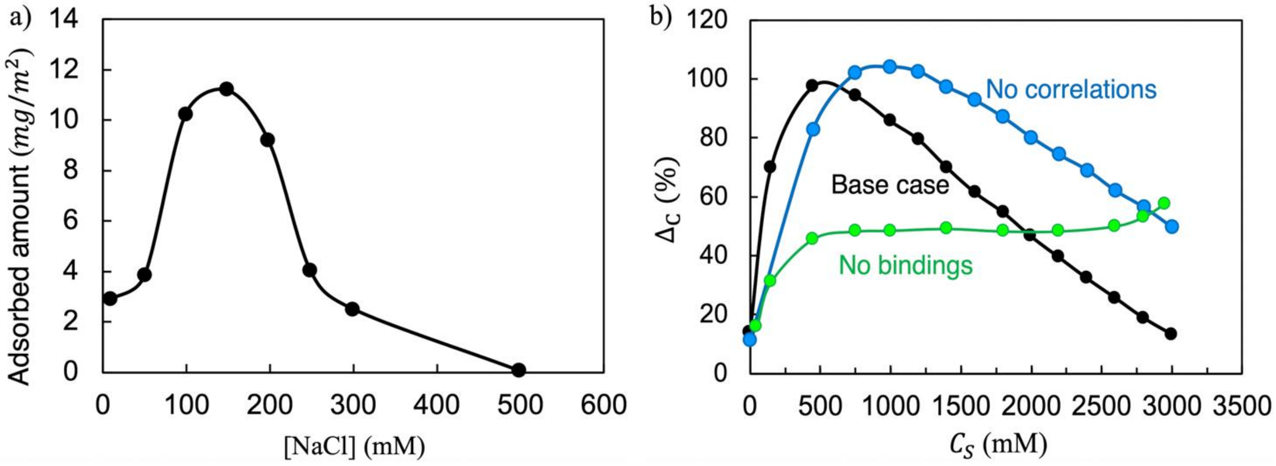


Figure 4. (a) Total polymer adsorbed in the build-up of a nine-layer QPVP (Mw 330K, DP 1600)/PMAA (7K, DP 65) films deposited at pH 8.4 at different added NaCl concentrations from ref. 54. (b) Overcharging predictions for our standard or the base case, where all free energy contributions are present (black), in the absence of the electrostatic correlations, where $f = f^{\mathrm{T}} + f^{\chi} + f^{\mathrm{rxn}} + f^{\mathrm{comb}}$ (blue), and in the absence of the ion binding with $f = f^{\mathrm{T}} + f^{\chi} + f^{\mathrm{corr}}$ (green). Note that when ion binding effects are considered as well as in our standard case, PEs get almost fully paired, leaving very few unpaired (or free) monomers. The binodal diagrams for the two cases of "No bindings" and "No correlations" of Figure 4b are available in Figure S3.

Effects of the strengths of ion-pairing and counterion binding

The types of salt ions and charged groups on polyelectrolytes affect the strengths of local ion binding, and therefore the degrees of ion-pairing and counterion binding, which in turn affect coacervation behavior. Most models and simulations of coacervation proposed so far treat polymers as strings of generic beads and salt ions as simple charged spheres, with no ion- or monomer-specific parameters. Our model is distinctive in that it captures, albeit phenomenologically, specific interactions that can reflect the local environment of ions and PE charged groups through assignment of binding free energies, i.e., $\Delta G_{\mathrm{A+}}$, $\Delta G_{\mathrm{C-}}$, and ΔG_{CA}. These binding parameters could be obtained experimentally, using spectroscopic techniques such as fluorescence spectroscopy,[64] or through atomistic simulations. We recently highlighted the role of ion-specificity effects in the doping and salt partitioning behavior of stoichiometric PECs.[42]

Figures 5a and 5b show, respectively, the binodal diagrams and polycation overcharging predictions as functions of salt concentration for various strengths of ion-pairing between charged groups on polyanion and polycation, ΔG_{CA}.

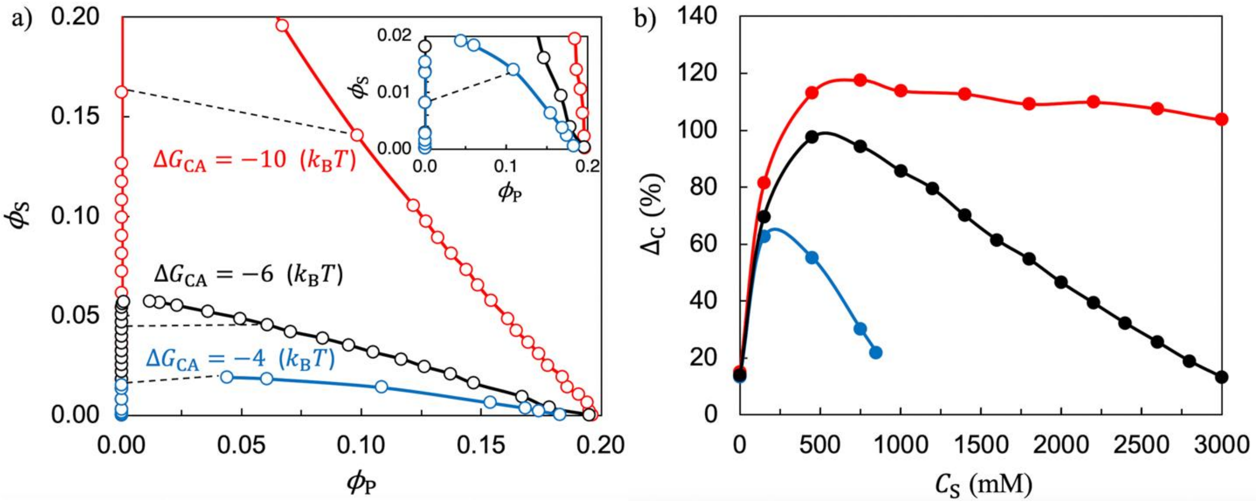


Figure 5. (a) Stoichiometric (binodal) phase diagrams with enlargement in the inset, and (b) degrees of polycation overcharging against added salt concentration (for the non-stoichiometric mixture) for various values of ion-pairing

strength ΔG_{CA}. The binodal diagrams for the ion-pairing strengths of $\Delta G_{\mathrm{CA}} = -10\ k_{\mathrm{B}}T$ and $\Delta G_{\mathrm{CA}} = -4\ k_{\mathrm{B}}T$ are available in Figure S4. The rest of parameters have standard values.

Increasing $-\Delta G_{\mathrm{CA}}$ leads to stronger associations of polyanion and polycation charged groups, and therefore increases the critical salt concentration (see Figure 5a). Correspondingly, the range of salt concentration over which adsorption of excess polycation can occur (i.e., the “overcharging window”) expands (see Figure 5b).

A higher ion-pairing strength in general increases the fraction of ion-pairing. But in the stoichiometric PEC this reduces the number of microstates (or configurations) for the polyelectrolytes, since most PE monomers get ion-paired, causing an unfavorable binding entropy. Similar to the mechanism of overcharging at low salt concentration discussed above, when the PEC is exposed to excess polycation solution, some of the polycations transfer into the PEC to increase the binding entropy, ΔS^{comb} (see Figure S5a). Thereby, stronger ion-pairing leads to higher overcharging. The concentration of the released counterions and the entropy gains upon overcharging are plotted in Figure S5 in the SI.

As shown in Figure S6 of the SI, the PEC volume fraction decreases with increasing strength of ion-pairing and becomes a linear function of salt concentration for very strong binding strengths. This prediction agrees well with the experimental findings of Dubas and Schlenoff that more strongly interacting polyelectrolyte pairs, such as polyallylamine hydrochloride (PAH) and PSS, form thinner multilayer films for the same polyelectrolyte mass and that their thicknesses increase linearly with salt concentration.[65]

In the following, we explore the effect of the strength of salt binding, or counterion binding ΔG_{CC}, on the adsorption of excess polyelectrolyte, while keeping fixed other parameters, including the size of salt ions as well as the ion pairing free energy. The strength of salt binding to polyelectrolytes should depend on salt type, for example following the Hofmeister series of $(\mathrm{F}^- < \mathrm{CH_3COO}^- < \mathrm{Cl}^- < \mathrm{Br}^- < \mathrm{I}^- < \mathrm{ClO}_4^-,\ )$ for anions;[47, 66] previous experiments have shown that the less hydrated salt ions bind more strongly to polyelectrolytes.[47, 66]

Here, for simplicity, we keep the strength of the cation binding to polyanions identical to that of the anion binding to polycations; i.e. $\Delta G_{\mathrm{CC}} \equiv \Delta G_{\mathrm{A+}} = \Delta G_{\mathrm{C-}}$.

