Ion Content of Polyelectrolyte Complex Coacervates and the Donnan Equilibrium

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Abstract

Oppositely-charged polyelectrolytes in solution spontaneously associate into hydrated complexes or coacervates, PECs. The morphology, stability and properties of PECs depend strongly on their ion content, which moderates the "sticky" reversible interactions between Pol^+ and Pol^- oppositely-charged repeat units. Here, it is shown that the distribution of ions between a PEC and the aqueous solution in which it is immersed is accurately predicted by the Donnan equilibrium. For ideal, stoichiometric mixing of polyelectrolytes, corresponding to an enthalpy of complexation $\Delta H_{PEC} \rightarrow 0$, the salt, MA, concentration inside the PEC, $[MA]_{PEC}$, is equal to the solution salt concentration, $[MA]_s$. Isothermal calorimetry measurements along a Hofmeister series show that if mixing is exothermic $[MA]_{PEC} < [MA]_s$, while for endothermic association of Pol^+ and Pol^- $[MA]_{PEC} > [MA]_s$. A set of simple self-consistent expressions illustrate PEC salt response without consideration of net Coulombic or electrostatic forces between charged species. ΔH_{PEC} exactly predicts deviations from ideal Donnan equilibria, which are connected to the equilibria between associated or intrinsic pairs of Pol^+Pol^- and extrinsic Pol^+A^- and Pol^-M^+ pairs, where counterions compensate polyelectrolyte charges. The equilibrium constant K_{pair} for Pol^+Pol^- pair formation is shown to be proportional to the volume charge density of the hydrated,

ion-free complex. K_{pair} may also be used to estimate the critical salt concentration at which polyelectrolytes completely dissociate.

Introduction

Phase separation of (bio)polyelectrolytes is induced by many stimuli, such as the "salting out" behavior of proteins reported by Hofmeister,[1] the addition of nonsolvents, and temperature change. Phase separation caused by oppositely-charged surfactants,[2] nanoparticles,[3] and polyelectrolytes (synthetic or natural) is termed "coacervation." The latter components yield polyelectrolyte complexes, or "complex coacervates," PECs.[4] Polyelectrolyte coacervation of proteins having opposite charge was reported by de Jong and Kruyt.[5] Mixing oppositely-charged synthetic polyelectrolytes, which typically have higher charge densities than proteins, first reported by Fuoss and Sadek[6], yields a hydrated complex which can have solid- or liquid-like properties.[7] The fascinating range of PEC properties has stimulated recent interest in these amorphous viscoelastic materials.

Early studies of spontaneous PEC formation from dilute solutions of individual polyelectrolytes noted two important properties.[4, 8] First, heats of complexation could not be detected, suggesting an entropy-driven process. Second, counterions were not found within the PEC, leading to the conclusion that the loss of counterions (the "escaping tendency of microions," as Michaels put it[4]) was the driving force for PEC formation summarized by Equation 1

$$Pol^-M^+{}_s + Pol^+A^-{}_s \rightarrow Pol^+Pol^-{}_{PEC} + M_s^+ + A_s^- \qquad [1]$$

where Pol^- , Pol^+ , M^+, A^- are respective polyanion repeat unit, polycation repeat unit, salt cation and salt anion, and subscripts "*s*" and "*PEC*" refer to solution and PEC phase. Equation 1 can be partially reversed by adding increasing amounts of salt to solution,

$$M_s^+ + A_s^- + Pol^+Pol_{PEC}^- \rightarrow Pol^+A_{PEC}^- + Pol^-M_{PEC}^+ \quad [2]$$

Depending on their ratio, which is controlled by this "doping" of salt into the condensed phase from dilute phase, the components of polyelectrolyte complexes/coacervates (PECs), polymers, salt and water, regulate an enormous range of physical properties. Efforts to understand the composition of PECs included early work by Overbeek and Voorn,[9] who inspired Veis to examine coacervation of gelatins.[10]

Theories of the composition of polyelectrolyte coacervates or complexes[11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26] have recently been summarized in reviews by Sing[27] and Muthukumar.[28] These theories include classical Flory-Huggins entropic terms for the free energy of mixing of ions, solvent and polyelectrolyte in the concentrated (PEC) phase along with electrostatic terms describing the variable range Coulombic repulsions/attractions among all charged components.

Many years after Michaels' pioneering work, sensitive calorimetry measurements[29, 30, 31, 32, 33, 34, 35, 36] detected heat signatures, apparently supporting the role of electrostatics as a driving force for PEC formation.[17] However, we recently showed, using Raman spectroscopic studies of water structure, that these enthalpies of complexation scaled with the degree of water structure disruption, suggesting that net changes in the hydration shells around ions and polyelectrolyte repeat units were actually responsible for measured enthalpy changes.[37]

Recognizing the influence of ions on PEC properties, there has been a surge of recent work to relate the ion content of these materials to solution concentration.[18, 21, 22, 24, 27, 38] In the present study, the Donnan equilibrium is used as a simple but rigorous expression of the balance of ion entropy between PEC and aqueous or dilute phase. Accurate measurements of PEC composition are combined with accurate calorimetry measurements of the heats of complexation to predict the effect of nonideal complexation ($\Delta H \neq 0$) on the PEC ion content.

Experimental

Materials. Poly(diallyldimethylammonium chloride) (PDADMAC, molar mass 400,000 – 500,000 g mol^{-1}) and poly(4-styrenesulfonic acid, sodium salt) (PSSNa, molar mass 75,000 g mol^{-1}) used in ITC experiments were from Sigma-Aldrich. PDADMAC (Ondeo-Nalco, molar mass ca. 400,000 g mol^{-1}) and PSSNa (AkzoNobel, VERSA TL 130, molar mass ca. 200,000 g mol^{-1}) were used to prepare compact tablets of PDADMA/PSS PEC for NaX doping experiments. Sodium chloride, sodium bromide, sodium acetate (NaAc, Ac = acetate), sodium iodide and sodium perchlorate ($NaClO_4$) were from Sigma-Aldrich. All salts were dried under vac at 110 °C for at least 24 h except for $NaClO_4$, which was dried at 140 °C under vac. All solutions were prepared by using deionized water (18 MΩ Barnstead, E-pure).

PDADMA(X) and PSSNa for Isothermal Titration Calorimetry. PDADMA(Br) and PDADMA(Ac) were prepared *via* ion exchange of PDADMA(Cl) dialyzed (3.5K molecular weight cutoff tubing, SnakeSkin, ThermoFisher) against 2.0 M solutions of NaBr and NaAc respectively. After 24 h the salt solution was replaced with a fresh salt solution, totaling 48 hours of exchange. Polyelectrolytes were then dialyzed against deionized water for 3 days, with water replacement every 24 h. Polyelectrolyte solutions were then freeze dried (Labconco, FreeZone 105). To ensure the polyelectrolytes were as dry as possible, following lyophilization the polyelectrolytes were heated at 120 ^{o}C for 4 h, sealed, then moved immediately into an argon filled glove box equipped with an analytical balance. The corresponding salts (NaCl, NaBr, NaAc) used in the solutions were likewise dried then weighed in the glovebox. Final salt solution concentrations for ITC were 0.100 M NaX.

Isothermal Titration Calorimetry of PDADMA(X) and PSSNa. ITC was performed using a VP-ITC (MicroCal Inc.) calorimeter. The ITC was calibrated with the internal y-axis calibration command followed by a standard titration between hydrochloric acid and Tris base to ensure the

enthalpy of neutralization was within 0.3% of the literature value.[39] Prior to each ITC experiment both the syringe solution and the sample cell solution were degassed for 10 min at room temp. Approximately 300 μl of 10 mM polyelectrolyte solution, based on the polyelectrolyte repeat unit, was loaded into the syringe. 25 μL of solution was manually discharged from the syringe to relieve any back pressure from purging and refilling the syringe. The sample cell (1.4545 mL) was filled with a 0.5 mM solution of the oppositely charged polyelectrolyte. The syringe was then placed into the sample cell, rotated at 270 rpm, and 4 μL aliquots were injected into the sample cell at a rate of 0.42 μL per sec, with 240 sec between injections. The heat flow was recorded as a function of time at 25.0 °C. The ITC data was exported into Excel and the enthalpies were calculated by summing the total heat generated. Dilution by the syringe solution was accounted for in the final enthalpy values.