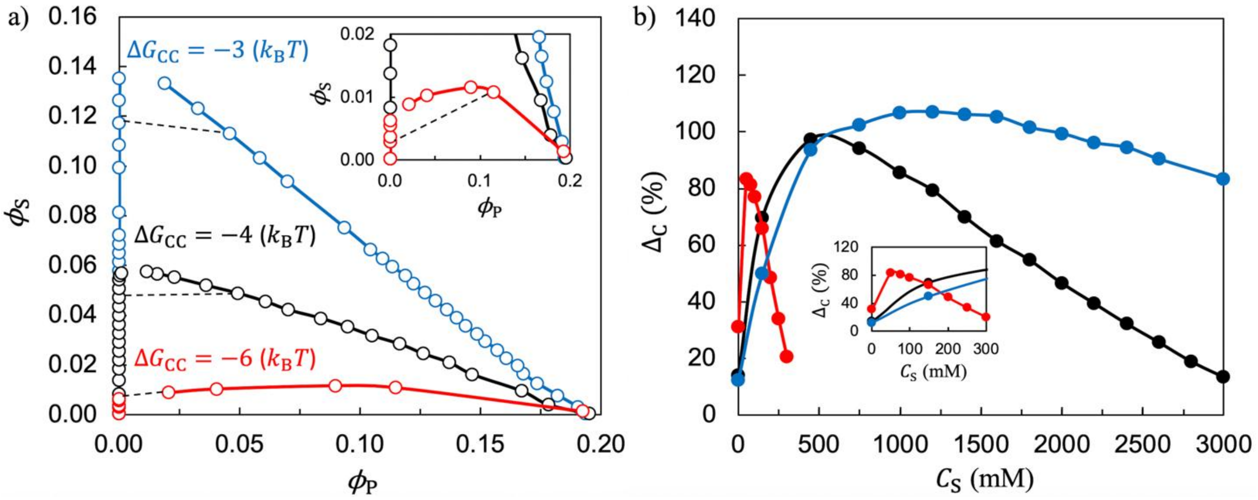


Figure 6. (a) Stoichiometric (binodal) phase diagrams, and (b) extents of polycation overcharging against added salt concentration (for the non-stoichiometric mixture) for various values of counterion binding strength ΔG_{CC}, with enlargements in insets. The rest of parameters have standard values.

Strong counterion binding reduces the critical salt concentration, as seen in the binodal diagrams in Figure 6a, and likewise, the overcharging window shrinks (Figure 6b). Generally, stronger binding of salt ions to polyelectrolytes acts qualitatively like adding more salt ions of fixed binding strength to the solution,[32] since both of these act to increase the driving force for counterion binding.

The inset plot of Figure 6b shows that, for stronger ΔG_{CC} (i.e., the red curve), the adsorption of polycations is higher at low salt, say less than 100 mM, while the adsorption is reduced at high salt concentrations. We will compare this prediction with relevant experimental observations shortly. The adsorption at low salt is increased when $-\Delta G_{\mathrm{CC}}$ is raised, because counterion binding becomes more pronounced for polycations in the supernatant (see Figure S7a), which leads to a greater release of counterions upon overcharging (see Figure S7b). At higher salt, however, the entropy gains driving the adsorption of excess polycation more easily weakens due to a drop in the translational entropies of salt ions and subsequent binding of salt ions to PEs, hence leading to smaller overcharging.

Higher strengths of PE-counterion binding also lead to larger volume fractions of the PEC phase (Figure S8) at a fixed salt concentration, the cause of which was discussed in our recent study.[42] This is in accord with several experimental studies that reported thicker multilayer films for more strongly binding salt ions in the Hofmeister series.[66, 67]

We note that the representative tie lines in the binodal diagrams of Figure 5a as well as in Figure 6a can tilt in either direction, depending on the ion binding strengths. A consistent trend between Figures 5a and 6a is observed, which falls in line with our recent doping study: easier dissociation of ion-pairs, which is the result of either weak ion-pairing or strong salt bindings to the polyelectrolytes, leads to preferential partitioning of salt ions into the PEC and positive tie-line slopes, while the reverse leads to for negative tie-line slopes. These trends accord with the experimental observations of Schlenoff et al., that the partitioning preference of salt ions between the PEC and the supernatant depend on the identities of salt ions and PE monomers, which influence their binding strengths.[48]

Comparison with experiments

In this section, we attempt to compare quantitatively our theory with experiments for overcharging. However, to the best of our knowledge, all experimental studies of overcharging in the literature are limited either to nanoscopic PECs obtained in titration studies[19] or polyelectrolyte multilayers (PEMU's),[20] rather than coacervates. Although the responses of multilayers and complex coacervates to external stimuli, such as salt concentration, are quite similar,[54] the difference in their morphologies (i.e., periodic vs. uniform composition) makes quantitative comparison between the two uncertain. Nevertheless, the order of magnitude of the degree of overcharging and its qualitative behavior are expected to be similar between multilayers and polyelectrolyte complexes, especially if the multilayers are well solvated and have been given time to equilibrate.

We explore overcharging of PDADMA/PSS in two different salt solutions: KBr and NaCl. In our recent study,[42] we used the following parameters and those listed in Table 1 (first row) to model *stoichiometric* PECs made of PDADMA/PSS in KBr, for which good agreement in doping and phase behavior of the PECs were found between the model and experiment. In that study, the ion-pairing strength between polyanion and polycation repeat units was set at $\Delta G_{\mathrm{CA}} = -6\ k_{\mathrm{B}}T$, and the FH parameter at $\chi_{\mathrm{PW}} = 0.6$. We used a degree of polymerization of N_{P}=1000 (which is large enough that results are rather insensitive to its value) and the experimental value of monomer size of $\omega_P = 6.5$ for both polyelectrolytes. The statistical segment length was set to $b_{\mathrm{P}} = 1.9\ d_{\mathrm{P}}$, with $d_{\mathrm{P}} = (\omega_{\mathrm{P}} v_{\mathrm{W}})^{1/3} \approx 5.8$ Å, giving $b_{\mathrm{P}} = 11.02$ (Å) which is the average statistical segment length of PSS in PDADMA/PSS PECs.[43, 68] There,[42] we highlighted the importance of the hydration shell of ions in the thermodynamics of PECs. Specifically, the effective size of ions was redefined as $\omega_{\mathrm{S}} = v_{\mathrm{S}}/v_{\mathrm{W}} + n$, with n being the number hydrating waters, and we treated these waters as part of the ions, increasing their effective sizes.

Table 1. Parameters used in the theory to model PECs of PDADMA/PSS in KBr solution (top row) and in NaCl (bottom row). $\Delta G_{\mathrm{A+}}$ and $\Delta G_{\mathrm{C-}}$ for KBr were obtained by fitting in previous work.

salt	ω_{S}	$\Delta G_{\mathrm{A+}}$ ($k_{\mathrm{B}}T$)	$\Delta G_{\mathrm{C-}}$ ($k_{\mathrm{B}}T$)
KBr	2.4	−3.85	−3.85
NaCl	3.2	−3.5	−2.0

Using the same parameters as used previously to model stoichiometric PECs of PDADMA/PSS in KBr,[42] in Figure 7a we find qualitative agreement between the experimental data and the predicted degree of overcharging of the PEC when it is exposed to 10 mM of polycation at different added salt concentrations. As mentioned earlier, we should not expect a perfect match, given the difference in the morphologies of multilayers and complex coacervates, and also the uncertainties in our parameter values and model assumptions. We note that, similar to our recent study,[42] agreement between theory and experiment is found only when using salt ion sizes larger than their bare sizes. Using $\omega_{\mathrm{S}} = 1$, i.e. assuming salt ions to occupy a molecular volume identical to that of water, generally leads to a higher maximum in overcharging ($>$ 100% such as in Figure 1b), as a function of salt concentration, than seen in typical LbL experiments. Nevertheless, an overcharging of even 100% has been reported for multilayers from PAH and PSS, which form especially strong ion-pairs.[69]

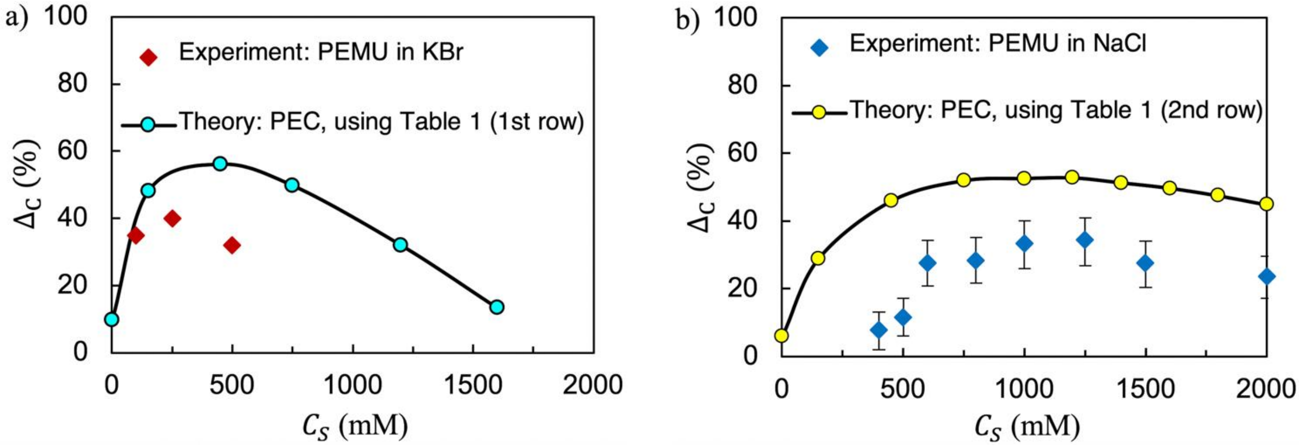


Figure 7. Predicted degree of overcharging Δ_{C} (line and circles) vs. experimental results for PDADMA/PSS multilayers (diamonds) in (a) KBr solution and in (b) NaCl solution. Here, the PEC in the theory, like the multilayer (PEMU) in the experiment, is in contact with a supernatant solution containing 10 mM polycation at different added salt concentrations.

Next, we focus on the same PDADMA/PSS mixture, but now in NaCl, rather than KBr. We assume that the ion-pairing strength, ΔG_{CA}, the FH interaction between polyelectrolytes and water, χ_{PW}, and the conformation of PEs remain the same as those in KBr solution. The only parameters that are allowed to change upon switching salt from KBr to NaCl are those listed in Table 1 that are directly related to the type of salt ions, i.e. the effective sizes of the salt ions and their binding strengths to PEs. Again, the effective sizes of sodium and chloride take into account their hydration shells; taking from literature the bare radii of sodium and chloride to be 0.95 Å and 1.81 Å, respectively, and their hydration numbers to be n = 3.7 and 2.0,[70] we get effective volumes of $\omega_{\mathrm{Na}^+} = 3.8$ and $\omega_{\mathrm{Cl}^-} = 2.6$ relative to water for sodium and chloride, respectively. Note that the bare volume of the ions is simply calculated as $\upsilon_{\mathrm{S}} = \frac{4}{3}\pi {r_{\mathrm{S}}}^3$with r_{S} being the radius of the bare ion, and that the effective volumes are given by $\omega_{\mathrm{S}} = \upsilon_{\mathrm{S}}/\upsilon_{\mathrm{W}} + n$. Here, for simplicity, we take both Na^+ and Cl^- to occupy an effective molecular volume of $\omega_{\mathrm{S}} = 3.2$, which is the average of the values for $\omega_{\mathrm{Na+}}$ and $\omega_{\mathrm{Cl-}}$.

It is believed that more hydrated ions generally bind more weakly to oppositely charged PEs; so, it is expected that $\mathrm{Na^+}$ and $\mathrm{Cl^-}$ (with average ω_S of 3.2) bind more weakly to PSS and PDADMA than do $\mathrm{K^+}$ and $\mathrm{Br^-}$ (with average ω_S of 2.4). This has been demonstrated in doping experiments of the Schlenoff group, where NaCl was found to be a weaker dopant than KBr.[47, 71]

Keeping this in mind, first, we treat $\Delta G_{\mathrm{A}+}$ and $\Delta G_{\mathrm{C}-}$ as free variables to match predictions with the experimental data for doping and salt partitioning of *stoichiometric* PDADMA/PSS PECs in NaCl. Then, we will use these parameters to find *a priori* overcharging predictions for the non-stoichiometric PECs. Following this procedure, we obtain values of $\Delta G_{\mathrm{A}+}$ and $\Delta G_{\mathrm{C}-}$ in Table 1 for NaCl that yield in Figures 8a and 8b good fits of the predictions of doping and salt partitioning of stoichiometric PECs of PDADMA/PSS in NaCl to experimental data. Interestingly, the obtained values for $\Delta G_{\mathrm{A}+}$ and $\Delta G_{\mathrm{C}-}$ accord with a recent work by the Sammalkorpi group showing that $\mathrm{Na^+}$ ions bind more strongly to PSS than $\mathrm{Cl^-}$ does to PDADMA.[72] Note that the match of predictions to the experimental results for KBr in Figure 8 is no surprise, since the parameters for KBr in Table 1 were fit to these data in previous work.[42]

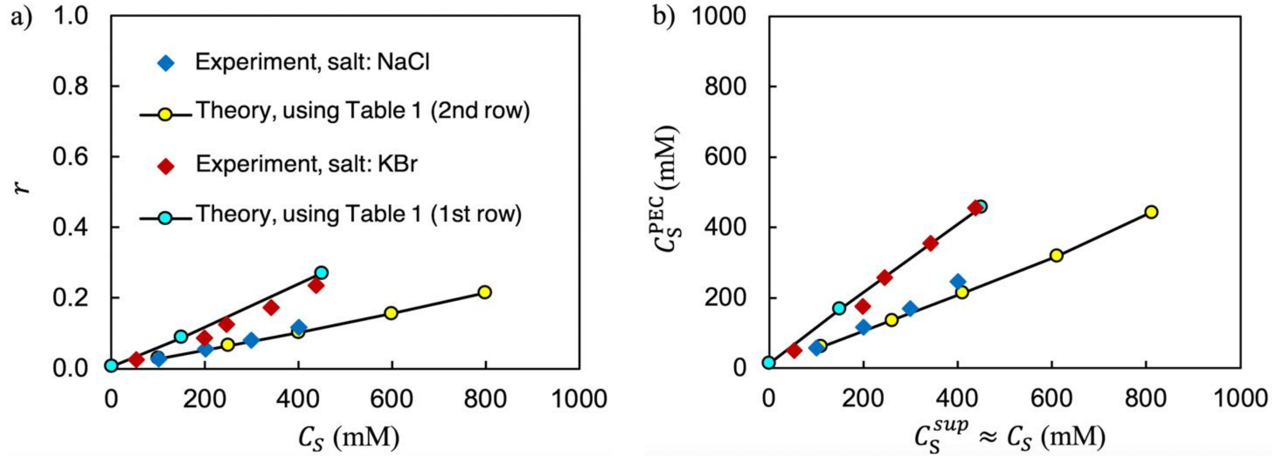


Figure 8. Theoretical predictions, lines and cyan-circles and lines and yellow-circles, using parameters in Table 1 (first row) and (second row) to model, respectively, *stoichiometric* PECs of PDADMA/PSS in KBr (dark red diamonds) and in NaCl (blue diamonds). (a) The molar ratio of salt to polyelectrolyte concentrations in the PEC, $r = [\mathrm{salt}]^{\mathrm{PEC}}/[\mathrm{PE}]^{\mathrm{PEC}}$, and (b) salt partitioning between the PEC and the supernatant. Note that the PEC occupies a very small volume out of the overall

volume both in experiment and theory, and hence the (added) salt concentration is almost the same as the salt concentration in the supernatant ($C_{\mathrm{S}}^{sup} \approx C_S$).