^{22}NaCl and ^{35}S-labeled Na_2SO_4 were from Perkin-Elmer Life Sciences. $^{22}Na^+$ (half-life 950 days, positron, γ emitter, E_{max} = 546 keV, produced with a specific activity of 914.66 Ci g^{-1}) was used as a "hot" $^{22}Na^+$ stock solution of 100 μCi in 1.0 g H_2O. ^{35}S-labeled SO_4^{2-} (half-life 87.4 days, β emitter, E_{max} = 167 keV, produced with a specific activity of 750 Ci mol^{-1}) was used as a "hot" ^{35}S stock solution of 1 mCi in 1.0 g H_2O.

Doping by the Radiotracer Method. PDADMA/PSS tablets with a diameter of 8 mm and thickness about 1 mm were cut from 2 cm wide flat tapes extruded as described previously.[40] These tablets were stored in 0.1 m NaCl before radiolabeling experiments. $^{22}Na^+$ labeled "hot" solutions of NaX with a molality of 1.0 m and a specific activity of 2.5 × 10^{-4} Ci mol^{-1} were prepared. For example, to prepare 1.0 m ^{22}NaCl "hot" solution, 2.9220 g NaCl (0.05 mol) was dissolved in a solution made by diluting 0.125 g "hot" $^{22}Na^+$ stock solution (12.5 μCi) with 49.875 g H_2O. To prepare 10 g of ^{22}NaCl "hot" solutions with a lower molality (e.g. 0.4 m), 4.1370 g of the 1.0 m ^{22}NaCl "hot" solution was diluted by adding 5.8630 g H_2O. The volume of "hot" solutions used for radiolabeling experiments (10 g, which is approximately 10 mL) was > 100

times of the volume of PDADMA/PSS tablet to ensure complete ion exchange. Standard tables were used to convert molality to molarity.

For scintillation counting, a 3 mm thick disk of plastic scintillator (SCSN-81, Kuraray) of diameter 38 mm was placed on top of a photomultiplier tube (PMT, RCA 8850) inside a light-tight black box. A drop of immersion oil between the disk and the PMT provided good optical contact. The PMT was powered to -2300 V by a Bertan 313B HV power supply and connected to a frequency counter (Philips PM6654C) to record the counts. The gate time on the counter was set at 10 s and the pulse threshold to -20 mV. Labview software was used to acquire data over the IEEE interface on the frequency counter.

For radiotracer experiments of each salt type, PDADMA/PSS tablets were first soaked in 10 g of the "hot" solution with the lowest concentration (e.g. 0.1 m ^{22}NaCl) for at least 3 h until the ion content equilibrated, indicated by a constant count rate *versus* immersion time. The tablets were then taken out of the "hot" solution, quickly dabbed dry with a lab wipe and weighed. Then each tablet was placed on top of the plastic scintillator disk for 15 min of counting. Tablets were then immersed in the following higher concentration "hot" solution (e.g. 0.2 m ^{22}NaCl) and the same procedure repeated. After a series of counts *versus* concentration NaX was completed for one X^-, tablets were immersed back into 0.1 m "cold" NaCl for 24 h before starting the next salt type radiotracer experiments. Calibration curves to convert counts to moles NaX were obtained by dispensing 3-15 μL aliquots of the 1.0 m "hot" solution on top of the plastic scintillator disk covered with an undoped PDADMA/PSS tablet. The stoichiometry of the PDADMA/PSS tablets was measured using the $^{22}Na^+$ and $^{35}SO_4^{2-}$ isotopes as described previously.[41] Finally, tablets were immersed in water for 24 h to remove any salt, dried at 120 °C for 8 h and weighed to obtain the total mass of polymers. For each data point, the total number of counts ranged from 1350 to 8550 with respective counting errors of 2.7% and 1.1%.

Results and Discussions

Ion Entropy and Equilibrium

Theories of PEC composition include an entropic contribution from ions to the free energy of a PEC. For example, Voorn and Overbeek (VO)[9] provided the following expression for the free energy of a coacervate of polycations and polyanions,[25] both of length *N*

$$f_{VO} = \frac{\emptyset_p}{N} ln \frac{\emptyset_p}{2} + \emptyset_s ln \frac{\emptyset_s}{2} + \emptyset_0 ln \emptyset_0 + f_{el} \quad [3]$$

A **B** **C** **D**

where $\emptyset_p$, $\emptyset_s$ and $\emptyset_0$ are the respective volume fractions of polymer, salt and solvent. Adhikari et al.[25] pointed out that the fraction of counterions released by polyelectrolytes should be included in $\emptyset_s$. In addition to the three mixing (entropic) terms **A**, **B**, **C** on the right side of Equation 3 an electrostatic (enthalpic) term **D** describes the net contributions from continuum electrostatics to the overall free energy. The electrostatic term is the most challenging from a theoretical viewpoint, requiring accurate summation of all Coulombic repulsive and attractive terms.[27, 28] One or more interaction parameters describing specific interactions between polymer, water and ions can also be added.[17, 25] VO theory, as initially presented, has been routinely criticized, although it is often used as a starting point and has, with appropriate parameterization, given acceptable fits to experimental data.[17]

Focusing on the ion content of PECs: while VO theory, and generalizations thereof,[19] predict $[MA]_{PEC} > [MA]_s$, the consensus with more modern approaches holds that $[MA]_{PEC} < [MA]_s$ [18, 21, 23, 25, 27, 38, 42], especially when chain connectivity is included,[24] a result supported by the experiments of Li et al.[38]

Expressions such as Equation 3 are based on volume fraction to adequately account for the configurational entropy of polymer chains. Although the statistical mechanics used in Flory-Huggins theory assigns polymer segments and other species each one lattice site, polymer segments usually have greater volumes than solvent and ions. Due to this lattice size mismatch, well known in polymer physics, using volume fractions underestimates the chemical potential of salt ions compared to the use of mole fractions, an issue recognized by Salehi and Larson, who introduced an additional weighting factor which was the ratio of molecule to solvent volumes.[19]

Equation 3 supports an approximation made in the present work: because of the 1/*N* dependence, the contribution to the total entropy from a polymer molecule (without counterions), term A on the right hand side, is much lower than that of small molecules/ions occupying the same volume. An estimate of the contribution from polymer configuration entropy on mixing, a few J, is provided in Supporting Information. We assume it to be negligible in the present work. As a PEC-specific example of the "entropy starved" contribution of the polymer chain, the osmotic pressure, π, of a PEC with high chain density, the same used in the present work, was estimated from the theory of Des Cloizeaux[43] to be at 100 times lower per repeat unit than π from the same number density (concentration) of NaCl.[41]

It is often assumed that when Pol^+ and Pol^- complex from solution the resulting PEC is close to stoichiometric and Equation 1 is an accurate representation of the overall stoichiometry. While this is true for most PEC systems when Pol^+A^- and Pol^-M^+ are carefully (and simultaneously!) mixed in stoichiometric quantities, an excess of one added polyelectrolyte often leads to an excess of that polyelectrolyte in the PEC. The phenomenon, known as *overcompensation* or *overcharging*, is represented by

$$(Pol^+Pol^-)_{PEC} + \sigma Pol^+A_S^- \rightarrow (Pol_{1+\sigma}^+A_\sigma^-Pol^-)_{PEC} \qquad [4]$$

where this example illustrates overcompensation of Pol^- by Pol^+ by a factor σ from excess Pol^+A^- in solution. The excess of polymer is revealed by the presence of its counterion in the PEC. Overcompensation/nonstoichiometry is actually essential in stabilizing (nano)particles of PEC such as "polyplexes" of DNA and a polycation, often used for gene delivery.[44] Overcompensation is also recognized to be central to the layer-by-layer buildup mechanism of ultrathin films of PEC.[45] Given the difficulty of mixing precisely stoichiometric quantities of Pol^+A^- and Pol^-M^+ it is probable that many PECs assumed to be stoichiometric are actually overcompensated by a few percent. The signature of nonstoichiometric PECs is significant swelling[46] as $[MA]_s \rightarrow 0$ because the trapped ions generate osmotic pressure.[41] There are many analytical methods to determine ion content in PECs. The use of radiolabeled ions is the most precise with the lowest detection limit while providing good accuracy.