We observe that the more weakly binding NaCl prefers to partition into the supernatant, as demonstrated by Schlenoff et al.[48] The slope of the molar ratio of salt to PE concentrations in the PEC, $r = [\mathrm{salt}]^{\mathrm{PEC}}/[\mathrm{PE}]^{\mathrm{PEC}}$, at low C_S in Figure 8a is a signature of the doping *strength* of the salt,[47] where higher slopes mean stronger doping of the PEC, while smaller slopes mean weaker doping. For the set of parameter values used in Table 1, it is seen in Figure 8 that our theory conveniently captures the dependence of doping strength on the type of salt through the ΔG_{CC} parameters.

Using the parameters in Table 1, we now show in Figure 7b that the degree of overcharging when the PEC is exposed to a large excess of polycation in NaCl solution is in qualitative agreement with the experimental data. Interestingly, for both KBr in Figure 7a and NaCl in Figure 7b, the non-monotonic dependence of overcharging Δ_{C} on salt concentration is correctly (although only qualitatively) predicted by the theory. Also predicted is the weaker dependence of Δ_{C} on NaCl than on KBr concentration, consistent with the higher binding strength of the latter, leading to a higher salt molarity for dissolution for NaCl than for KBr. In addition, overcharging is higher at low molarities in KBr solution than in NaCl. Both of these observations are depicted in Figure 6 (and its inset plot), and thus can be attributed directly to the different strengths of binding of KBr ions compared to NaCl ions to the polyelectrolytes.

Note that at $[\mathrm{NaCl}] < 500$ mM, there is almost no overcharging in the experiment, while the predicted overcharging is appreciable. This can perhaps be attributed, at least in part, to the fixed Gaussian conformation assumed for the polyelectrolytes in the model; at low salt concentrations, it is expected that polyelectrolytes in the supernatant solution take more rod-like conformations. The Gaussian coil, rather than rod-like, structure in the supernatant enhances counterion binding on the polyelectrolytes,[41] and hence leads to increased transfer of excess polyelectrolyte into the complex to liberate their counterions (i.e. more gain in translational entropy). In the case of KBr, however, a coil-like conformation seems to yield better predictions at low molarity, which can perhaps be attributed to the stronger binding of KBr ions to their respective polyelectrolytes. The RPA approach used here could be improved upon by incorporating adaptive chain structure effects, allowing the chain conformation to respond to solution conditions. It remains to be seen if this modification of the theory will lead to better predictions at low molarities of weakly binding salt ions. Nevertheless, we note that neutron scattering results have revealed Gaussian PE conformations in the *PEC* of PDADMA/PSS in NaCl solution.[68] In addition, more experiments including spectroscopic measurements that reveal local interactions between salt ions and polyelectrolytes, as well as molecular simulations, could clarify the sources of these differences.

A notable observation in the comparison of overcharging predictions and experimental data for both KBr and NaCl solutions in Figures 7 is that, except at low molarities of NaCl where chain structure may play an important role, all predictions are higher than the data by around $\Delta_{\mathrm{C}} \approx 20\%$ at any fixed added salt molarity. In addition to the uncertainties in our parameter values and model assumptions, part of this disagreement is perhaps related to the number of layers of the multilayers of PDADMA/PSS in the experiments, since an overcharging increase of $\Delta_{\mathrm{C}} \approx 10\%$, measured by a radiolabeling technique, was found when the number of layers of the multilayers was increased from 20 to 40.[20] It is possible that multilayers with larger number of layers have a weaker influence of the substrate surface and so behave more similarly to complex coacervates, assuming that the thicker multilayers are equally equilibrated as the thinner layers. Yet, other assumptions, such as the neglect of correlations of ion-pairing along the contour of the polyelectrolyte, might be responsible for the deviation between theory and experiment.

Finally, the effect of the hydrophobicity of polyelectrolytes on overcharging is investigated in the SI for interested readers, where it is found that more hydrophobic polyelectrolytes adsorb more efficiently on PECs and promote overcharging.

Conclusions and Prospective

To explore the driving forces of “overcharging” i.e., adsorption of excess polyelectrolyte by a stoichiometric complex from a large excess of supernatant, we have employed a polyelectrolyte solution theory that accounts for the effects of ion-specific ion pairing, counterion binding, electrostatic correlation free energy with polymer charge connectivity, backbone hydrophobicity, as well as translational and ion binding entropies. We find that the adsorption of excess polyelectrolyte is mainly driven by two entropic mechanisms: (1) release of counterions, and (2) an increased combinatorial binding entropy of the polyelectrolyte in the complex as new excess chains enter the complex from the surrounding supernatant. The first mechanism is consistent with earlier attribution of overcharging to entropy gain from counterion release, while the second, combinatorial entropy driving force, is similar to that identified by Sing and co-workers.[17] Our assessment of the sources of overcharging provides a rationalization for overcharging and formation of multilayer films at salt-free condition, where counterion release effects are negligible.

Insights from the present study are expected to aid in the rational design of overcharged PECs for a myriad of novel applications, such as layer-by-layer multilayer film formation,[22, 23] and DNA transfection[25, 26, 73, 74] where DNA is complexed with excess, biocompatible polycations. For example, among other factors, the efficiency of DNA transfection is known to strongly depend on the degree of overcharging of DNA-polycation complexes, as the lower positive polyelectrolyte charge of the complex leads to inefficient cell-PEC bindings, while strongly positively (over)charged PECs cause structural damage to, and even death of, cells.[26, 73] The work reported here may help in controlling this charge.

Our study shows that overcharging is non-monotonic both in salt concentration and in binding strength between salt ions and polyelectrolytes. At very low salt, overcharging is dominated by an increase in the combinatorial binding entropy. At intermediate salt levels, gains in both entropy of released counterions and in combinatorial entropy of binding of oppositely charged species to polyelectrolytes drive excess polyelectrolyte into the complex. At high salt, however, the gains in these entropies diminish, thus reducing the driving force for overcharging. As a rule of thumb, for a given salt, the maximum overcharging lies roughly halfway between zero salt and the dissolution salt concentration, beyond which the complex dissolves into the supernatant. Interestingly, experimental work by Salehi and coworkers shows a maximum rate of growth of Layer-by-Layer deposited multilayer films that is also at a salt concentration roughly half-way between zero and the dissolution salt concentration.[52]

Using the same set of parameters that we used in our recent study to model doping and phase behavior of stoichiometric PECs of PDADMA/PSS PECs in KBr solution, in the present work we could find qualitative agreement for overcharging between the model and experimental data for multilayers made of PDADMA/PSS in KBr solution. Estimating the effective size of salt ions and taking as fitting parameters the binding strengths of Na^{+} and Cl^{-} to, respectively, PSS and PDADMA, we rationalized doping and salt partitioning data for PDADMA/PSS PECs in NaCl. Using this set of fitting parameters, with no further fitting our a priori predictions of the degree of overcharging match only qualitatively the data for multilayers made of PDADMA/PSS in NaCl solution. A quantitative match is perhaps not expected due to the use of multilayers rather than coacervates in the experiments and to approximations in the theory. In particular, treating polyelectrolytes as Gaussian chains in the supernatant solution of NaCl could help explain the overprediction of overcharging. If so, this could be resolved by a more sophisticated approach for treating chain structure of PEs in the supernatant.

The present work has neglected correlations between ion-pairs which have been shown to play an important role in PEC formation.[17, 75] Our model might be extended to account for such correlations, if such correlations can be incorporated within the framework of the model in tractable form.

It would also be very interesting to measure the concentration of released counterions upon overcharging using coarse-grained molecular simulations to test the theory in more detail.[76] Another intriguing study would be to employ molecular simulations to investigate the binding free energies of Na^{+} and Cl^{-} to, respectively, PSS

and PDADMA modeled in this study, or those of K^{+} and Br^{-} to, respectively, PSS and PDADMA modeled in our previous study.[42] A number of studies have highlighted the significance of a correlation between an ion's hydration and its binding affinity,[46, 77, 78] indicating that more hydrated ions generally bind more weakly to the PE charged groups.[47, 66] From molecular simulations, one could obtain molecular-level information such as diffusivity of ions near PEs or radial distribution functions, and how they are correlated with the ions' hydration. One could then identify the "bound" ions that possess negligible diffusivity and mixing entropy, perhaps enabling calculation of the "binding" free energy from simulations.

Overall, this work nicely bridges responses of polyelectrolyte multilayers (PEMUs) and PECs to external stimuli, such as salt concentration, and highlights the role of specificity of salt ions and polyelectrolyte monomers in the overcharging behavior of PEMUs and PECs.

Acknowledgement

We are grateful to the National Science Foundation (NSF) for supporting this research under grant DMR 1707640. We are also grateful for helpful comments by Dr. Jian Qin. Any opinions, findings, and conclusions or recommendations expressed in this work are those of the authors and do not necessarily reflect the views of NSF.

Appendix A: Concentration of released counterions and newly formed ion-pairs upon overcharging

Using the equilibrium volume fractions and fractions of counterion binding and ion-pairing for polyelectrolytes in the co-existing phases for the stoichiometric and non-stochiometric mixtures, one can calculate the concentrations of released counterions and newly formed ion-pairs upon overcharging. We do this calculation under the assumption that there is a huge excess of polycations, so that transfer of a few polycation chains into the relatively tiny PEC phase does not change the properties of the remaining polycations in the supernatant phase. At the same time, the polyanions are fully bound to the polycations in the PEC phase that essentially none of them deplete into the supernatant phase until the salt concentration is close to the dissolution salt concentration. These assumptions greatly simplify calculations and are consistent with the experimental work on overcharging by Schlenoff and coworkers.

The following expression gives the concentration of released small cations from polyanions as a result of in-migration of polycations.

$$C_+^{\mathrm{released}} = \frac{n_+^{\mathrm{released}}}{V\,N_{\mathrm{Avo}}} = \left(\alpha_{\mathrm{A+}}^{\mathrm{PEC,\,st}} - \alpha_{\mathrm{A+}}^{\mathrm{PEC,\,non-st}}\right) \times \text{ overall concentration of polyanion monomers}$$

$$= \left(\alpha_{\mathrm{A+}}^{\mathrm{PEC,\,st}} - \alpha_{\mathrm{A+}}^{\mathrm{PEC,\,non-st}}\right) \times \frac{\phi_{\mathrm{P}}^{\mathrm{PEC,\,st}} v^{\mathrm{PEC,\,st}}}{\omega_{\mathrm{A}}\, v_{\mathrm{W}}\, N_{\mathrm{Avo}}} \tag{A1}$$

$$= \left(\alpha_{\mathrm{A+}}^{\mathrm{PEC,\,st}} - \alpha_{\mathrm{A+}}^{\mathrm{PEC,\,non-st}}\right) \times \frac{\phi_{\mathrm{A}}^{\mathrm{PEC,\,non-st}} v^{\mathrm{PEC,\,non-st}}}{\omega_{\mathrm{A}}\, v_{\mathrm{W}}\, N_{\mathrm{Avo}}} \tag{A2}$$

with n_+^{released} being the (net) number of released cations from the polyanions, and V the total volume of solution including both phases. All “concentrations” here are number concentrations, and $\phi_{\mathrm{P}}^{\mathrm{PEC,\,st}} = \phi_{\mathrm{A}}^{\mathrm{PEC,\,st}} = \phi_{\mathrm{C}}^{\mathrm{PEC,\,st}}$ is the polymer volume fraction in the stoichiometric complex.

Calculation of the concentration of released anions, however, should be performed with care. Upon adsorption, a portion of the anions of the incoming, excess, polycation are released. The total concentration of released anions from the incoming, excess, polycation is given by,

$$C_-^{\mathrm{released-t}} = \frac{n_-^{\mathrm{released-t}}}{V\,N_{\mathrm{Avo}}} = \left(\alpha_{\mathrm{C-}}^{\mathrm{sup,\,non-st}} - \alpha_{\mathrm{C-}}^{\mathrm{PEC,\,non-st}}\right) \times \text{ overall concentration of adsorbing, excess polycation monomers}$$

$$= \left(\alpha_{\mathrm{C-}}^{\mathrm{sup,\,non-st}} - \alpha_{\mathrm{C-}}^{\mathrm{PEC,\,non-st}}\right) \times \frac{\left(\phi_{\mathrm{C}}^{\mathrm{PEC,\,non-st}} v^{\mathrm{PEC,\,non-st}} - \phi_{\mathrm{P}}^{\mathrm{PEC,\,st}} v^{\mathrm{PEC,\,st}}\right)}{\omega_{\mathrm{C}}\, v_{\mathrm{W}}\, N_{\mathrm{Avo}}} \tag{A3}$$

However, not all of these anions are truly released; some of them get adsorbed onto the existing polycations already present in the stoichiometric complex. (Note that, in our approach, all polycations in the complex at thermodynamic equilibrium are identical.) The corresponding concentration of adsorbed anions can be written as,

$$C_-^{\text{adsorbed}} = \frac{n_-^{\text{adsorbed}}}{V\, N_{\text{Avo}}}$$

$$= \left(\alpha_{\text{C}-}^{\text{PEC, non-st}} - \alpha_{\text{C}-}^{\text{PEC, st}}\right) \times \text{ overall concentration of polycation monomers present in the stoichiometric complex}$$

$$= \left(\alpha_{\text{C}-}^{\text{PEC, non-st}} - \alpha_{\text{C}-}^{\text{PEC, st}}\right) \times \frac{\phi_{\text{P}}^{\text{PEC, st}} v^{\text{PEC, st}}}{\omega_{\text{C}}\, v_{\text{W}}\, N_{\text{Avo}}} \tag{A4}$$

In the equations above, $n_-^{\text{released-t}}$ and n_-^{adsorbed} show the number of total anions released and adsorbed, respectively. The net concentration of released anions from the adsorbing, excess polycations can thus be simply calculated using,

$$C_-^{\text{released}} = \frac{n_-^{\text{released}}}{V\, N_{\text{Avo}}} = C_-^{\text{released-t}} - C_-^{\text{adsorbed}} \tag{A5}$$

where n_-^{released} denotes the (net) number of released anions.

Finally, the concentration of newly formed ion-pairs between adsorbing, excess polycations and the complex polyanions can be written as,

$$C_{\text{ip}}^{\text{formed}} = \frac{n_{\text{ip}}^{\text{formed}}}{V\, N_{\text{Avo}}} = (\text{concentration of ion pairs in the nonstoichiometric complex})$$

$$- (\text{concentration of ion pairs in the stoichiometric complex})$$

$$= \beta_{\text{C}}^{\text{PEC, non-st}} \times \frac{\phi_{\text{C}}^{\text{PEC, non-st}}\, v^{\text{PEC, non-st}}}{\omega_{\text{C}}\, v_{\text{W}}\, N_{\text{Avo}}} - \beta_{\text{P}}^{\text{PEC, st}} \times \frac{\phi_{\text{P}}^{\text{PEC, st}} v^{\text{PEC, st}}}{\omega_{\text{C}}\, v_{\text{W}}\, N_{\text{Avo}}} \tag{A6}$$

In the equations above, $n_{\text{ip}}^{\text{formed}}$ represents the number of the newly formed ion-pairs, and $\beta_{\text{P}}^{\text{PEC, st}}$ and $\beta_{\text{C}}^{\text{PEC, non-st}}$ are the fractions of ion-pairs for polyelectrolytes in the stoichiometric and in the non-stoichiometric complexes, respectively. These concentrations can be normalized with the overall concentration of the minor polyelectrolyte (polyanion here), since it remains in the PEC phase until very close to critical salt.