We have investigated the equilibrium ion content of PECs in response to $[MA]_s$ for many years.[47, 48] In the "doping" experiment here, MA = NaA and the $^{22}Na^+$ radiolabel is used to track the exact quantity of NaA entering the PEC as a function of solution NaA concentration. Since the starting amount of polyelectrolyte is known, the experimental data is presented as the ratio *r* of moles of MA to moles of polyelectrolyte, which is also the ratio of their concentration in the PEC phase:

$$r = \frac{moles\ MA\ in\ PEC}{moles\ polyelectrolye} = \frac{[MA]_{PEC}}{[PE]_{PEC}} \qquad [5]$$

$[PE]_{PEC}$ is the total concentration of Pol^+Pol^- in whatever form; i.e. for a stoichiometric PEC

$$[PE]_{PEC} = [Pol^+Pol^-] + \frac{[Pol^+A^-]_{PEC} + [Pol^-M^+]_{PEC}}{2} \qquad [6]$$

Examples of *r* versus $[MA]_s$ in a PEC of PDADMA/PSS for various A^- along a Hofmeister series are shown in Figure 1. These data are along the lines of previous results[48] but they have been

collected using radiolabeled salts which permit a greater degree of accuracy and they reveal, unlike measurements of released ions, whether any ions remain in the PEC even as $[MA]_s \rightarrow 0$.

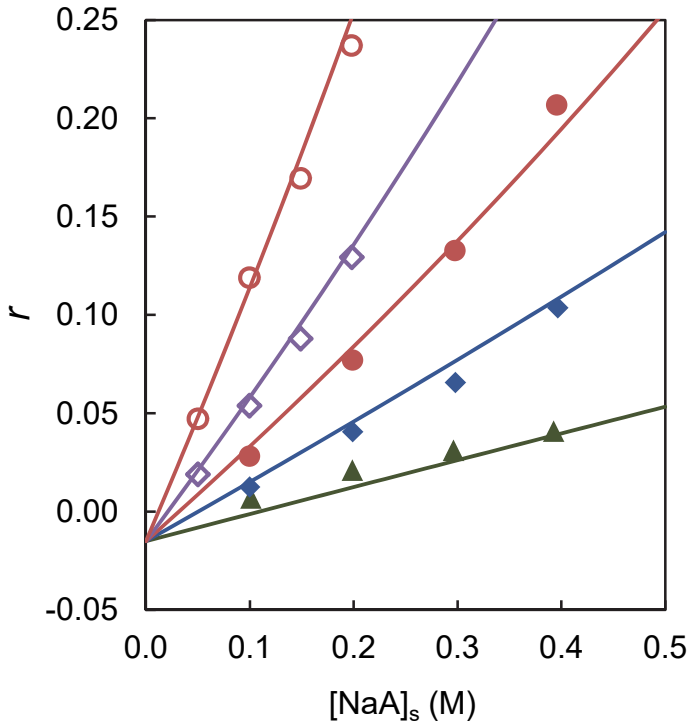


Figure 1. Ratio *r,* $[MA]_{PEC}/[PE]_{PEC}$*, versus* the concentration of NaA in solution for a PDADMA/PSS PEC at room temperature**.** Acetate$^-$ (▲); Cl^- (♦); Br^- (●); I^- (◊); and ClO_4^- (○). The lines are fits using Equation 32.

The remainder of this paper focuses on quantitative predictions for ion content. First to be addressed is the "ideal" case where $\Delta H \rightarrow 0$ and only entropic contributions determine the equilibrium PEC ion content. This is followed by a treatment of nonideal cases where the influence of $\Delta H \neq 0$ on the equilibrium must be considered. Finally, the value of the simple equilibrium expressions derived in predicting PEC composition will be shown.

PEC Ion Concentration, Ideal Case

The Donnan equilibrium,[49] in use for over 100 years,[50] considers 2 phases, ***i*** and ***R***, with small ions M^+ and A^- that can populate both phases and larger macroions (colloid, polyelectrolyte, protein) that are restricted to one phase. Under ideal conditions

$$[M^+]_{\boldsymbol{i}}[A^-]_{\boldsymbol{i}} = [M^+]_{\boldsymbol{R}}[A^-]_{\boldsymbol{R}} \quad [7]$$

The Donnan equilibrium is a simple way of leveling the net entropic contributions from ions partitioned between two phases. Though simple and claimed to be "not applicable" to PECs,[22] the expression is neither phenomenological nor empirical. Philipse and Vrij provide a detailed account of the thermodynamic foundation of the Donnan equation of state.[49]

In our systems, phase ***i*** is PEC and ***R*** is the aqueous solution, *s*, (sometimes called the "dilute phase")

$$[M^+]_{PEC}[A^-]_{PEC} = [M^+]_s[A^-]_s \quad [8]$$

If the PEC is stoichiometric,

$$[M^+]_{PEC} = [A^-]_{PEC} \quad [9]$$

and the solution contains only MA

$$[M^+]_s = [A^-]_s \quad [10]$$

then

$$K_{Donnan} = \frac{[M^+]_{PEC}[A^-]_{PEC}}{[M^+]_s[A^-]_s} = \frac{[MA]^2_{PEC}}{[MA]^2_s} = 1 \; for \; ideal \; PEC \quad [11]$$

and $[MA]_{PEC} = [MA]_s$ [12]

i.e. $\Delta G^0 = -RTlnK = 0$. This is for an *ideal* system that is driven by entropy alone. The concept of *ideal* has its usual implications: there are no net changes in specific interactions whether an

ion is in the PEC or aqueous phase (i.e. the ion doesn't "care" where it is). Equation 11 is the simplest result, possible for stoichiometric PECs only. (The Donnan equilibrium was previously used in an attempt to rationalize nonstoichiometric or overcompensated PEC, in which case $[M^+]_{PEC} \neq [A^-]_{PEC}$.[51]) An additional simplification here is that concentration has been used in place of activities, implying that the activity coefficients cancel. For an ideal system this is the case (by definition, activity coefficients represent the degree of nonideality). If osmotic pressure is determined mainly by the ions, one would also expect the condition $[MA]_{PEC} = [MA]_s$ to balance the (ideal) osmotic pressure between PEC and solution phases.

In our extensive experience with PECs made from PDADMA and PSS we have found one set of conditions that leads to nearly athermal mixing: using KBr as MA. Data previously collected[7] for a phase diagram is reworked here in Figure 2 to present $[MA]_{PEC}$ versus $[MA]_s$. The weight% of KBr was accurately determined, as were the water and polymer weight %. These were translated to $[KBr]_{PEC}$ using accurate values for the density of Pol^+Pol^- (ρ = 1.27 g cm^{-3}), KBr (ρ = 2.75 g cm^{-3}), and H_2O (ρ = 1.00 g cm^{-3}), and Equation 5 and the following:

$$[PE]_{PEC} = 1000/V_m \qquad [13]$$

For any *r*

$$V_m = \frac{M_{Pol^+Pol^-}}{\rho_{PEC}} + 18m_r + r\frac{M_{MA}}{\rho_{MA}} \qquad [14]$$

V_m is the molar volume (cm^3 mol^{-1}) of the PEC normalized to the total moles of PE (Equation 6). $M_{Pol+Pol-}$, M_{MA} and 18 are the respective formula weights of Pol^+Pol^- (dry), MA, and water, while m_r is the number of water molecules per PE (which is a function of *r*).

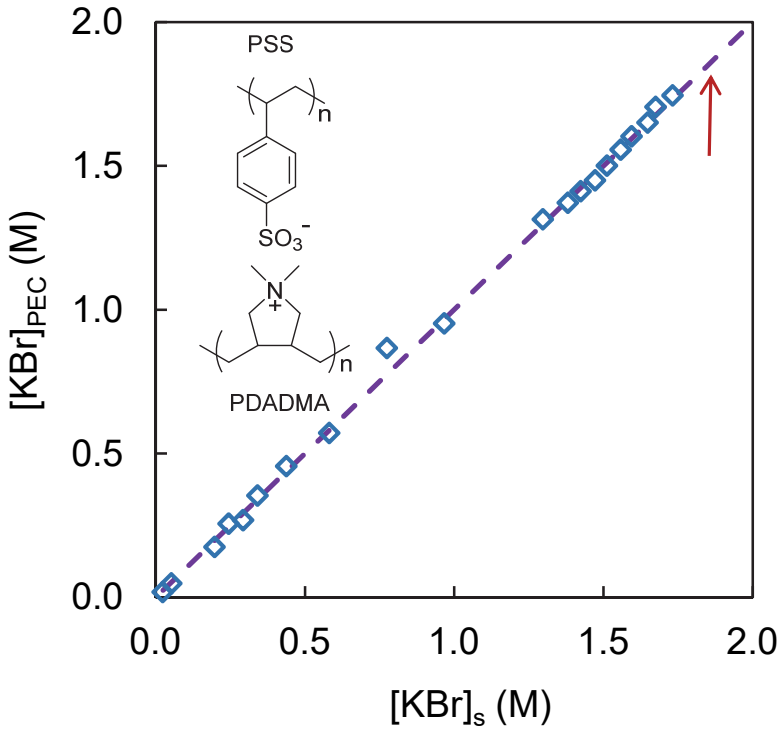


Figure 2. The dependence of $[KBr]_{PEC}$ on $[KBr]_s$ for a PDADMA/PSS PEC at room temperature. The morphology of the PEC, in equilibrium with $[KBr]_s$, proceeds from a solid (with a glass transition), to a rubber, to a liquid-like coacervate with increasing $[KBr]_s$. At experimental $[KBr]_s > [KBr]_c$, indicated with an arrow (1.8 M), the coacervate dissolves and the system is single phase. Data adapted from reference [7]. The dashed line represents $[KBr]_{PEC} = [KBr]_s$. Inset shows the PDADMA/PSS unit.