Appendix B: Change in translational and combinatorial entropies of the system upon overcharging

The translational entropy of all free salt ions in an arbitrary phase α is

$$S^{\mathrm{T}}(\text{all salt ions in phase } \alpha) = -k_{\mathrm{B}}\, n_{-}^{\mathrm{f},\alpha}\, \ln \phi_{-}^{\mathrm{f},\alpha} - k_{\mathrm{B}}\, n_{+}^{\mathrm{f},\alpha}\, \ln \phi_{+}^{\mathrm{f},\alpha} \tag{B1}$$

with $n_{\pm}^{\mathrm{f},\alpha}$ and $\phi_{\pm}^{\mathrm{f},\alpha}$ denoting the number and volume fraction of free salt ions in the phase, respectively. In the formulism above, each logarithm term is the translational entropy of each single ion of the corresponding ion type.

Upon overcharging, some of the counterions from the polyelectrolytes are released, as discussed earlier. Neglecting the translational entropy of the polyelectrolytes (due to high degree of polymerization), the (translational) entropy change ΔS^{T} of the solution, which is mainly due to counterion release upon overcharging, can be written as,

$$\Delta S^{\mathrm{T}} = -k_{\mathrm{B}}\, n_{-}^{\mathrm{released}} \ln \phi_{-}^{\mathrm{f,\,PEC,\,non-st}} - k_{\mathrm{B}}\, n_{+}^{\mathrm{released}} \ln \phi_{+}^{\mathrm{f,\,PEC,\,non-st}} \tag{B2}$$

which can be transformed into (molar) concentration units with,

$$\frac{\Delta S^{\mathrm{T}}}{N_{\mathrm{Avo}}\, k_{\mathrm{B}} V} = -C_{-}^{\mathrm{released}} \ln \phi_{-}^{\mathrm{f,\,PEC,\,non-st}} - C_{+}^{\mathrm{released}} \ln \phi_{+}^{\mathrm{f,\,PEC,\,non-st}} \tag{B3}$$

Here $\phi_{\pm}^{\mathrm{f,\,PEC,\,non-st}}$ is the volume fraction of free counterions (or salt ions) ($\pm$) in the overcharged PEC and $\mathrm{N_{Avo}}$ is Avogadro's number. To obtain eq B3, we have assumed that each freed counterion remains in the complex at equilibrium and contributes a fixed amount of $-k_{\mathrm{B}} \ln \phi_{\mathrm{S}}^{\mathrm{f,\,PEC,\,non-st}}$ to the translational entropy with $\mathrm{S} = +$ or $-$. This requires that $\phi_{\mathrm{S}}^{\mathrm{f,\,PEC,\,non-st}}$ remain roughly constant during the release process, and we take $\phi_{\mathrm{S}}^{\mathrm{f,\,PEC,\,non-st}}$ to be the value at the end of the polycation adsorption process (i.e. at the equilibrium state).[14] Also, the results are not sensitive to the phase where the freed counterions are considered to reside in at equilibrium, since for our standard set of parameters the concentration of free salt ion in the PEC is close to, albeit not exactly the same as, that in the supernatant (and so, one can use $\phi_{\mathrm{S}}^{\mathrm{f,\,sup,\,non-st}}$ instead of $\phi_{\mathrm{S}}^{\mathrm{f,\,PEC,\,non-st}}$ in equation B3); see Figure 9.

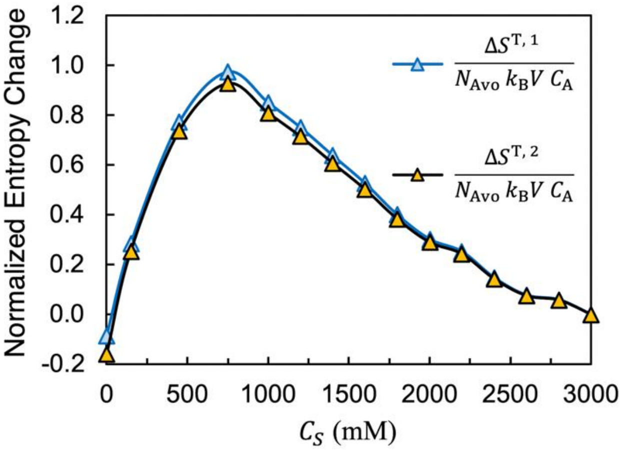


Figure 9. Change in the normalized translational entropy upon overcharging at different added salt concentrations, calculated by eq B3 using either the concentration of free salt ions in the overcharged PEC, shown as ($\Delta S^{\mathrm{T,\,1}}$, blue symbols), or the concentration of salt ions in the supernatant phase, shown as ($\Delta S^{\mathrm{T,\,2}}$, yellow symbols). The entropies are normalized by the solution concentration of polyanion ($C_{\mathrm{A}} = 0.1$ mM).

Next, we develop a simple expression for the change in combinatorial entropy, ΔS^{comb}, resulting from the numbers of ways of arranging counterions, ion pairs, and free charges along the polyelectrolytes, during polycation adsorption as,

$$\frac{\Delta S^{\mathrm{comb}}}{N_{\mathrm{Avo}}\,k_{\mathrm{B}}V} = \left[\left(S_{\mathrm{A}}^{\mathrm{comb,\,PEC,\,non-st}} - S_{\mathrm{A}}^{\mathrm{comb,\,PEC,\,st}}\right) + \left(S_{\mathrm{C}}^{\mathrm{comb,\,PEC,\,non-st}} - S_{\mathrm{C}}^{\mathrm{comb,\,PEC,\,st}} - S_{\mathrm{excess-C}}^{\mathrm{comb,\,sup,\,non-st}}\right)\right]/N_{\mathrm{Avo}}\,k_{\mathrm{B}}V \qquad \text{(B4)}$$

In eq B4, $S_{\mathrm{P}}^{\mathrm{comb},\,\alpha,\,j}$ denotes the entropy of binding of oppositely charged species to polyelectrolytes of type P (with $\mathrm{P} = \mathrm{A}, \mathrm{C}$, or "$\mathrm{ads,\ excess-C}$") in phase α ($\alpha =$ "PEC" or "sup") and for case j ($j =$ "st" or "non − st"). For instance, $S_{\mathrm{C}}^{\mathrm{comb,\,PEC,\,non-st}}$ represents the entropy of binding to all polycations in the non-stoichiometric (or overcharged) complex. In addition, we note that the last term with the subscript "$\mathrm{ads,\ excess-C}$" represents the combinatorial entropy of only the excess polycations adsorbing from the supernatant.

To calculate each term on the RHS of eq B4, we simply use eq 7 for polyanion and eq 8 for polycation, which can be generally expressed as,

$$-\frac{v_{\mathrm{W}}\, S_{\mathrm{P}}^{\mathrm{comb}}}{k_{\mathrm{B}} V} = \frac{\phi_{\mathrm{P}}}{\omega_{\mathrm{P}}}[\alpha \ln \alpha + \beta \ln \beta + (1-\alpha-\beta)\ln(1-\alpha-\beta)] \tag{B5}$$

or,

$$\frac{S_{\mathrm{P}}^{\mathrm{comb}}}{k_{\mathrm{B}} V} = -\frac{\phi_{\mathrm{P}}}{\omega_{\mathrm{P}} v_{\mathrm{W}}}[\alpha \ln \alpha + \beta \ln \beta + (1-\alpha-\beta)\ln(1-\alpha-\beta)] \tag{B6}$$

We note that equation B6 represents the combinatorial entropy of polyelectrolytes of type P in units of k_{B} with the volume fraction ϕ_{P}, *within the total solution volume V*. Therefore, in order to calculate each term of eq B4, we need to find the relevant polyelectrolyte volume fraction within the total volume V, which is the volume fraction of the polymer within a given phase, multiplied by the volume of that phase within the entire solution.

According to this, the combinatorial entropies in eq B4 are given by,

$$\frac{S_{\mathrm{A}}^{\mathrm{comb,\,PEC,\,non-st}}}{N_{\mathrm{Avo}}\, k_{\mathrm{B}} V} = -\frac{\phi_{\mathrm{A}}^{\mathrm{PEC,\,non-st}} v^{\mathrm{PEC,\,non-st}}}{\omega_{\mathrm{A}}\, v_{\mathrm{W}}\, N_{\mathrm{Avo}}} \times [\alpha_{\mathrm{A+}} \ln \alpha_{\mathrm{A+}} + \beta_{\mathrm{A}} \ln \beta_{\mathrm{A}} + (1-\alpha_{\mathrm{A+}}-\beta_{\mathrm{A}})\ln(1-\alpha_{\mathrm{A+}}-\beta_{\mathrm{A}})]^{\mathrm{PEC,\,non-st}} \tag{B7}$$

$$\frac{S_{\mathrm{A}}^{\mathrm{comb,\,PEC,\,st}}}{N_{\mathrm{Avo}}\, k_{\mathrm{B}} V} = -\frac{\phi_{\mathrm{P}}^{\mathrm{PEC,\,st}} v^{\mathrm{PEC,\,st}}}{\omega_{\mathrm{P}}\, v_{\mathrm{W}}\, N_{\mathrm{Avo}}} \times [\alpha_{\mathrm{PS}} \ln \alpha_{\mathrm{PS}} + \beta_{\mathrm{P}} \ln \beta_{\mathrm{P}} + (1-\alpha_{\mathrm{PS}}-\beta_{\mathrm{P}})\ln(1-\alpha_{\mathrm{PS}}-\beta_{\mathrm{P}})]^{\mathrm{PEC,\,st}} \tag{B8}$$

$$\frac{S_{\mathrm{C}}^{\mathrm{comb,\,PEC,\,non-st}}}{N_{\mathrm{Avo}}\, k_{\mathrm{B}} V} = -\frac{\phi_{\mathrm{C}}^{\mathrm{PEC,\,non-st}} v^{\mathrm{PEC,\,non-st}}}{\omega_{\mathrm{C}}\, v_{\mathrm{W}}\, N_{\mathrm{Avo}}} \times [\alpha_{\mathrm{C-}} \ln \alpha_{\mathrm{C-}} + \beta_{\mathrm{C}} \ln \beta_{\mathrm{C}} + (1-\alpha_{\mathrm{C-}}-\beta_{\mathrm{C}})\ln(1-\alpha_{\mathrm{C-}}-\beta_{\mathrm{C}})]^{\mathrm{PEC,\,non-st}} \tag{B9}$$

$$\frac{S_{\mathrm{C}}^{\mathrm{comb,\,PEC,\,st}}}{N_{\mathrm{Avo}}\, k_{\mathrm{B}} V} = \frac{S_{\mathrm{A}}^{\mathrm{comb,\,PEC,\,st}}}{N_{\mathrm{Avo}}\, k_{\mathrm{B}} V} \tag{B10}$$

$$\frac{S_{\mathrm{excess-C}}^{\mathrm{comb,\,sup,\,non-st}}}{N_{\mathrm{Avo}}\, k_{\mathrm{B}} V} = -\frac{\left(\phi_{\mathrm{C}}^{\mathrm{PEC,\,non-st}}\, v^{\mathrm{PEC,\,non-st}} - \phi_{\mathrm{P}}^{\mathrm{PEC,\,st}} v^{\mathrm{PEC,\,st}}\right)}{\omega_{\mathrm{C}}\, v_{\mathrm{W}}\, N_{\mathrm{Avo}}} \times [\alpha_{\mathrm{C-}} \ln \alpha_{\mathrm{C-}} + \beta_{\mathrm{C}} \ln \beta_{\mathrm{C}} + (1-\alpha_{\mathrm{C-}}-\beta_{\mathrm{C}})\ln(1-\alpha_{\mathrm{C-}}-\beta_{\mathrm{C}})]^{\mathrm{sup,\,non-st}} \tag{B11}$$

For economy in notation in eqs B7 through B11, we specify in the superscripts whether the mixture is stoichiometric or non-stoichiometric ("st" or "non − st") and what phase, PEC or supernatant ("PEC" or "sup"), is considered; the values of the α's and β's are calculated for the situation corresponding to that specified by the superscripts on the relevant brackets on the right sides of the above equations.

Avogadro's number appearing in the equations above simply transforms the entropy concentrations into Molar units. In addition, in the present study, since the minor polyelectrolyte (polyanion) almost always remain in the PEC phase, one can normalize these entropy concentrations with the total polyanion concentration ($C_A = 0.1$ mM).

Supporting Information

The Supporting Information is available free of charge on the Royal Society of Chemistry website at:

https://www.rsc.org/

References

1. W. C. Blocher and S. L. Perry, *WIREs Nanomedicine and Nanobiotechnology*, 2017, **9**, e1442.
2. R. A. Kapelner and A. C. Obermeyer, *Chemical Science*, 2019, **10**, 2700-2707.
3. B. Valley, B. Jing, M. Ferreira and Y. Zhu, *ACS Applied Materials & Interfaces*, 2019, **11**, 7472-7478.
4. S. Bouhallab and T. Croguennec, in *Polyelectrolyte Complexes in the Dispersed and Solid State II*, ed. M. Müller, Springer, Berlin, Heidelberg, Berlin, Heidelberg, 2013, vol. 256, ch. Spontaneous Assembly and Induced Aggregation of Food Proteins pp. 67-101.
5. S. Kroschwald, S. Maharana, D. Mateju, L. Malinovska, E. Nüske, I. Poser, D. Richter and S. Alberti, *eLife*, 2015, **4**, e06807.
6. S. F. Banani, H. O. Lee, A. A. Hyman and M. K. Rosen, *Nature Reviews Molecular Cell Biology*, 2017, **18**, 285-298.
7. C. P. Brangwynne, P. Tompa and R. V. Pappu, *Nature Physics*, 2015, **11**, 899-904.
8. A. A. M. André and E. Spruijt, *Biophysical Journal*, 2018, **115**, 1837-1839.
9. Q. Wang and J. B. Schlenoff, *Macromolecules*, 2014, **47**, 3108-3116.
10. C. H. Porcel and J. B. Schlenoff, *Biomacromolecules*, 2009, **10**, 2968-2975.
11. Y. Marcus, *The Journal of Physical Chemistry B*, 2005, **109**, 18541-18549.
12. C. G. Sinn, R. Dimova and M. Antonietti, *Macromolecules*, 2004, **37**, 3444–3450.
13. M. Muthukumar, *The Journal of Chemical Physics*, 2004, **120**, 9343-9350.
14. Z. Ou and M. Muthukumar, *The Journal of Chemical Physics*, 2006, **124**, 154902.
15. J. Fu and J. B. Schlenoff, *Journal of the American Chemical Society*, 2016, **138**, 980-990.
16. X. Xu, Q. Ran, P. Dey, R. Nikam, R. Haag, M. Ballauff and J. Dzubiella, *Biomacromolecules*, 2018, **19**, 409-416.