It is clear that this nearly athermal system (ΔH_{PEC} = -0.30 kJ mol^{-1})[37] nearly follows the ideal $[KBr]_{PEC} = [KBr]_s$. No other system where $\Delta H_{PEC} \approx 0$ has been reported in the ITC literature, although it should certainly not be unique in this respect. For reference, all the weight fractions, mole fractions, volume fractions etc. have been tabulated in Supporting Information Table S1.

If m_r is approximately constant, which is the case for the system in Figure 2 for $r < 0.2$, (see Table S1, Supporting information)

$$V_{m,r\to 0} = \frac{M_{Pol^+Pol^-}}{\rho_{PEC}} + 18m_{r\to 0} \quad [15]$$

It should be emphasized that the volume used to determine $[KBr]_{PEC}$ is assumed to *include* the volume of the polyelectrolytes. Given the good agreement of the Donnan equilibrium theory with experiment our assumption appears to be justified. All ions, whether or not they are counterions associated with Pol^+ or Pol^-, have the opportunity to explore all the space in a PEC. This is because ion/polyelectrolyte place exchange occurs rapidly at a rate of 10^6 to 10^9 s^{-1}.[52] Polymer chains do not exclude water or ions.

Ions versus Counterions

Equation 2 shows the transformation of paired (intrinsic) to unpaired (extrinsic) polyelectrolyte repeat units. At any instant in time, not all MA that enters the PEC breaks Pol^+Pol^- pairs. This point is clearly illustrated by further analysis of the data for PDADMA/PSS doped with KBr: at sufficiently high $[MA]_s$ the salt concentration $[MA]_{PEC}$ far exceeds the concentration of PE in the complex. The ratio *r* (= $[MA]_{PEC}/[PE]_{PEC}$, Equation 5) is plotted versus $[MA]_{PEC}$ in Figure 3. For example, at the highest $[MA]_{PEC}$ measured (1.75 M, see Supporting Information Table S1) the PE concentration is only 0.33 M, *r* = 5.3, which means there are 5 times as many ions as polyelectrolyte repeat units. It is clear that at higher $[MA]_s$ only a fraction of ions that enter the PEC to balance the Donnan equilibrium actually break Pol^+Pol^- pairs and end up as counterions for polyelectrolyte as represented in Equation 2. The ions in the PEC are distributed as follows:

$$[MA]_{PEC} = [MA]_{PEC,count} + [MA]_{PEC,co} \quad [16]$$

where $[MA]_{PEC,count}$ represents the concentration of MA acting as counterions and $[MA]_{PEC,co}$ represents those MA not associated with Pol^+ or Pol^-, called co-ions in ion exchange terminology.[53] Defining *f* as the fraction of MA acting as counterions

$$\frac{[MA]_{PEC,count}}{[MA]_{PEC}} = f \qquad [17]$$

$$\frac{[MA]_{PEC,co}}{[MA]_{PEC}} = 1 - f \qquad [18]$$

Counterions and co-ions are expected to exchange rapidly, far faster than exchange between adjacent pairs of Pol^+Pol^-.

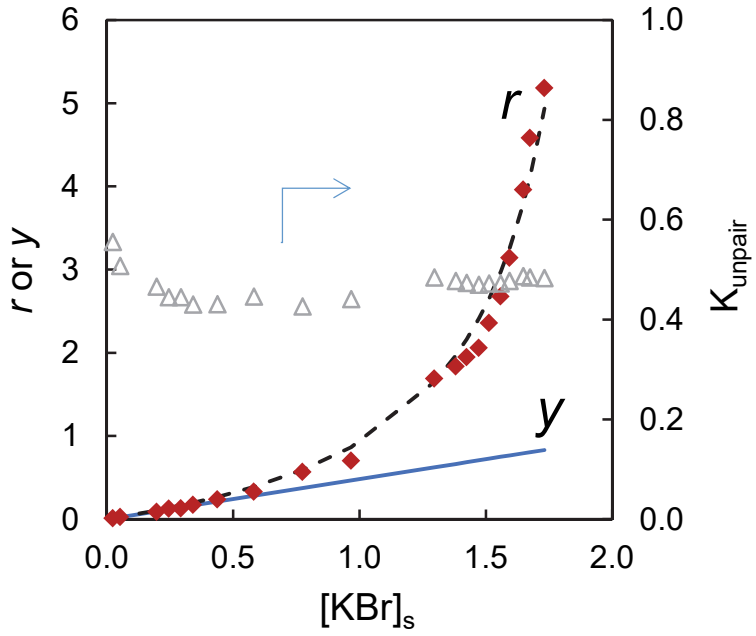


Figure 3. Graph to show the difference between *r* and *y*. *r* (♦) is obtained directly from the data (Supporting Information Table S1. Adapted from reference[7]). For all values of *y* (solid line) from 0 to 1, $y = K_{unpair}[KBr]_s$ where $K_{unpair} = 0.48$. *r* calculated from Equation 25 is also shown (dashed line). Alternatively, K_{unpair} was calculated from Equation 23 using the data in Table S1, Supporting Information. Note that *y* and *r* converge as $r \rightarrow 0$. In this case $y \approx r$ for $r < 0.2$, implying all ions entering the PEC over this range of *r* actually separate Pol^+Pol^- pairs and become counterions, as illustrated in Equation 2. At $r > 0.2$, corresponding to $[MA]_s > 0.5$ M, the co-ion population becomes significant.

The reason there are counter- and co-ions is simple: if ions were excluded from anywhere but next to the polyelectrolyte in the expanding PEC their entropy would not be maximized. By the same reasoning, it is argued that at low levels of *r* the ions are forced to be counterions – the only locations for them are next to Pol^+ and Pol^-. Exchange of a charged polymer segment for a counterion yields a net zero change in Coulombic interaction energy. The distinction between counter- and co-ions is important because the counterions reflect the extent to which Pol^+Pol^- pairs have been broken (which occurs, in an ideal system, isoenthalpically)

The equilibrium between paired and unpaired polyelectrolyte may be written as follows:

$$K_{unpair} = \frac{[Pol^+A^-]_{PEC}[Pol^-M^+]_{PEC}}{[Pol^+Pol^-]_{PEC}[MA]_S^2} \quad [19]$$

If *y* is the fraction of PE in the "extrinsic" or unpaired form (Pol^+A^- and Pol^-M^+)

$$f = \frac{y}{r} \quad [20]$$

and

$[Pol^+A^-]_{PEC} = [Pol^-M^+]_{PEC} = fr[PE]_{PEC}$ and $[Pol^+Pol^-]_{PEC} = (1-fr)[PE]_{PEC}$.

Thus,

$$K_{unpair} = \frac{(y)^2[PE]_{PEC}}{(1-y)[MA]_S^2} = \frac{(fr)^2[PE]_{PEC}}{(1-fr)[MA]_S^2} \quad [21]$$

The following relationship is also consistent

$$y = K_{unpair}[MA]_s \quad [22]$$

which means

$$K_{unpair} = \frac{1}{[PE]_{PEC}+[MA]_s} \quad [23]$$

K_{unpair} is a quantitative measure of the efficiency of MA at breaking Pol^+Pol^- pairs; or K_{unpair}^{-1} = K_{pair} may be interpreted as the strength of complex formation. K_{unpair} reflects the ability of the polyelectrolyte (with their waters of hydration) to accommodate ions. K_{pair} values for different pairs of polyelectrolytes provides a useful library of Pol^+Pol^- interaction strengths.[54]

A useful limit of Equation 19 is $[MA]_s \rightarrow 0$ and r, y $\rightarrow$ 0, in which case

$$K_{unpair} = \frac{1}{K_{pair}} = \frac{1}{[PE]_{PEC,r\rightarrow 0}} = \frac{\frac{M_{Pol^+Pol^-}}{\rho_{PEC}} + 18m_{r\rightarrow 0}}{1000} \quad [24]$$

Note that K_{unpair}, as written in Equation 19 should not vary with *y* (or *r*), so Equation 24 provides a convenient and reliable estimate of K_{unpair} for an ideal PEC. All that is needed is an estimate of the molar volume of undoped hydrated PEC.

The relationships between *y* and *r* as a function of solution concentration are depicted in Figure 3, along with values for K_{unpair} using experimental values of $[PE]_{PEC}$, $[MA]_s$ and *y* (from *y* = $0.48[MA]_s$). It is evident that K_{unpair} remains ≈ 0.5 over the whole range of PEC composition. Supporting Information Figure S1 shows a zoom-in of the *r* ≈ *y* region in Figure 3.

For an ideal PEC, the relationship between y and r is given by

$$r = \frac{[PE]_{PEC,r\rightarrow 0}}{[PE]_{PEC}} = \frac{y}{1-y} \quad [25]$$

Predicted ideal responses of PECs having various hydrated molar volumes giving K_{unpair} values according to Equations 22, 24, and 25 are shown in Figure 4. The range of molar volumes for hydrated undoped PEC $V_{m,r\rightarrow 0}$ is from 200 cm^3 to 700 cm^3, representing a range from the most compact (e.g. poly(allylamine)/polyvinylsulfonate) to less compact complexes. Exothermic complexation would push the data closer to the x-axis (stronger Pol^+Pol^- association) whereas endothermic PEC formation would move the data closer to the y-axis (weaker complexes).

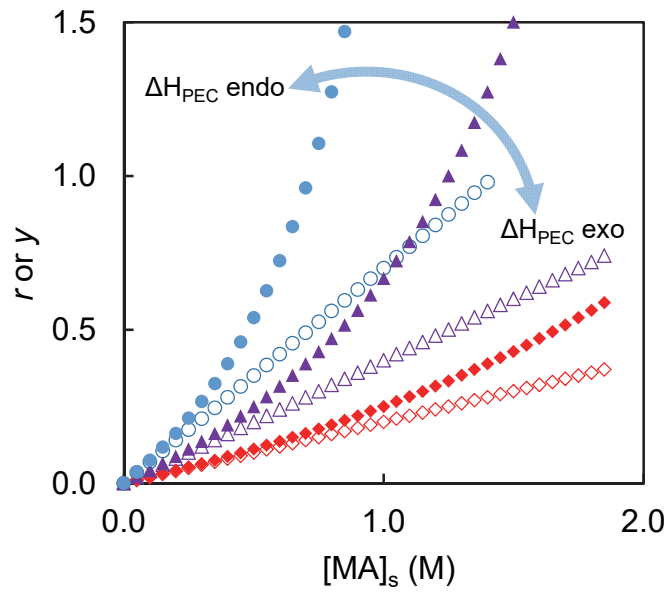


Figure 4. Predicted relationship between *r* and *y* as a function of $[MA]_s$ for ideal PECs having various undoped molar volumes, V_m (Equation 15) in the case of $\Delta H_{PEC} = 0$. ● (*r*), ○ (*y*) for V_m = 200 cm^3; ▲ (*r*), Δ (*y*) for V_m = 400 cm^3; ♦ (*r*), ◊ (*y*) for V_m = 700 cm^3. The effect of endo- or exothermic complexation is indicated.

PEC Ion Concentration, Nonideal Case

Almost all PEC complexation involves $\Delta H \neq 0$. The heat flow is believed to come from a change in water structure around charged units shown in Equation 1.[37] A net enthalpic contribution shifts the ideal equilibrium as follows:

$$K_{unpair,\Delta H\neq 0} = K_{unpair,\Delta H=0} e^{\Delta H_{PEC}/RT} = \frac{1}{[PE]_{PEC,y\to 0}} e^{\Delta H_{PEC}/RT} \quad [26]$$

and Equation 22 is modified in the same way

$$y = [MA]_s K_{unpair,\Delta H=0} e^{\Delta H_{PEC}/2RT} \quad [27]$$

Note that $[PE]_{PEC,r\to 0}$ does not depend on the nature of MA (i.e the sign and magnitude of ΔH_{PEC}): for the same Pol^+Pol^-, all $[PE]_{PEC}$ as a function of *r* converge to the same value for undoped $[PE]_{PEC}$.

In the same way, since $K_{Donnan,\Delta H=0} = 1$

$$K_{Donnan,\Delta H\neq 0} = e^{\Delta H_{Donnan}/RT} \quad [28]$$

The enthalpy change for the Donnan equilibrium applies to the entire salt population within the PEC, not just the ions that are serving as counterions. The enthalpy change is proportional to the fractions of ions that are acting as counterions

$$\Delta H_{Donnan} = f_{count}\Delta H_{PEC} + f_{co}\Delta H_{transfer} \approx f_{count}\Delta H_{PEC} = \frac{y}{r}\Delta H_{PEC} \quad [29]$$

where $\Delta H_{transfer}$ is the enthalpy change on moving MA from solution to the co-ion state in PEC. $\Delta H_{transfer}$ is assumed to be zero as the co-ions are assumed to be hydrated in the same way as their solution counterparts, the hydration environment not extending past about one shell of hydration water.

$$K_{Donnan,\Delta H\neq 0} = e^{\frac{y}{r}\Delta H_{PEC}/RT} \quad [30]$$

At low *r*, $y \approx r$

$$[MA]_{PEC} = [MA]_s e^{\frac{y}{r}\Delta H_{PEC}/2RT} \approx [MA]_s e^{\frac{\Delta H_{PEC}}{2RT}} \; as \; r \to 0 \quad [31]$$

$$r \approx \frac{[MA]_s e^{\Delta H_{PEC}/2RT}}{[PE]_{PEC}} \quad [32]$$

Equation 31 is a quantitative statement of enthalpy-entropy compensation.[55]

Examples of PDADMA/PSS with theoretical athermal, exothermic and endothermic ΔH_{PEC}, using *r* values from the KBr system, are sketched in Figure 5

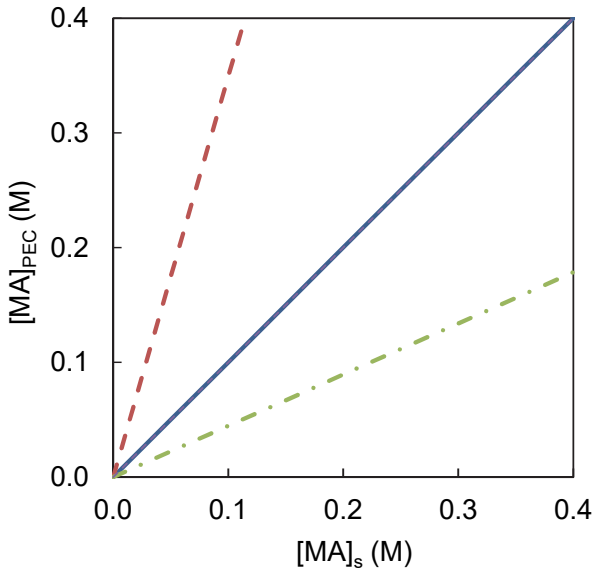


Figure 5. Calculated $[MA]_{PEC}$ versus $[MA]_s$ for r ≈ y < 0.2 using Equation 31 for a PDADMA/PSS PEC, with isothermal (ΔH_{PEC} = 0, $[MA]_s = [MA]_{PEC}$, solid line), negative deviation (exothermic, ΔH_{PEC} = -4000 J mol^{-1} in this example, dot-dash line), positive deviation (endothermic, ΔH_{PEC} = +5000 J mol^{-1}) heats of complexation. If ΔH_{PEC} = 0, $[MA]_{PEC} = [MA]_s$, an ideal Donnan equilibrium.

Figure 5 makes it clear that the salt content of a PEC made from a specific pair of polyelectrolytes can experience *either* negative *or* positive deviation, depending on the (hydration) characteristics of the (counter)ions.

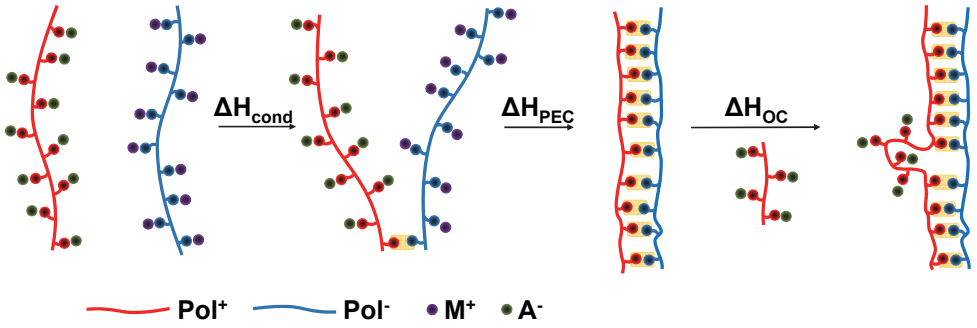
Determination of Accurate ΔH_{PEC}

Using isothermal calorimetry, ITC,[29, 30, 31, 32, 33, 34, 35, 36] the apparent ΔH_{PEC} component of $\Delta G_{PEC} = \Delta H_{PEC} - T\Delta S_{PEC}$ may be measured with great sensitivity. Because entropic driving forces are small, even small enthalpy changes impact the formation of PECs. Thus, Laugel et

al. measured increasingly negative ΔH_{PEC} for increasingly-strongly associating pairs of polyelectrolytes.[31] Although modern ITC systems come with a variety of software to analyze binding energies (mostly between biomolecules) results are model-dependent and geared more towards characterizing specific individual (protein) binding sites. Thus, we have used only experimental ΔH_{PEC} and not extracted additional quantitative information from the form of the titration curve.

Before proceeding further, some clarification of the relevant steps in complexation is needed. When solutions of individual polyelectrolytes are brought together they first encounter each other, then associate, summarized by Equation 1. The cartoon in Scheme 1 breaks down these two steps, the first labeled “condensation,” where the polyelectrolytes come together, and the second, “complexation.” It is clear that ITC, such as those titrations shown in Supporting Information Figures S2 and S3, provides the sum of the two steps and not the actual enthalpy of complexation. The response of the PEC ion content to solution ion content, Equation 2, is *almost* the reverse of Equation 1. We and others have, without proof, made the implicit assumption that there are no enthalpy changes on condensing polyelectrolytes (first step) i.e. that ΔH measured by ITC = ΔH_{PEC}.

In Scheme 1, one Pol^+Pol^- pair has formed between the condensed polyelectrolytes. This “first contact” defines the point when polyelectrolyte goes from solution to PEC phase. First contact requires loss of polyelectrolyte translational entropy (understood to be negligible), ΔH for the formation of one Pol^+Pol^- pair, a very small fraction of the ΔH provided by *n* repeat units (*n*, degree of polymerization, >> 1), and an unknown change of the environment around the chain as it approaches other chains. In the actual complexation step the rest of the Pol^+ and Pol^- units pair and lose counterions (in less than 1 mS[56, 57]). Therefore, $\Delta H_{PEC} = \Delta H_{PEC,U} - \Delta H_{cond}$ where $\Delta H_{PEC,U}$ is the ITC measured, uncorrected, complexation enthalpy.



Scheme 1. Showing the initial condensation of solution polyelectrolytes into a complex where one Pol^+Pol^- pair is formed between two molecules (the "first contact"). This is rapidly followed by more extensive complexation of the rest of the polyelectrolyte. If one of the solution polyelectrolytes is in excess, overcompensation occurs (last step), known to be up to 40% for PDADMA(X) or PSSNa.

Also added to Scheme 1 is the known property that excess polyelectrolyte adds to *overcompensate* stoichiometric material. In recent ITC studies, Požar and Kovačević were the first to point out that going beyond 1:1 or stoichiometric complexes produces/uses minimal heat.[33] A follow-up study[35] showed that overcompensated PEC resulted in nanoparticles stabilized by excess polyelectrolyte, confirming Dautzenberg's model.[58] In other words, taking polyelectrolyte from dilute solution and concentrating it within the PEC is nearly athermal.

In the system studied here, overcompensation by either PSS(Na^+) or PDADMA(Cl^-) was found to be about 40%.[51] There is no reason to expect it to be 40% for all PECs. Overcompensation has neither been studied for a variety of Pol^+Pol^- combinations nor as a function of A^- ion, so it is assumed here to be similar for Br^- and Cl^-. Experimentally, the heat of overcompensation, ΔH_{oc}, is taken directly from the ITC data of heat versus mole ratio of Pol^+/Pol^- or Pol^-/Pol^+ (depending on which is being added to the cell) in the region ratio = 1.0 → 1.4. Such an example is shown in Figure 5 for NaBr. Interestingly, overcompensation with

PDADMA(Br) generates a small amount of heat $\Delta H_{oc,PDADMABr}$ whereas overcompensation with PSSNa does not ($\Delta H_{oc,PSSNa} \approx 0$).

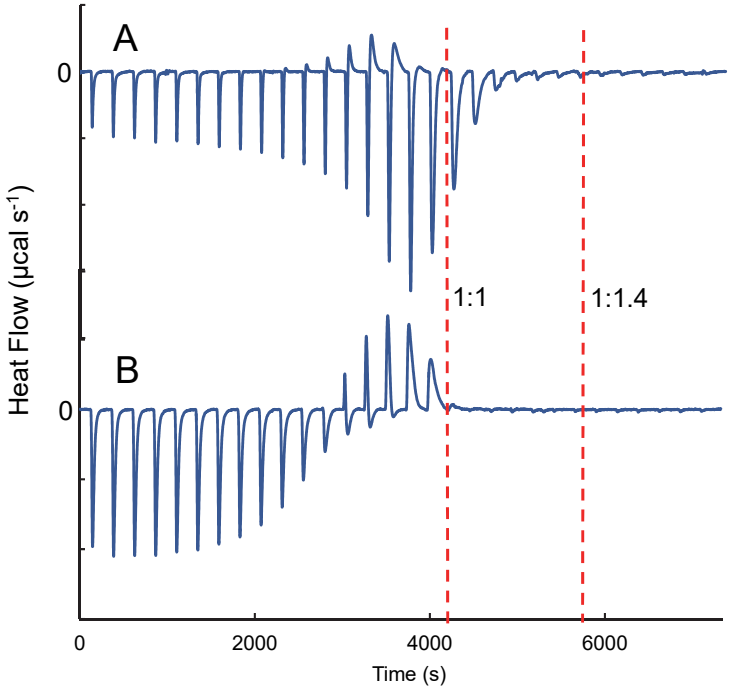


Figure 6. ITC titration of PDADMA(Br) into PSSNa (A); and PSSNa into PDADMA(Br) (B). A 1:1 stoichiometry is indication by the left-most dotted line. Overcompensation to a level of 1.4 is indicated by the second dotted line. No significant heat flow is observed with PSSNa overcompensation (region from 1:1 to 1:1.4, B), whereas exotherms are observed when PDADMA(Br) overcompensates (A). Exothermic spikes point downwards.

It is reasonable to assume that the additional overcompensating polyelectrolyte from solution experiences a change in environment similar to the initial complexation (step 1, Scheme 1). Thus, any heat generated after 1:1 stoichiometry represents condensation of the added polyelectrolyte (but not complexation, because all the polyelectrolyte in the cell has been used). If the overcompensation level is about 40%[51]

$$\Delta H_{PEC} = \Delta H_{PEC,U} - \frac{\Delta H_{oc,PDADMA(X)}}{0.4} - \frac{\Delta H_{oc,PSSNa}}{0.4} \quad [33]$$

ΔH_{PEC} values for various NaX are listed in Table 1. Unfortunately, counterions on the hydrophobic side of the Hofmeister series, SCN^-, I^-, ClO_4^-, caused $PDADMA^+$ to precipitate. We were thus unable to collect $\Delta H_{PEC,U}$ for these ions. ΔH_{PEC} values for I^- and ClO_4^-, were estimated by the fitting described later.

Table 1. Enthalpies of complexation and condensation for PDADMA(X) with PSSNa at 25 °C.

Anion, X^-	[a]$\Delta H_{PEC,U}$ J mol^{-1}	[b]r at $0.1M$	ΔH_{oc} J mol^{-1}	[d]ΔH_{PEC} J mol^{-1}
CH_3COO^-	-5985	0.007	[c]0	-6027
Cl-	-2160	0.012	[c]0	-2186
Br^-	-774	0.028	-383	+189
I^-	n.d.	0.054	n.d.	[e]+2200
ClO_4^-	n.d.	0.119	n.d.	[e]+5000

[a]experimental $\Delta H_{PEC,U}$ in 0.1M NaX

[b]from Figure 1.

[c]magnitude is less than 10 J mol^{-1}

[d]$\Delta H_{PEC} = \frac{\Delta H_{PEC,U} - \Delta H_{oc}/0.4}{(1-r)}$

[e]fit to data

The Hofmeister series trend in Table 1 has been observed in other works for different PECs. Požar and Kovačević[33] demonstrated that ΔH_{PEC} for PSS/PAH complexation can go from exothermic to endothermic depending on the counterion (they were able to use ClO_4^-). Oppermann and Schulz[29] found less variation in ΔH_{PEC} with poly(methacryloyloxyethyl trimethylammonium)/PSS in MCl where M^+ was Li^+, Na^+, K^+, Rb^+ and ΔH_{PEC} = -1.7 to -0.7 kJ mol^{-1} in accord with the general observation that variation of cations in polyanions has less effect on specificity or Hofmeister trends.

Calculated $[MA]_{PEC}$ versus $[MA]_s$

Equations 5 and 13 provide $[NaX]_{PEC} = 1000r/V_m$, which allows data from the doping graph to be transformed into $[NaX]_{PEC}$, presented in Figure 7. The water content m_r was measured experimentally as the total PEC mass – (mass PE + mass ion) (see Supporting Information Table S2 for corresponding weight%). The solid lines are a plot of Equation 31 (including a small intercept, discussed below). For NaCl, NaBr and NaAc, only experimentally determined ΔH_{PEC} and $[PE]_{PEC}$ values (approximately constant see Supporting Information Table S1) were used with no freely adjustable parameters. Because $\Delta H_{PEC,0}$ could not be determined for $NaClO_4$ and NaI, respective ΔH_{PEC} of +5000 and +2200 J mol^{-1} were fit to the data.

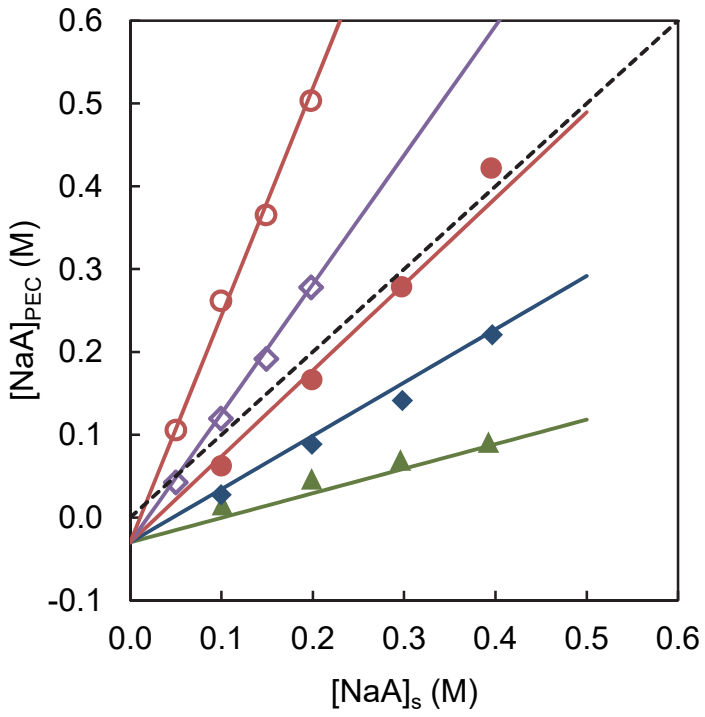
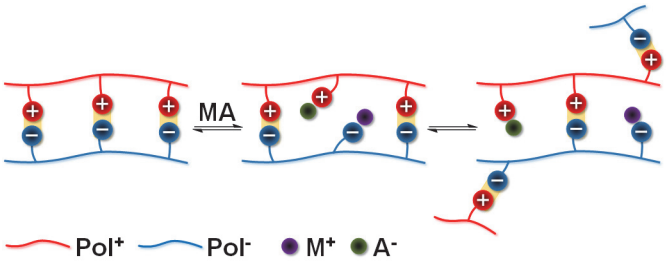


Figure 7. Salt NaA concentration in PSS/PDADMA PEC, $[NaA]_{PEC}$, *versus* solution salt concentration $[NaA]_s$. For all salts, cation $M^+ = Na^+$. Results for five anions in the Hofmeister Series $ClO_4^- < I^- < Br^- < Cl^- <$ acetate$^-$. The dotted line indicates $[NaA]_{PEC} = [NaA]_s$. Cl^- (♦), and acetate (▲), towards the "hydrophilic" end of the Series, exhibit negative deviations $[NaA]_{PEC} <$

$[NaA]_s$. ClO_4^- (○), and I^- (◊), at the "hydrophobic" end of the Series, show positive deviation $[NaA]_{PEC} > [NaA]_s$. Br^- (●), $\Delta H_{PEC} = +0.189$ kJ mol^{-1}, is close to $[NaA]_{PEC} = [NaA]_s$. Solid lines for acetate, Cl^- and Br^- are predicted from Equation 31 using ΔH_{PEC} values from Tables 1. Solid lines for ClO_4^- and I^- are given by Equation 31 with respective fitted ΔH_{PEC} of +5000 and +2200 kJ mol^{-1}. The intercept, c', on the y-axis, -0.03, is thought to be mainly from the osmotic pressure of the polyelectrolyte chains.

The fact that hydrophobic ions I^- and ClO_4^- also precipitated the polycation suggests that the number of stable coacervates containing these ions in high concentration might be limited: coacervation with Pol^- would compete with precipitation by A^-.

The excellent fit of the data in Figures 2 and 7 to the Donnan equilibrium implies that after doping, ions are decoupled, represented in Scheme 2, which would be required to maximize their entropy.



Scheme 2. After doping MA into the PEC (step 1) and breaking a Pol^+Pol^- pair, ions M^+ and A^- are decoupled from each other (step 2) and are free to travel throughout the PEC alone.

Intercept

A nonzero intercept -c' of $[MA]_{PEC}$ *versus* $[MA]_s$ is consistently seen in Figure 7 for all salts: it appears that a minimum concentration of any salt in solution is needed before MA starts to dope into the PEC. This negative intercept on the $[MA]_{PEC}$ axis could be a result of nonstoichiometry: there is a slight excess of PDADMA, measured using $^{35}SO_4^{2-}$, to be about 0.7

mol%, corresponding to 0.016 M Cl^- in the PEC, which is equivalent to 0.008 M ideal salt. Excess PSS was found to be less than 0.0006 mol% measured using $^{22}Na^+$.[41]

It is believed most of c' comes from the osmotic pressure of the polyelectrolyte chains themselves, which was previously estimated to be the equivalent of 0.045 M (ideal) salt.[41] To maintain a balance of osmotic pressure, the PEC does not admit MA until a minimum $[MA]_s$ is reached, otherwise the osmotic pressure of the PEC would exceed that of the solution. The actual value of the PEC osmotic pressure, about 0.022 M salt, appears to be a little lower than that calculated. The PEC osmotic pressure, and therefore the magnitude of c', depends on the chain density. PECs with high water content will have a smaller c' but it has little influence on the compositions of PECs that are well doped with salt (i.e. this small correction to the composition can be safely ignored at higher $[MA]_s$).

"Strength" of Complexes, Charge Density, and the Critical $[MA]_s$ for PEC Dissolution

In theoretical treatments of polyelectrolytes the distance between charges on the backbone, ℓ, is an important parameter.[28] This distance is presented as linear charge density, (charges per m), ℓ^{-1}. Since most synthetic polyelectrolytes have 2 carbons in the backbone repeat unit, ℓ for a fully charged polymers is about 0.25 nm. A comparison of the binding strengths between Pol^+Pol^- pairs showed little correlation with ℓ.[54] On the other hand, Equation 24 shows that K_{pair} (= $1/K_{unpair}$) is simply the charge density of hydrated PEC (moles of charge per L). The molar volume V_m, which yields K_{unpair}, depends on the molecular weight of the Pol^+Pol^- unit and its density, which should remain in the range of 1.2 g cm^{-3} for a number of common polyelectrolytes, and its starting hydration level $m_{r\rightarrow 0}$. Thus, the *volume charge density* of Pol^+Pol^- is a determining factor of the association strength of Pol^+ and Pol^-.

The higher the molar volume, the less salt per cm^3 can be accommodated within the PEC, the weaker the association. The water content (m_r), in the same way, also controls

Pol^+Pol^- association by its contribution to V_m. This is where the concept of "hydrophilicity/phobicity" may be applied. The entropic component of hydrophobicity is given (and measurable) by the number of water molecules per Pol^+Pol^-, m_r, whereas the enthalpic one, measured by ΔH_{PEC} reflects changes in water structure. For example, polycarboxylates are "hydrophilic," contain a high proportion of water (large m_r) yielding weak PECs which are easily doped to produce liquid-like complexes/coacervates.[17, 34, 35, 54, 59] Protonated poly(primary amines) especially those with low molecular weight Pol^+Pol^-, like polyallylamine, PAH, or polyvinylamine, have low water content (hydrophobic) and provide strongly-associating PECs by virtue of their small V_m (see Figure 4 also) supplemented by any exothermic ΔH_{PEC}. [31, 54]

Random copolymers are often made with mixtures of charged and neutral (hydrophilic) repeat units. An estimate of K_{unpair} can be made in such a case as follows, assuming each Pol^+ and Pol^- carry respective numbers x and z neutral units of molecular weight $M_{neut,x}$ and $M_{neut,y}$ and average density ρ_{neut}

$$K_{unpair,ideal} \approx \frac{\frac{M_{Pol+Pol-}}{\rho_{PEC}} + \frac{xM_{neut,x} + zM_{neut,z}}{\rho_{neut}} + 18m_{r\to 0}}{1000} \quad [34]$$

Neutral hydrophilic units would make m_r much larger, increasing K_{unpair} substantially, meaning the PEC is much easier to dope and destabilizes by reaching y ≈ 1 at low $[MA]_s$, a behavior recently observed by Huang et al.[60] Charged/neutral block copolymers would behave differently since the monomer types can phase separate.

Michaels et al. noted the swelling and plasticization of poly(vinylbenzyltrimethylammonium)/PSS PECs by salt[61] (Equation 2) and the eventual dissolution of PECs with sufficiently high $[MA]_s$, which is termed the critical salt concentration $[MA]_c$,[62] also called "salt resistance" by Bungenberg de Jong for PECs from biopolymers.[63] The

value for $[MA]_c$ should be the point where y = 1: the 2-phase PEC/solution system should become one phase as all Pol^+Pol^- pairs are broken.

The calculated $[MA]_c$ for the ideal PEC in Figure 2 is 2.1 M (= $1/K_{dop}$) The experimental value for $[MA]_c$ is somewhat lower: 1.80 M. In fact, $[MA]_c$ may be closer to the critical overlap concentration c* for chains in the PEC. Neutron scattering measurements of the conformation of deuterated PSS chains in a PDADMA/PSS PEC suggested c* was near the PE chain density of 0.31 M seen here.[7] Given that it would be difficult to make a continuous PEC phase without overlap of chains, it is likely that $[MA]_c$ is either achieved when y = 1 or when c* is reached, whichever requires the lower $[MA]_s$.

It may not be possible to reach true solutions of individual dissolved polyelectrolytes if one of the polyelectrolytes is insoluble at most [MA] (e.g. PDADMA in I^-, ClO_4^-, SCN^-) or if $[MA]_c$ is beyond the solubility limit of MA itself, or if MA_{aq} becomes a nonsolvent before $[MA]_c$ is reached. One is more likely to avoid solubility limitations or nonsolvent conditions with weaker PECs, such as those made from carboxylates, since $[MA]_c$ is lower.

The "Dilute Phase"

The formation of PECs is often described as a phase separation into a dilute or solution phase and a concentrated phase (complex or coacervate). Phase diagrams predict boundaries or binodals between these phases.[17] The concept of a dilute phase having dissolved PE has troublesome practical and conceptual implications. Prior reports, including the one further analyzed in Figure 2, have relied on equilibration of PECs in sealed/closed systems to establish phase diagrams.[7, 17] If the dilute phase is constantly being replenished (by circulation), the PEC would be constantly shedding polyelectrolyte. For biomedical applications this means constant leaching of toxic polycation. Fortunately, the rate of polymer equilibration for some PECs is exceedingly slow at sufficiently low $[MA]_s$.

Conceptually, it is hard to imagine single chains of polyelectrolyte can exist in the dilute phase at y<1. In our view, the PE is more likely to be found as diffuse clusters of macromolecules associated by a few contacts of the kind seen in Scheme 1.

Theory: Quo Vadis?

It is reasonable that the vast quantities of work invested in trying to understand the solution properties of polyelectrolytes[28] should be leveraged in descriptions of complexes made from these polymers. In the first experiments on synthetic polyelectrolytes, Fuoss and Sadek stressed the importance of the "strong electrostatic field."[6] There is no doubt that PECs, particularly as dry samples, are held together by electrostatic forces of the kind one would expect between charges in a vacuum. There are some (persistent) pitfalls in extending vacuum electrostatics to a description for the *driving force* of formation of equilibrium hydrated PECs from polyelectrolytes in solution. First, the assumption of weakly charged chains, allowing linear Debye-Hückel approximations, are not appropriate for systems with dense charges. The full Poisson Boltzmann treatment can rectify this deficiency to some extent. Molecular simulations are only as good as the parameters used. For example, net electrostatics are typically approximated by a particle mesh Ewald method.[64] But a small error in ΔH from a small error in Coulomb energy would significantly influence the predicted PEC composition, given the delicate entropic nature of the driving force for PEC formation. The role of screening length must be questioned in the high ionic strength environment of PECs. For example, the density of charges, $[Pol^+Pol^-] + [MA]_{PEC}$, is high and remains so, for the PDADMA/PSS system, at around 2 M over all $[KBr]_s$ (0 to 1.8 M KBr) as seen in Supporting Information Figure S5.

Deeper fundamental understanding can be gained by predicting the hydration level of all charged species within the PEC, a challenge that was raised decades ago and that still remains a focus of solution physical chemistry. Theory should (re)focus on water structuring and

interactions.[65] Short range "ion specific" interactions of the sort considered by Frank and Wen,[66] Samoilov,[67] Hirata and coworkers,[68] Collins,[69] Vlachy and Dill,[70, 71] Ninham,[72] and others have been used to tackle the Hofmeister series. ΔH_{PEC} should reflect the change of water structure around charged species before and after complexation. The enthalpy of PEC formation can be stated in terms of interaction parameters χ between all charged species and solvent. e.g.

$$\chi_{Pol^+Pol^-} = (\chi_{Pol^+A^-} + \chi_{Pol^-M^+}) - (\chi_{M^+} + \chi_{A^-}) \quad [35]$$

where all species have their equilibrium hydration levels (i.e. are immersed in water). The use of continuum electrostatics forecloses on the discussion of counterion-polymer interaction parameters. The word "counterion" is used here to indicate close proximity (within a couple of hydration spheres) of ion to repeat unit. Specific interactions are often included as an afterthought to explain deviations from electrostatic theory. It would be possible to insist on an electrostatic interaction energy (term D, Equation 3) and construe $\Delta H_{PEC} = 0$ to represent exact cancelling of electrostatic and specific interactions. However, it would still be desirable to know the origin and size of these specific interactions.

Conclusions

The same issues that complicate the theory of solutions of individual polyelectrolytes -- their charges and counterions -- substantially simplify the description of complexes made from them. The Donnan equilibrium accurately predicts the salt content of a PEC. It also predicts the pairing strength of oppositely charged segments as the polyelectrolytes phase separate from solution. Of the three components in a PEC – water, polyelectrolyte and salt – the polymer itself has a modest role in the driving force for formation by participating in net charge neutrality and the volume fraction. Polyelectrolyte chains do not contribute net coulombic free energy going

from solution to PEC phases, nor do they contribute appreciable entropy, but they may provide small enthalpic changes *via* the interaction parameters in Equation 29. The entropic driving force for PEC formation comes from rearranging (releasing) the counterions. The osmotic pressures (water mole fractions) of the PEC, which includes a small contribution from the polymer chains, and solution should also balance. The roles of entropy and enthalpy may be clarified by emphasizing that the association of PE is *driven* by entropy but *moderated* by enthalpy.

The researcher wishing to rationalize the distribution of salt in their PECs is advised to use the following approach: first, prepare PECs that are as close to stoichiometric as possible. Then measure PEC water content by drying PEC equilibrated with an aqueous solution of MA at reasonably low $[MA]_s$ (low enough to yield minimal doping but high enough to prevent inflation of the PEC from the osmotic pressure of the polymer chains and any residual salt) – 0.1 M NaCl is recommended. Use Equation 24 to obtain K_{unpair} for an ideal system. Then measure ΔH_{PEC} by calorimetry to obtain K_{unpair} using Equation 26. Finally, Equation 31 should yield a good estimate for $[MA]_{PEC}$ as a function of $[MA]_s$ for low levels of doping.

SUPPORTING INFORMATION

Estimate of $T\Delta S_{config}$ for mixing polymer chains in PEC; tables of data used in Figures 2 and 7; ITC titrations of PDADMA(Cl) and PDADMA(Ac) with PSSNa; graph showing m_R, number of water molecules per PE; graph showing mole fractions of water inside and outside PEC; graph showing approximately constant concentration of (PE + KBr) (~2 M) in PEC.

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Notes

The authors declare no competing financial interest

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