17. T. K. Lytle and C. E. Sing, *Soft Matter*, 2017, **13**, 7001-7012.
18. T. K. Lytle and C. E. Sing, *Molecular Systems Design & Engineering*, 2018, **3**, 183-196.
19. T. Kremer, D. Kovačević, J. Salopek and J. Požar, *Macromolecules*, 2016, **49**, 8672-8685.
20. H. M. Fares and J. B. Schlenoff, *Macromolecules*, 2017, **50**, 3968-3978.
21. J. Gummel, F. Cousin and F. Boué, *Journal of the American Chemical Society*, 2007, **129**, 5806-5807.
22. S. Correa, N. Boehnke, E. Deiss-Yehiely and P. T. Hammond, *ACS Nano*, 2019, **13**, 5623-5634.
23. G. B. Sukhorukov, E. Donath, H. Lichtenfeld, E. Knippel, M. Knippel, A. Budde and H. Möhwald, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 1998, **137**, 253-266.
24. M. Müller, in *Polyelectrolyte Complexes in the Dispersed and Solid State II*, Advances in Polymer Science, Springer, Berlin, Heidelberg, Berlin, Heidelberg, 2012, vol. 256, ch. Sizing, Shaping and Pharmaceutical Applications of Polyelectrolyte Complex Nanoparticles, pp. 197-260.
25. P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold and M. Danielsen, *Proceedings of the National Academy of Sciences*, 1987, **84**, 7413-7417.
26. O. Boussif, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix and J. P. Behr, *Proceedings of the National Academy of Sciences*, 1995, **92**, 7297-7301.
27. B. P. Mead, P. Mastorakos, J. S. Suk, A. L. Klibanov, J. Hanes and R. J. Price, *Journal of Controlled Release*, 2016, **223**, 109-117.
28. D. Volodkin and R. von Klitzing, *Current Opinion in Colloid & Interface Science*, 2014, **19**, 25-31.
29. G. Liu, Y. Hou, X. Xiao and G. Zhang, *The Journal of Physical Chemistry B*, 2010, **114**, 9987-9993.
30. A. E. El Haitami, D. Martel, V. Ball, H. C. Nguyen, E. Gonthier, P. Labbé, J.-C. Voegel, P. Schaaf, B. Senger and F. Boulmedais, *Langmuir*, 2009, **25**, 2282-2289.
31. J. E. Wong, H. Zastrow, W. Jaeger and R. von Klitzing, *Langmuir*, 2009, **25**, 14061-14070.
32. S. T. Dubas and J. B. Schlenoff, *Macromolecules*, 1999, **32**, 8153-8160.
33. B. Wu, C. Li, H. Yang, G. Liu and G. Zhang, *The Journal of Physical Chemistry B*, 2012, **116**, 3106-3114.
34. P. Kujawa, P. Moraille, J. Sanchez, A. Badia, S. Centre-ville and C. Hc, *Journal of the American Chemical Society*, 2005, **127**, 9224-9234.
35. P. Nestler, M. Paßvogel and C. A. Helm, *Macromolecules*, 2013, **46**, 5622−5629.
36. C. B. Bucur, Z. Sui and J. B. Schlenoff, *Journal of the American Chemical Society*, 2006, **128**, 13690-13691.
37. J. Požar and D. Kovačević, *Soft Matter*, 2014, **10**, 6530-6545.
38. R. A. Ghostine, M. Z. Markarian and J. B. Schlenoff, *Journal of the American Chemical Society*, 2013, **135**, 7636-7646.
39. Y. E. Ghoussoub, M. Zerball, H. M. Fares, J. F. Ankner, R. von Klitzing and J. B. Schlenoff, *Soft Matter*, 2018, **14**, 1699-1708.
40. A. Salehi and R. G. Larson, *Macromolecules*, 2016, **49**, 9706-9719.
41. S. Friedowitz, A. Salehi, R. G. Larson and J. Qin, *The Journal of Chemical Physics*, 2018, **149**, 163335.
42. M. Ghasemi, S. Friedowitz and R. G. Larson, *Macromolecules*, 2020, **53**, 6928-6945.

43. H. M. Fares, Y. E. Ghoussoub, J. D. Delgado, J. Fu, V. S. Urban and J. B. Schlenoff, *Macromolecules*, 2018, **51**, 4945-4955.
44. J. McCarty, K. T. Delaney, S. P. O. Danielsen, G. H. Fredrickson and J.-E. Shea, *The Journal of Physical Chemistry Letters*, 2019, **10**, 1644−1652.
45. K. Shen and Z.-G. Wang, *The Journal of Chemical Physics*, 2017, **146**, 84901.
46. A. Chremos and J. F. Douglas, in *Gels and Other Soft Amorphous Solids*, American Chemical Society, 2018, vol. 1296, ch. 2, pp. 2-15.
47. R. A. Ghostine, R. F. Shamoun and J. B. Schlenoff, *Macromolecules*, 2013, **46**, 4089−4094.
48. J. B. Schlenoff, M. Yang, Z. A. Digby and Q. Wang, *Macromolecules*, 2019, **52**, 9149-9159.
49. Y. Zhang, P. Batys, J. T. O'Neal, F. Li, M. Sammalkorpi and J. L. Lutkenhaus, *ACS Central Science*, 2018, **4**, 638-644.
50. A. Kudlay, A. V. Ermoshkin and M. Olvera de la Cruz, *Macromolecules*, 2004, **37**, 9231-9241.
51. J. Hua, M. K. Mitra and M. Muthukumar, *The Journal of Chemical Physics*, 2012, **136**, 134901.
52. A. Salehi, P. S. Desai, J. Li, C. A. Steele and R. G. Larson, *Macromolecules*, 2015, **48**, 400-409.
53. H. Mjahed, J.-C. Voegel, A. Chassepot, B. Senger, P. Schaaf, F. Boulmedais and V. Ball, *Journal of Colloid and Interface Science*, 2010, **346**, 163-171.
54. S. A. Sukhishvili, E. Kharlampieva and V. Izumrudov, *Macromolecules*, 2006, **39**, 8873-8881.
55. I. Koltover, T. Salditt and C. R. Safinya, *Biophysical Journal*, 1999, **77**, 915-924.
56. P. A. Patel, J. Jeon, P. T. Mather and A. V. Dobrynin, *Langmuir*, 2006, **22**, 9994-10002.
57. S. Schwarz, J. Nagel and W. Jaeger, *Macromolecular Symposia*, 2004, **211**, 201-216.
58. A. Lodi and G. Ponterini, *Thin Solid Films*, 2006, **496**, 585-594.
59. T. Radeva, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2002, **209**, 219-225.
60. J. B. Schlenoff, H. Ly and M. Li, *Journal of the American Chemical Society*, 1998, **120**, 7626-7634.
61. N. Korolev, A. P. Lyubartsev and A. Laaksonen, *Journal of Biomolecular Structure and Dynamics*, 2004, **22**, 215-226.
62. S. Y. Park, R. F. Bruinsma and W. M. Gelbart, *Europhysics Letters*, 1999, **46**, 454-460.
63. M. Quesada-Pérez, E. González-Tovar, A. Martín-Molina, M. Lozada-Cassou and R. Hidalgo-Álvarez, *ChemPhysChem*, 2003, **4**, 234-248.
64. V. D. Suryawanshi, L. S. Walekar, A. H. Gore, P. V. Anbhule and G. B. Kolekar, *Journal of Pharmaceutical Analysis*, 2016, **6**, 56-63.
65. S. T. Dubas and J. B. Schlenoff, *Langmuir*, 2001, **17**, 7725-7727.
66. M. Salomäki, P. Tervasmäki, S. Areva and J. Kankare, *Langmuir*, 2004, **20**, 3679-3683.
67. S. Dodoo, R. Steitz, A. Laschewsky and R. von Klitzing, *Physical Chemistry Chemical Physics*, 2011, **13**, 10318-10325.
68. M. Z. Markarian, H. H. Hariri, A. Reisch, V. S. Urban and J. B. Schlenoff, *Macromolecules*, 2012, **45**, 1016-1024.
69. H. Riegler and F. Essler, *Langmuir*, 2002, **18**, 6694-6698.
70. V. P. Korolev, *Journal of Structural Chemistry*, 2011, **52**, 737.
71. J. Fu, H. M. Fares and J. B. Schlenoff, *Macromolecules*, 2017, **50**, 1066−1074.
72. R. Zhang, Y. Zhang, H. S. Antila, J. L. Lutkenhaus and M. Sammalkorpi, *The Journal of Physical Chemistry B*, 2017, **121**, 322-333.
73. M. A. Mintzer and E. E. Simanek, *Chemical Reviews*, 2009, **109**, 259-302.
74. S. Taheri-Araghi, D.-W. Chen, M. Kohandel, S. Sivaloganathan and M. Foldvari, *Nanoscale*, 2019, **11**, 1037-1046.

75. M. Radhakrishna, K. Basu, Y. Liu, R. Shamsi, S. L. Perry and C. E. Sing, *Macromolecules*, 2017, **50**, 3030-3037.
76. L. Li, S. Srivastava, M. Andreev, A. B. Marciel, J. J. de Pablo and M. V. Tirrell, *Macromolecules*, 2018, **51**, 2988-2995.
77. A. Chremos and J. F. Douglas, *The Journal of Chemical Physics*, 2017, **147**, 241103.
78. M. Andreev, J. J. de Pablo, A. Chremos and J. F. Douglas, *The Journal of Physical Chemistry B*, 2018, **122**, 4029-4034.

For Table of Contents use only: