

Salt Resistance as a Measure of the Strength of Polyelectrolyte Complexation

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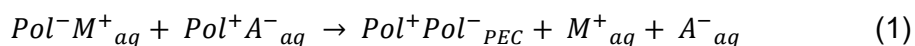
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Abstract

When mixed, solutions of positive and negative polyelectrolytes may spontaneously phase separate into blended, hydrated complexes or coacervates, PECs. Charge-pairing interactions between oppositely-charged polyelectrolytes within PECs are weakened with the addition of salt MA. With a sufficiently high concentration of MA, the PEC may dissociate back into the individual polyelectrolytes, reversing the liquid-liquid phase separation induced by charge pairing and other interactions. This critical salt concentration, CSC, or “salt resistance,” has been extensively used to compare the stability and strength of association in PECs. However, the CSC is not always observed and it shows a strong dependence on the type of ions comprising MA. In addition, the CSC is more likely to be observed with PECs assembled from polycarboxylates, a weak polyelectrolyte. Here, it is shown a lack of experimental CSC is correlated with the preferred role of ions M^+ and A^- in the PEC, counterion *versus* co-ion, or the specificity of a particular ion for a particular polyelectrolyte repeat unit, revealed by calorimetric measurements. The importance of the enthalpy of ionization of weak polyelectrolytes in providing an experimentally measurable CSC is quantitatively demonstrated.

Introduction

When a polyanion (Pol^-) and polycation (Pol^+) are mixed in solution, a polyelectrolyte complex or coacervate (PEC) may form which is an amorphous blend of the two polyelectrolytes.¹⁻² The complexation event, a type of liquid-liquid phase separation, LLPS, can be represented as follows:



where A^- and M^+ are ions of salt MA. The entropic release of counterions is a major driving force for complexation.³⁻⁵

Adding salt to the solution phase partially reverses Equation 1 in a process known as “doping.” Doping breaks Pol^+Pol^- pairing interactions and additional water molecules usually accompanies doping. These effects reversibly plasticize the polymer

(“saloplasticity”), which softens the material and provides a spectrum of solid-like to liquid-like morphologies.⁶ If sufficient salt is added to solution, Equation 1 may be fully reversed and the PEC may completely dissociate back into a single phase mixture. The point where this occurs is known as the critical salt concentration, CSC, or the “salt resistance,” a term coined by Bungenberg de Jong in his extensive pioneering work on PECs.⁷⁻⁸ The effect of salt concentration on the population of Pol⁺Pol⁻ pairs may be interpreted using classical electrostatic screening arguments,⁹⁻¹⁰ or by a more charge-specific competition between pairing of polyelectrolyte segments and (counter)ions.¹¹

The salt resistance is an important point on phase diagrams of PEC composition.⁹ A sketch of a binary PEC phase diagram, showing salt and polymer concentrations, is given in Figure 1.

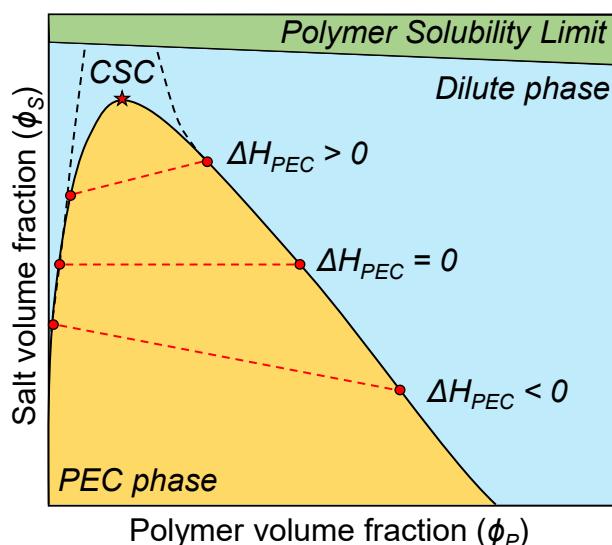


Figure 1. Typical phase diagram for liquid-liquid phase separation of PECs from oppositely-charged polyelectrolytes. The PEC phase is rich in polymer whereas the dilute phase contains little, or no, polymer. The critical salt concentration or salt resistance is shown by the point “CSC.” Tie lines (red dotted) are for conditions where the enthalpy of complexation, ΔH_{PEC} , is positive (> 0), negative (< 0) or $= 0$ (isothermal). If ΔH_{PEC} is > 0 the salt concentration is greater in the PEC phase and vice versa if ΔH_{PEC} is < 0 .¹² For $\Delta H_{PEC} = 0$ the PEC and dilute phase salt concentrations are equal. In cases where the CSC is not obtained, the top portion of the phase diagram will instead appear to follow the black dotted lines, not showing an apex. With sufficient added salt polyelectrolytes may become insoluble.

The salt resistance is commonly used as a measure of the interaction strength of the Pol⁺Pol⁻ pairs within a PEC. The CSC, which varies strongly with the identities of Pol⁺ and Pol⁻ and *also* on the nature of MA, is at (for $\Delta H_{PEC} = 0$) or near the maximum in the binodal, as shown in Figure 1. The “tie lines,” examples shown in Figure 1, connecting the binodals, or boundary between PEC and dilute phase, are also of interest.¹³ Negative tie lines indicate a lower concentration of salt in the PEC, $[MA]_{PEC}$, than in the dilute phase, $[MA]_s$; positive tie lines the reverse; and level tie lines mean $[MA]_{PEC} = [MA]_s$.¹⁴

The free energy of PEC formation/phase separation, ΔG_{PEC} is given by $\Delta H_{PEC} - T\Delta S_{PEC}$. ΔS_{PEC} is always positive at low salt concentrations. The ΔH term reports the sum of all specific interactions: electrostatic, hydration, hydrophobic, hydrogen

bonding, and dipolar. Calorimetry studies of polyelectrolyte complexation rarely reveal an athermal process,^{3-4, 12, 15-21} although this condition is almost met by a system comprising poly(diallyldimethylammonium), PDADMA, and poly(styrene sulfonate), PSS in KBr.⁶

It has been demonstrated previously that when the enthalpy of complexation between two polyelectrolytes is endothermic, a polyelectrolyte multilayer made with those two polymers is likely to grow exponentially.⁴ When interactions are coupled to charges, the ion distribution between the solution phase and PEC phase follows a Donnan equilibrium modified by the complexation enthalpy.¹¹⁻¹² The Donnan equilibrium, describing the distribution of small ionic species across a semipermeable membrane that has macroions restricted to one phase,²² accurately predicts the distribution of several ionic species.¹²

Though the CSC has been accepted as a general phenomenon for PECs, there are some instances where it has not, or cannot, be observed. A survey of the literature indicates that PECs using polycarboxylates as Pol⁻ show a CSC at relatively low salt concentration, whereas a higher, or unattainably high, concentration of salt is needed to completely separate PSS from polycations. Curiously, for the same PDADMA/PSS PEC, a minor switch from NaCl to KBr makes the difference between achieving a measurable CSC or not.²³ The difference is: with Cl⁻ ΔH_{PEC} is exothermic while with Br⁻ $\Delta H_{\text{PEC}} \approx 0$.¹² In a site-specific model it is theorized that when complexation is endothermic, the salt ions have a preference to act as *counterions* for charged repeat units within the PEC, breaking Pol⁺Pol⁻ pairs. Conversely when complexation is exothermic, the salt ions prefer to act as *co-ions* and not break pairs.¹¹ PEC stability against added salt is further complicated if at least one of the polyelectrolytes is a weak polyacid/base (i.e. has a pH dependent degree of ionization).^{17, 24-25} Weak polyelectrolytes such as polyacrylic acid, PAA, have been extensively used to prepare PECs and thin films of polyelectrolyte complex made by the “multilayer” method. The opportunity to vary the solution charge density while constructing multilayers was exploited by Rubner and coworkers^{24, 26} and others,¹⁷ who observed pK_a shifts on complexation and suggested potential applications.²⁷⁻²⁸ Usually, bulk PECs from weak polyelectrolytes have been investigated under conditions where the polyelectrolyte is nearly fully ionized.²⁹⁻³⁰

The term “coacervate” was used to describe the polymer-dense phase for LLPS of biopolymers.⁹ Bungenberg de Jong’s earliest work on coacervates⁸ recognized the potential importance of LLPS (by charge pairing of oppositely-charged biopolymers) in the formation of biologically relevant structures. Oparin carried on this idea (and the term) in his postulates on the origin of life.³¹ Charge pairing in biomolecules occurs between pH dependent units (carboxylate, amine, phosphate, imidazole). Shifts in pK_a of peptide residues due to changes in environment, including the proximity of oppositely charged groups, in folded proteins have been extensively investigated by biochemists.³² Only histidine and cysteine residues have pK_a values around 7, suggesting at physiological pH they would be the only catalytically active amino acids.³³ However, it has been shown that folded protein environments can shift the pK_as of nearly all ionizable groups close to physiological pH.³⁴ The pK_a shift of these groups can be extreme, for example certain lysine residues have been shown to shift by 4.7 pH units.³⁵

The purpose of the present work is twofold: first, potential limitations of defining PEC stability by the salt resistance are highlighted. Second, the essential contribution of ionization enthalpies of weak polyelectrolytes to PEC salt resistance measurements is quantitatively explored. When PAA is complexed with PDADMA, the degree of PAA ionization within the PEC is always greater than the degree of ionization of

uncomplexed PAA at the same solution pH and [salt]. These changes in ionization make it more difficult to dissolve the PEC with salts.

Experimental

Materials. Poly(diallyldimethylammonium chloride) (PDADMAC, molar mass 200,000–350,000 g mol⁻¹) and poly(4-styrenesulfonic acid, sodium salt) (PSSNa, molar mass 75,000 g mol⁻¹) were from Sigma-Aldrich. Prior to use both polyelectrolytes were dialyzed (3,500 molecular weight cutoff tubing, SnakeSkin™, ThermoFisher) against deionized water for 48 h, with water replacement every 12 h. Polyelectrolyte solutions were then freeze-dried (Labcono, FreeZone 105). Poly(acrylic acid) (PAA, molar mass 250,000 g mol⁻¹) was from Polysciences, Inc. and used without further purification. Sodium chloride and sodium bromide were supplied by Sigma-Aldrich and dried at 110 °C for 24 h. Hydrochloric acid (VWR Chemicals BDH, 1.0 N) and sodium hydroxide standard (Hach, 1.00 N) were used as received. 2-(N-morpholino)ethanesulfonic acid (MES), 3-morpholinopropane-1-sulfonic acid (MOPS), N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS), N-cyclohexyl-2-aminoethanesulfonic acid (CHES), and N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) were from Sigma-Aldrich and used as received. All solutions were prepared using deionized water (18 MΩ cm Barnstead, Nanopure).

Isothermal Calorimetry, ITC. ITC was performed using a VP-ITC (MicroCal Inc.) calorimeter. The ITC was calibrated with an internal y-axis calibration followed by a standard titration between hydrochloric acid and Tris base. Prior to loading, both syringe and sample cell solutions were matched in pH using dilute NaOH or HCl. All samples were degassed for 10 min at room temp. Approximately 300 μL of a 10 mM polycation in 0.05 M NaCl was loaded into the syringe. 10 μL of the syringe solution was manually discharged from the syringe to relieve any back pressure from the loading process. Prior to filling, the sample cell (1.4138 mL) was washed with 0.5 mM polyanion in 0.05 M NaCl. The syringe was rotated at 260 rpm in the sample cell with an injection size of 4 μL per aliquot at a rate of 0.50 μL s⁻¹, with 240 s between injections. The heat flow was recorded as a function of time at 25.0 °C for all samples. Enthalpies were calculated by summing the total heat generated to the 1:1 end point with a correction for the background dilution enthalpy. Acidic conditions below pH 4 could not be probed without risk of damage to the instrument.

pH Titrations of PAA. The potentiometric titration was performed with a glass pH/reference electrode, calibrated with buffer solutions of pH 4.00, 7.00, and 10.00. The titration was performed from the alkaline region, starting from a solution of 0.01 M PAA, 0.02 M NaOH and varying NaCl concentration. To these solutions, 0.100 M standard HCl solution was added with a micropipet at room temperature. The initial volume of the polymer solution was 15 mL.

PEC Tablets. PECs of PDADMA/PAA and PDADMA/PSS were complexed using equivolume amounts of 0.125 M polyelectrolyte in 0.25 M NaCl. The resulting PECs were allowed to stir for 24 h at room temp, followed by a water wash every 12 h for 72 h, to remove ions. The PECs were stirred until the conductivity of the water solution was less than 10 μS cm⁻¹. PECs were then dried for 24 h at 110 °C and ground into a fine powder. The powders were placed into an 8 mm diameter stainless steel mold with a drop of water. A stainless steel weight of approximately 8 kg was placed onto the mold and the PEC pressed into a circular tablet over 24 h under pressure. These tablets were used for ATR-FTIR and radiolabeling experiments.

ATR-FTIR. ATR-FTIR spectra were collected using a ThermoScientific Nicolet iS20 with a Pike MIRacle universal ATR attachment fitted with a single reflection

diamond/ZnSe crystal and high pressure clamp. A stainless steel well was machined to fit onto the crystal plate to allow solid samples immersed in solution to be pressed onto the crystal while preventing evaporation of water from the samples. All PEC spectra were taken with the PEC tablet immersed in either 0.05 M or 0.30 M NaCl at each specified pH. The pH listed was recorded after 24 h of immersion in the specified salt concentration. Background for all spectra was ambient air, and a spectrum of 0.05 M or 0.30 M NaCl was subtracted from all spectra. To find α_2 , the degree of PAA ionization in PDADMA/PAA PEC, PDADMA/PAA tablets were placed into the reservoir filled with a solution of known pH and pressed onto the ATR crystal with a high-pressure clamp. Pressing the tablets while they were immersed in the solutions ensured that they remained fully hydrated. Two experimental challenges limited the extremes of pH. First, PEC tablets at lower pH expanded and became more fragile, which occasionally resulted in splitting of the PEC when pressed with the clamp. At high pH tablets became viscous and liquid-like while not swelling as much.

UV-Vis. UV-Vis experiments were conducted on a Cary 100 Bio UV-Vis spectrometer to determine the CSC of PECs. PDADMA/PAA PECs were dissolved in a 4 M NaCl at pH 12, resulting in a final PEC concentration of 0.1 M. A portion of the resulting solution was placed in a quartz cuvette with a reference solution of equal NaCl concentration and small aliquots of 1.0 M HCl were added to both cuvettes until an increase in scattering was observed at 390 nm and the pH was then recorded. Other cuvettes were pH adjusted from 5 - 12 and water was added to dilute the NaCl concentration until an increase in scattering was observed at 390 nm and the pH was then recorded. Similar experiments were conducted using PDADMA/PSS in NaBr and NaCl, however the resulting PEC in NaCl formed a solid that stuck to the sides of the cuvette.

Dynamic Light Scattering, DLS. PDADMA/PAA aggregate sizes at low pH were determined by dynamic light scattering using a goniometer system (ALV CGS-3-A0-111, Langen, Germany) equipped with a He-Ne laser ($\lambda = 632.8$ nm, 22 mW) and vertically polarized light. At an angle of 30° , measurements were taken in 10 mm capped cylindrical borosilicate glass tubes through a reservoir filled with a refractive index matching liquid (toluene). The polymer samples of 12.5 mM concentration at pH 1.15 were prepared by diluting a stock solution of dissolved PEC in 4.0 NaCl, with a pH adjustment with 1.0 M HCl. By pseudo-cross-correlation of the signals from two photomultipliers, the intensity autocorrelation function $g(2)(q, \tau)$ where $q = 4\pi nD \sin(\theta/2)/\lambda$ was obtained with suppressed noise by using ALV correlator software V.3.0. The hydrodynamic radius R_h was calculated along with the distribution of R_h .

Radiolabeling. A radiolabeling technique was used to determine the stoichiometry of as-prepared PEC tablets. Radiolabeled ions label the extrinsic or counterion-compensated sites of undoped PECs with high precision and sensitivity. Thus, ^{22}Na labeled NaCl “hot” stock solution was prepared by adding 1 mL water into 100 μCi $^{22}\text{NaCl}$ (γ -emitter, half-life 950 days, $E_{\text{max}} = 511$ keV, PerkinElmer), whereas ^{35}S labeled Na_2SO_4 “hot” stock solution was prepared by adding 1 mL water into 1 mCi $\text{Na}_2^{35}\text{SO}_4$ (β -emitter, half-life 87.4 days, $E_{\text{max}} = 167$ keV, PerkinElmer). 5 mL 0.1 M NaCl hot solution was prepared by adding 0.25 mL NaCl hot stock solution (25 μCi) into 4.75 mL water mixed with 0.0292 g NaCl (0.0005 mol), which gave a specific activity of 0.05 Ci mol $^{-1}$. Similarly, 5 mL 0.1 M Na_2SO_4 hot solution was prepared by adding 0.05 mL Na_2SO_4 hot stock solution (0.05 mCi) into 4.95 mL water mixed with 0.071 g Na_2SO_4 (0.0005 mol), which gave a specific activity of 0.1 Ci mol $^{-1}$. A 10^{-5} M NaCl or Na_2SO_4 rinse solution was prepared by adding 10 μL of the 0.1 M hot solution to 100 mL water. To determine the amount of excess polyanion, PEC tablets were first immersed in 10 mL non-labeled 0.1 M NaCl solution for 24 h to allow complete ion exchange. After that, each PEC tablet was immersed in 5 mL 0.1 M NaCl hot solution for 24 h to allow radiotracers to label the PSS extrinsic sites. Then the radiolabeled PEC tablet was rinsed with 2 batches of 5 mL NaCl hot rinse solution for 24 h each (48

h total) to remove any residual isotopes that were not involved in radiolabeling of extrinsic sites (for example, in pores). The rinsed PEC tablet was then immersed in 5 mL non-labeled 0.1 M NaCl solution for 24 h to extract $^{22}\text{Na}^+$ associated with the extrinsic sites. Finally, a mixture of 500 μL extracted solution and 5 mL liquid scintillation cocktail (LSC, MP Biomedicals) was prepared in a 20 mL plastic vial. Once the mixture turned transparent, this vial was mounted on top of an RCA 8850 photomultiplier tube in a dark box and counted for at least 15 min. A calibration curve was obtained by adding known amounts of hot solution into 5 mL LSC to convert counts per second, cps, to moles of extrinsic sites. The same radiolabeling procedure was repeated with $\text{Na}_2^{35}\text{SO}_4$ hot solution to determine the amount of excess polycation. Finally, PEC tablets were collected and rinsed in water for 24 h, dried at 120 °C in a vacuum oven for 24 h, and weighed to obtain total polymer dry weight. The total counts ranged between 54000 and 720000 with respective counting errors of 0.4 and 0.1%.

Results and Discussion

Defining the CSC for pH-independent PECs

Figure 1 shows a typical salt resistance measurement for a PEC made from a strongly-dissociated (i.e. pH-independent) pair of polyelectrolytes. In this case, PSS and PDADMA have been complexed from dilute solution, yielding a cloudy suspension of particles rather than a mass that settles to the bottom of the container (which is produced from concentrated polyelectrolyte solutions). The suspension scatters light and is detected via turbidimetry using a wavelength of light that is longer than any of the specific UV-vis absorption features (i.e. peaks from electronic transitions) from either polymer. In this classical method, as salt is added the solution becomes clear at the CSC.^{7, 36} Dynamic light scattering is also a sensitive method for detecting complete dissolution of solid PEC.

Two closely-related sodium salts, NaBr and NaCl, have been used in an attempt to reach the CSC for the PDADMA/PSS complex. Figure 1 shows that CSC_{NaBr} is at 2.6 M NaBr, but CSC_{NaCl} cannot be achieved up to 3.4 M NaCl. A similar result was seen by Ali and Prabhu comparing KBr and NaCl for PDADMA/PSS.²³ The CSC is often explained using the continuum electrostatics arguments of salt “screening,” where higher ionic strengths weaken the electric fields between charges. Classical screening arguments are unable to account for the substantial difference in PEC response to NaBr versus NaCl seen in Figure 2.

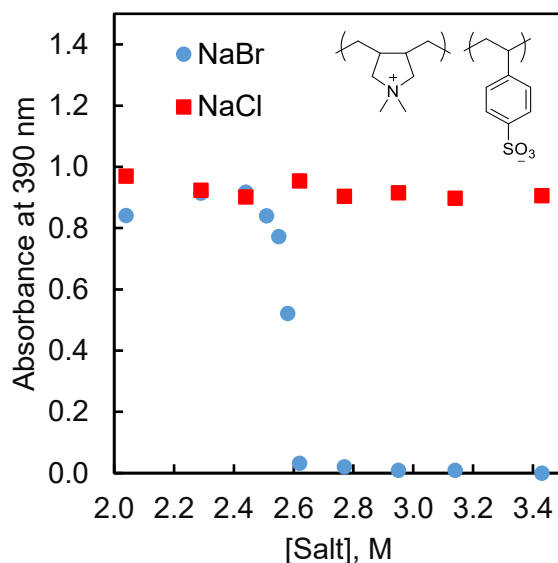
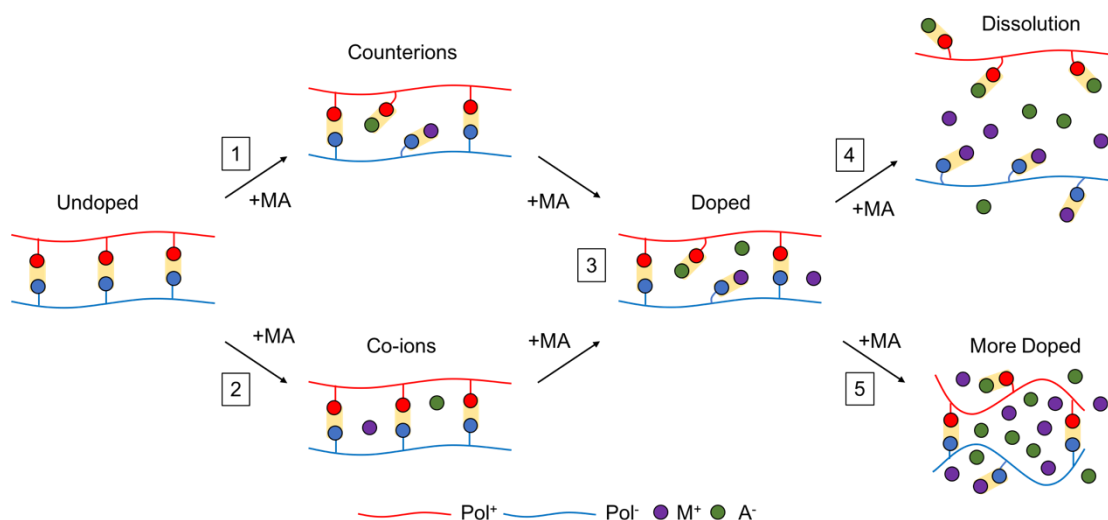


Figure 2. Turbidimetric measurements to detect the complete dissociation of Pol^+ and Pol^- at the critical salt concentration, CSC, at room temp. Absorbance at 390 nm of PDADMA/PSS suspension *versus* the concentration of added NaBr or NaCl. Decrease of absorbance above 2.6 M NaBr is from the decrease in scattering due to the transition above the CSC.

Scheme 1, Path 1, illustrates the simple assumption that ions break Pol^+Pol^- pairing *via* a specific site-exchange mechanism between Pol^+Pol^- pairs and $\text{Pol}^+\text{A}^- + \text{Pol}^+\text{M}^+$ (partial reversal of Equation 1).



Scheme 1. Representation of PEC behavior with added salt, MA. The counterion environment for salt includes specific interactions of ions with the polyelectrolytes. The co-ion environment is similar to that in bulk solution.

Scheme 1 also shows some updated concepts related to the fate of ions entering a PEC. If there is sufficient volume within the PEC, instead of breaking Pol^+Pol^- pairs, ions M^+ and A^- simply occupy space in the PEC as co-ions (Path 2). At equilibrium (State 3) the PEC contains a mixture of co- and counter-ions. The fraction of MA within the PEC taking Path 1 is f . f is not necessarily = 1 at any point in the doping of Pol^+Pol^- or complexation of Pol^+ and Pol^- (as in Equation 1), which decreases the efficiency of added salt in breaking Pol^+ and Pol^- apart. The additional two paths in

Scheme 1 illustrate a final dilemma: the more salt that takes Path 1 the more volume is created within the PEC that can be occupied as co-ions and the more f decreases as the [salt] nears the CSC, rapidly inflating the PEC with water and salt as it does so.⁶ Thus, although Path 4 is anticipated to reach the CSC, Path 5 becomes more favored.

The preferred path may be understood using ΔH_{PEC} as a measure of the “preference” of an ion to locate next to a polymer repeat unit as a counterion rather than exist as a PEC co-ion, which is assumed to be in a (hydration) environment similar to that of the bulk solution. When the source of ΔH_{PEC} is attributed to ion specificity, exothermic ΔH_{PEC} (complexation is in the *opposite* direction to that shown in Scheme 1) indicates the ion prefers a co-ion environment whereas an endothermic ΔH_{PEC} shows the ion prefers to be a counterion. Because ions enter a stoichiometric PEC in pairs (to maintain charge neutrality) the preferences of both ions are convoluted, but a series of anions showed a systematic trend of ΔH_{PEC} along a Hofmeister series.¹² Raman spectroscopy studies of PDADMA bearing counterions along this series showed excellent correlation with a change in water network hydrogen bonding,³ emphasizing the specificity of the hydration environment for different ions.

The near-CSC scenarios of Paths 4 versus 5 may now be used to understand the strong differences between similar salts seen in Figure 2. Complexation in Br⁻ is nearly athermal (about +200 J) and about 2.4 kJ more endothermic than in Cl⁻ ($\Delta H_{PEC} \approx -2.2$ kJ)¹² implying Cl⁻ prefers the co-ion environment more than Br⁻ does. As the PEC becomes more doped with NaCl, f remains low, allowing the PEC to retain Pol⁺Pol⁻ pairs (Path 5). At sufficiently high concentration the salt may even dehydrate the PEC *via* osmotic pressure, making it impossible to dissolve.³⁷ Even -2.4 kJ mol⁻¹ (about 1 kT) of ΔH_{PEC} is enough to suppress the observation of the CSC.^{12, 23} Thus, salt resistance and the top part of the phase diagram (Figure 1) may not be measurable or achievable and will depend strongly on the nature of MA. Values of f computed by Ghasemi et al.³⁸ decreased substantially with salt addition to PDADMA/PSS, leading to inflation of the PEC near the CSC.

Weak Complexes: pH dependent complexation enthalpy

The ease of breaking pairs along Path 1, crucial to dissolving PECs with salt, is described by an unpairing equilibrium constant, K_{unpair}

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

using concentration in place of activities (i.e. assuming activity coefficients = 1 or, more likely, they cancel). Greater values of K_{unpair} means weaker complexes. As a measure of stability against added salt, K_{unpair} may be preferred to the CSC because the composition is not changing as drastically with [salt] at low [salt] as it is near the CSC. On the other hand, salt doping measurements are more time-consuming than simply increasing [salt] until the complex dissolves. The PEC in Figure 2 between aromatic sulfonate and quaternary ammonium happens to be of “medium” strength in the combinations of different polyanions and polycations.³⁹ Polycarboxylates form weaker complexes that are more likely to be liquid-like.³⁹ For this reason, early work on PECs, which focused on bio-, or bio-derived, polyelectrolytes of low charge density, tended to report fluid-like coacervates with an emphasis on spontaneous droplet formation, compartmentalization, and possible connections with origin of life.^{7-8, 31} Recent works have focused on potential applications of pH dependent complexes such as drug delivery systems⁴⁰ or self-healing materials.⁴¹

PDADMA/PAA has a lower salt resistance³⁹ (see Supporting Information Figure S1) than that of PDADMA/PSS. When PAA is fully ionized, the complexation turns out to be endothermic (about +2 kJ mol⁻¹, *vide infra*), reinforcing Paths 1 and 4 due to a

specific preference of the counterions for the polyelectrolytes. Of course, measuring ΔH_{PEC} only provides the enthalpic part of the free energy, whereas measuring K_{unpair} yields ΔG_{PEC}^0 via $\Delta G_{\text{PEC}}^0 = -RT \ln(1/K_{\text{unpair}})$.

Influence of pH on the CSC

The stability of PECs with pH-insensitive charge is indifferent to the solution pH (except for additional doping induced by added ionic strength at extremes of pH). Likewise, ΔH_{PEC} does not depend on pH. As an example, ΔH_{PEC} for PDADMA/PSS formation is about -2.2 kJ mol^{-1} over the pH range 6 to 10 (see Supporting Information Figure S2).

Unlike PDADMA/PSS, the CSC for PDADMA/PAA shows a distinct pH-dependent response (Figure 3). Above about pH 7 the CSC is found at about 0.5 M NaCl. At lower pH, the CSC rises sharply and exceeds experimentally accessible [NaCl], limited by the solubility of PAA.

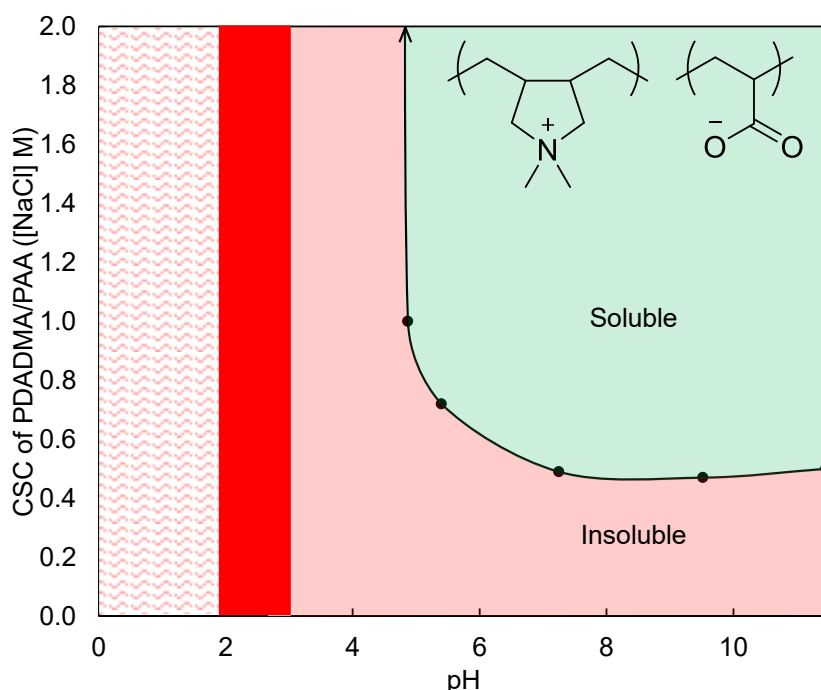


Figure 3. CSC of PDADMA/PAA as a function of pH. The black line represents the CSC boundary determined via turbidimetry. In the shaded area at pH < 2 suspended particles of PEC are found. Inset: repeat units of polyelectrolytes.

It was surprising to discover clear solutions could not be obtained at pH < 2 for any [NaCl]. Under sufficiently acidic conditions, no charges should reside on PAA and complexation should thus be intuitively “turned off.” In fact, though solutions were less scattering at low pH, polyelectrolyte association was still observed for nominally neutral PAA, indicated by milky solutions. DLS showed particles with hydrodynamic radius, R_h , of about $1 \mu\text{m}$ and rather narrow size distribution (see Supporting Information Figures S3 and S4). Previous literature has suggested that when PAA has little to no charge, it can still bind to other molecules via hydrogen bonding⁴²⁻⁴³ and/or hydrophobic interactions not coupled to ions. From Figure 3, whichever interaction may dominate is not sensitive to [salt]. Complexation of neutral species via dehydration has been termed “water-mediated complex coacervation.”⁴⁴

Both Alonso et al.¹⁷ and Vitorazi et al.¹⁸ investigated complexation between

PDADMAC and PAA via ITC. Alonso et al. demonstrated that when PAA was injected into PDADMAC at high pH, complexation was endothermic, while at low pH complexation was exothermic. Vitorazi et al., investigating complexation at pH 7 and 10, suggested the use of (unknown) buffers and a high salt concentration (0.5 M NaCl) in the work of Alonso et al. may have resulted in unreliable enthalpy values.¹⁸ Using Good's buffers, which are known to not interact significantly with bio based polymers, the PDADMA/PSS complexation enthalpy was reduced presumably due to doping (see Supporting Information Figure S2). In addition, the enthalpy of buffer ionization contributes to the measured ΔH_{PEC} . Therefore, calorimetric measurements in this work focused on titrations of PDADMAC into PAA at low salt concentrations in solutions carefully pH-adjusted using HCl or NaOH but using no buffer.

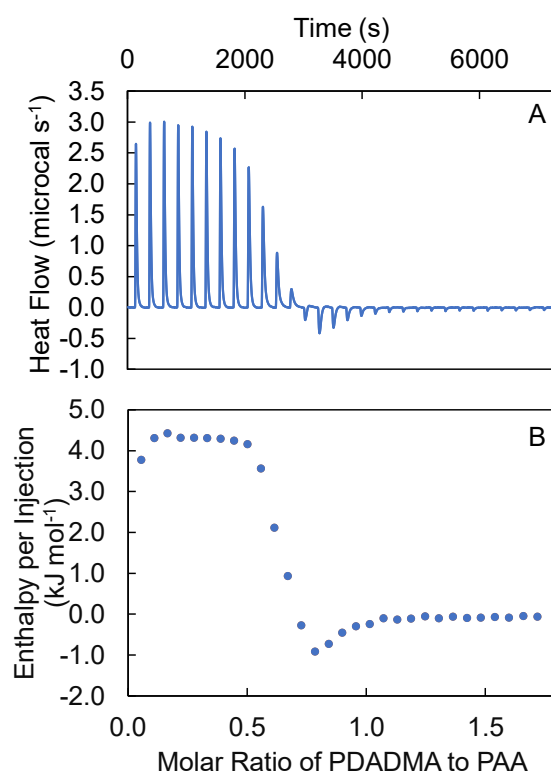


Figure 4. ITC of 10 mM PDADMAC into 0.5 mM PAA, at pH 10 in 0.05 M NaCl. Panel A shows the raw heat flow as a function of time. Panel B shows the enthalpy per injection as a function of the molar ratio.

Figure 4 displays a typical ITC thermogram. Complexation enthalpies for each addition were summed to a stoichiometric ratio between polycation and polyanion (Figure S5). A low concentration of NaCl was used (0.05 M, 10% of the CSC) to define [NaCl] and to accelerate the rate of Pol⁺ pairing with Pol⁻. ITC experiments of PDADMAC added to PAA in 0.05 M NaCl from pH 4 to 13 showed that below pH 5 ΔH_{PEC} turns *exothermic* and at the same point the CSC soars to unmeasurable values. CSC and ΔH_{PEC} versus pH are compared in Figure 5.

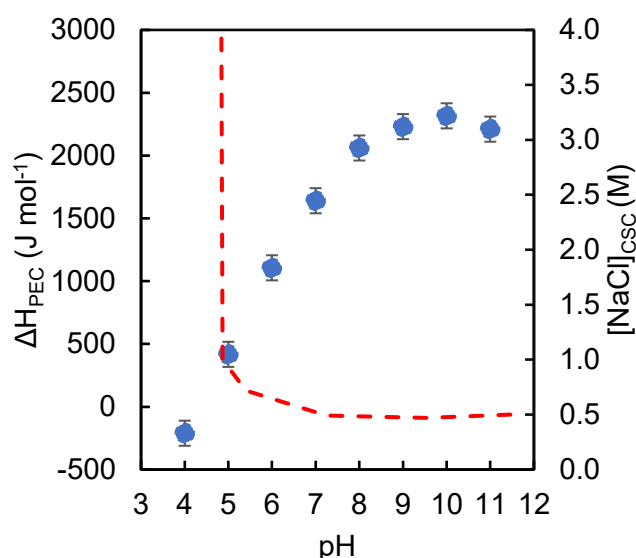
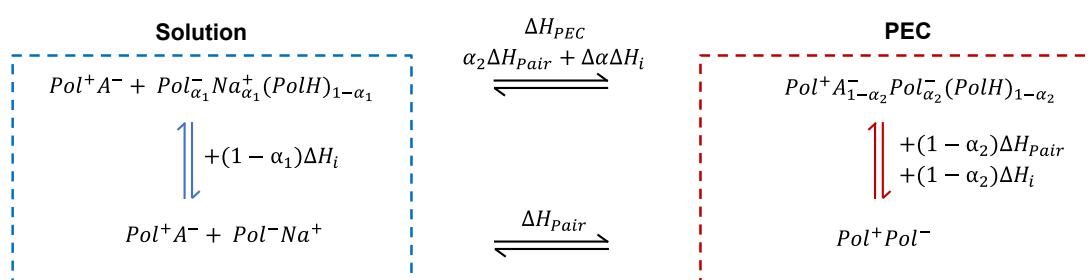
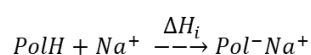


Figure 5. Measured enthalpy of complexation, ΔH_{PEC} , of PDADMA with PAA as a function of pH in 0.05 M NaCl. For reference, the CSC from Figure 3 is shown as a dotted line (right axis).

During complexation, pH sensitive polyelectrolytes are known to undergo pK_{app} shifts.^{24-25, 28, 45} This forced ionization of functional groups is also known to be induced by oppositely-charged amino acids in proteins and is considered central to the catalytic ability of enzymes.^{35, 46}

Scheme 2 summarizes the way complexation-induced shifts in ionization occur. The example shown is for a negative weak acid polyelectrolyte, $PolNa^+$ when completely ionized and $PolH$ when fully protonated, with solution degree of ionization α_1 . Complexation with pH-independent strong polyelectrolyte Pol^+A^- results in a PEC wherein the degree of ionization of Pol^- is α_2 . ΔH_{PEC} therefore includes a component $\Delta\alpha\Delta H_i$ where ΔH_i is the enthalpy of ionization, represented by



Scheme 2. Associations and enthalpies for pH-sensitive polyanion in solution and in PEC. For simplicity, salt doping equilibria are not shown and M^+ is represented by a sodium ion. Solution phase polyelectrolytes are in the blue box and PEC phase polyelectrolytes are in the red box.

Scheme 2 also breaks out the enthalpy of charge pairing ΔH_{pair} between fully ionized Pol^+ and Pol^- . The contribution of this pairing enthalpy to ΔH_{PEC} is $\alpha_2\Delta H_{pair}$. The net enthalpy measured by the ITC gives ΔH_{PEC} ,

$$\Delta H_{PEC} = \alpha_2\Delta H_{pair} + \Delta\alpha\Delta H_i \quad (3)$$

where $\Delta\alpha = \alpha_2 - \alpha_1$

Equation 3 shows ΔH_{PEC} is determined by the sign and magnitudes of ΔH_{Pair} , ΔH_i , α_1 and α_2 . At high pH, $\alpha_2 \rightarrow 1$ and $\Delta\alpha \rightarrow 0$ so $\Delta H_{PEC} \rightarrow \Delta H_{Pair}$. From Figure 5 ΔH_{Pair} was estimated to be 2320 J mol⁻¹.

Scheme 2 does not include doping by salt, which effectively removes Pol⁺Pol⁻ from the cycle shown. If salt doping were included in Scheme 2, then the equations would be modified by a factor of y , where y is the fraction of Pol⁺Pol⁻ converted to Pol⁺A⁻ + Pol⁻M⁺ in the PEC phase. Scheme S1 in Supporting Information presents this more complex situation. The main point is that salt doping reduces the magnitude of ΔH_{PEC} but does not change the sign.

Finding α_1

The α_1 values for synthetic polyacids such as PAA as a function of pH were investigated by Kern,⁴⁷ and later by Katchalsky and Spitnik,⁴⁸ who found that a simple Henderson-Hasselbalch, H-H, equation did not fit the broad titration curves observed. A rearranged form of Katchalsky's extended H-H equation for a fixed [NaCl] is^{25, 49}

$$pH = pK_{app} + n \log \left(\frac{\alpha_1}{1-\alpha_1} \right) \quad (4)$$

Where pK_{app} is the apparent pK_a (pH for 50% neutralization) and n is an interaction parameter between neighboring ionized groups on the polyelectrolyte.⁴⁹ In certain cases it also may be related to a conformational change in the polyelectrolyte,⁴⁹⁻⁵⁰ but not for PAA.^{49, 51} This interaction parameter, independent of molecular weight⁵² and polyelectrolyte concentration,⁴⁸ depends on the salt concentration of the surrounding solution.⁴⁸ Extended H-H plots for PAA at different [NaCl] are given in Figure S6. The pK_{app} and n values are summarized in Table 1.

Table 1. pK_{app} and n values for PAA neutralized by 1 M HCl in fixed concentrations of NaCl determined by extended Henderson-Hasselbach plots.

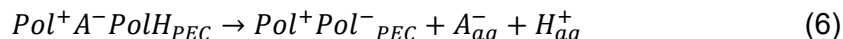
[NaCl] M	pK_{app}	n
0.05	6.08	2.04
0.15	5.76	2.00
0.30	5.62	1.96
0.50	5.41	1.90
1.00	5.17	1.74
1.50	5.11	1.73

Table 1 shows that as the salt concentration increases there is a decrease the pK_{app} and n . Few previous works have looked at more than five concentrations of NaCl with PAA: some of the most comprehensive are those of Kodama et al.⁵³ and Dickhaus et al.⁵⁴ The pK_{app} of 5.41 for 0.5 M NaCl is nearly identical to the value of 5.4 reported by Petrov et al.²⁵ and the pK_{app} value of 5.17 for 1.0 M NaCl is close to the value of 5.2 reported by Kim et al.⁴⁹ The values reported by Dickhaus et al. at identical polyelectrolyte and salt concentrations appear to be approximately half a pK_a unit lower than those shown in Table 1.⁵⁴ Figure 6 displays both the experimental α_1 points along with a solid line representing the fitted α_1 at any pH. Good agreement is shown throughout the fit except for extremes of the experimental titration curve.

According to Kodama, for PAA in solutions of NaCl,

$$pK_{app} = pK_0 + \log[Na^+]_p - \log[Na^+]_{aq} \quad (5)$$

where pK_0 is the monomer pK_a and $\log[Na^+]_p$ is the Na^+ concentration inside the polymer coil.⁵³ So adding more solution NaCl shifts the solution pK_{app} lower. Similarly, the ionization of Pol in the PEC reads as follows



so more NaCl shifts the PEC pK_{app} higher. At sufficiently high $[NaCl]$ $pK_{app,PEC} \rightarrow pK_{app,solution} \rightarrow pK_{a,monomer}$ (i.e. acetic acid $pK_a = 4.75$). The maximum salt concentration is limited by the fact that 1.5 M NaCl is near the Θ condition for PAA.⁵⁵

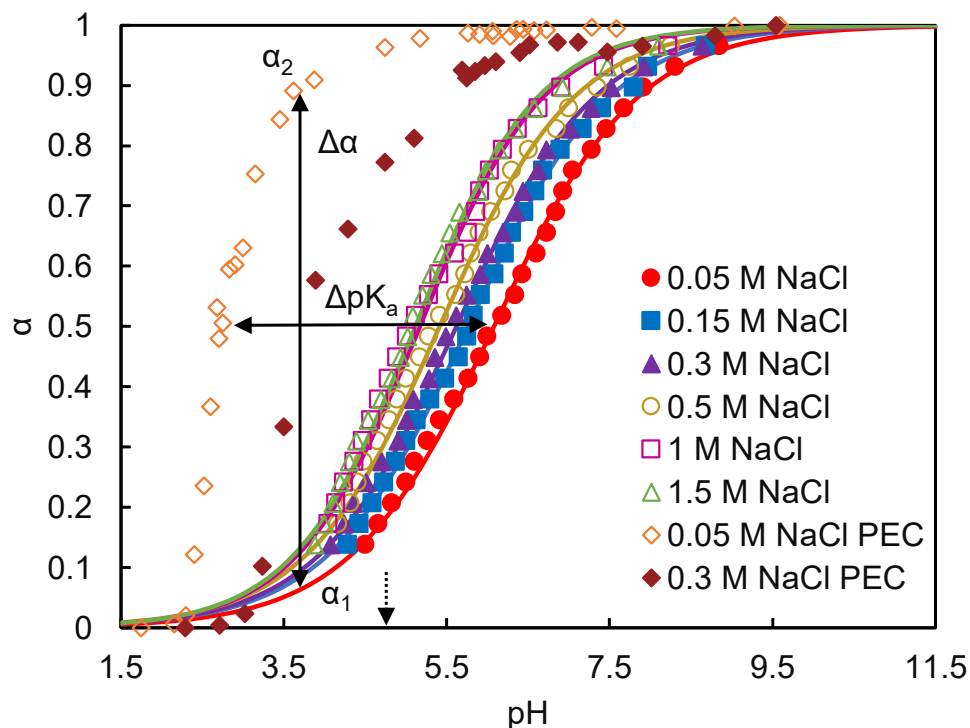


Figure 6. PAA degree of ionization α versus solution pH: α_1 , for 0.01 M PAA (Na-form) neutralized by HCl in solutions of various $[NaCl]$. Solid lines are fits to equation 4 using pK_{app} and n values in Table 1. Diamond shaped points are for the neutralization of PAA, degree of ionization α_2 , within PDADMA/PAA PEC. ΔpK_a is the shift in pK_{app} between PAA in solution and within PEC. The difference in degree of ionization of PAA in solution and within PEC is $\Delta\alpha$, which depends on pH. Dashed arrow shows the pK_a of acetic acid. Note that adding salt moves pK_{app} in solution lower, towards pK_0 , while pK_{app} in the PEC increases.

Finding α_2

To solve for $\Delta\alpha$ and ultimately ΔH_i in Equation 3, α_2 is needed. Various methods of measuring the ionization inside a PEC have been used. Petrov et al., using potentiometric titrations, found that α_2 and pK_{app} values were identical in both bulk PECs of PDADMA/PAA and in multilayer shells.²⁵ They reported pK_{app} values of 3.6 and 4.0 in water and 0.5 M NaCl, respectively, and pK_{app} shifts (ΔpK_a) of 2.85 and 1.4 respectively. Using computational models, Salehi and Larson⁵⁶ employed a system of specific charge-charge interactions connected by equilibria including those shown in Scheme 2 and were able to model the bulk titration curve reported by Petrov et al. for PDADMA/PAA.²⁵ Burke and Barrett using zeta potential measurements of colloidal particles coated with multilayers, reported PAA pK_{app} shifts of almost 4 units in PAH/PAA.⁵⁷ Using FTIR, Choi and Rubner demonstrated pK_{app} values of PAA in PEC multilayers of 2.2 with PAH and 3 with PDADMAC.²⁴ Cho and Zacharia using ATR-

FTIR of a multilayer film of linear poly(ethylene imine) and PAA, reported a pK_{app} of PAA between 2.3 and 2.5.⁵⁸

The internal state of ionization within PDADMA/PAA PECs as a function of solution pH was determined here using ATR-FTIR. To obtain reference spectra of PDADMAC, fully ionized ($\alpha_1 = 1$) PAA and fully protonated ($\alpha_1 = 0$) PAA, concentrated solutions of PDADMAC, and PAA at high (pH 13) and low (pH 2.5) were drop cast directly on a single reflection diamond ATR crystal. Figure 7 shows two distinct peaks associated with the carboxylic acid group of PAA. The C=O bond stretching in neutral PAA, PAAH, at pH 2.5 appears at 1700 cm^{-1} while at pH 13 the asymmetric stretching band of the ionized carboxylate appears from 1610-1500 cm^{-1} .

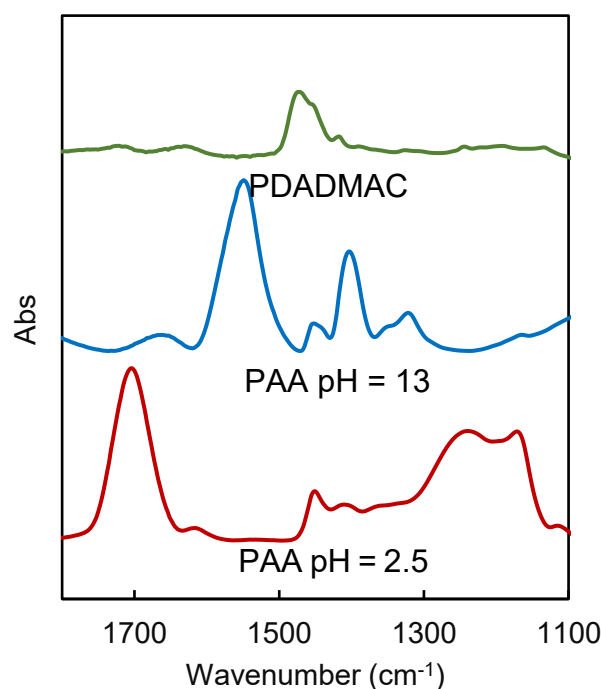


Figure 7. ATR-FTIR spectra of PAA dried from high (pH 13) and low pH (pH 2.5) PAA solutions; and PDADMAC at 25°C.

α_2 was calculated by Equation 7, using a ratio of the protonated carboxylic acid C=O stretching to the PDADMA peak at 1475 cm^{-1} , with half peak integrations from 1760-1705 and 1495-1470 cm^{-1} , respectively. Judging from the titration curve of PDADMA/PAA reported by Petrov et al., it was assumed that for the PEC in 0.05 M NaCl at pH 9.59 PAA is fully ionized and at pH 1.75 PAA is fully protonated.²⁵

$$\alpha_2 = 1 - \frac{\frac{\int PAAH_{Measured}}{\int PDADMA_{Measured}} - \frac{\int PAAH_{Max}}{\int PDADMA_{Max}}}{\frac{\int PAAH_{Min}}{\int PDADMA_{Min}}} \quad (7)$$

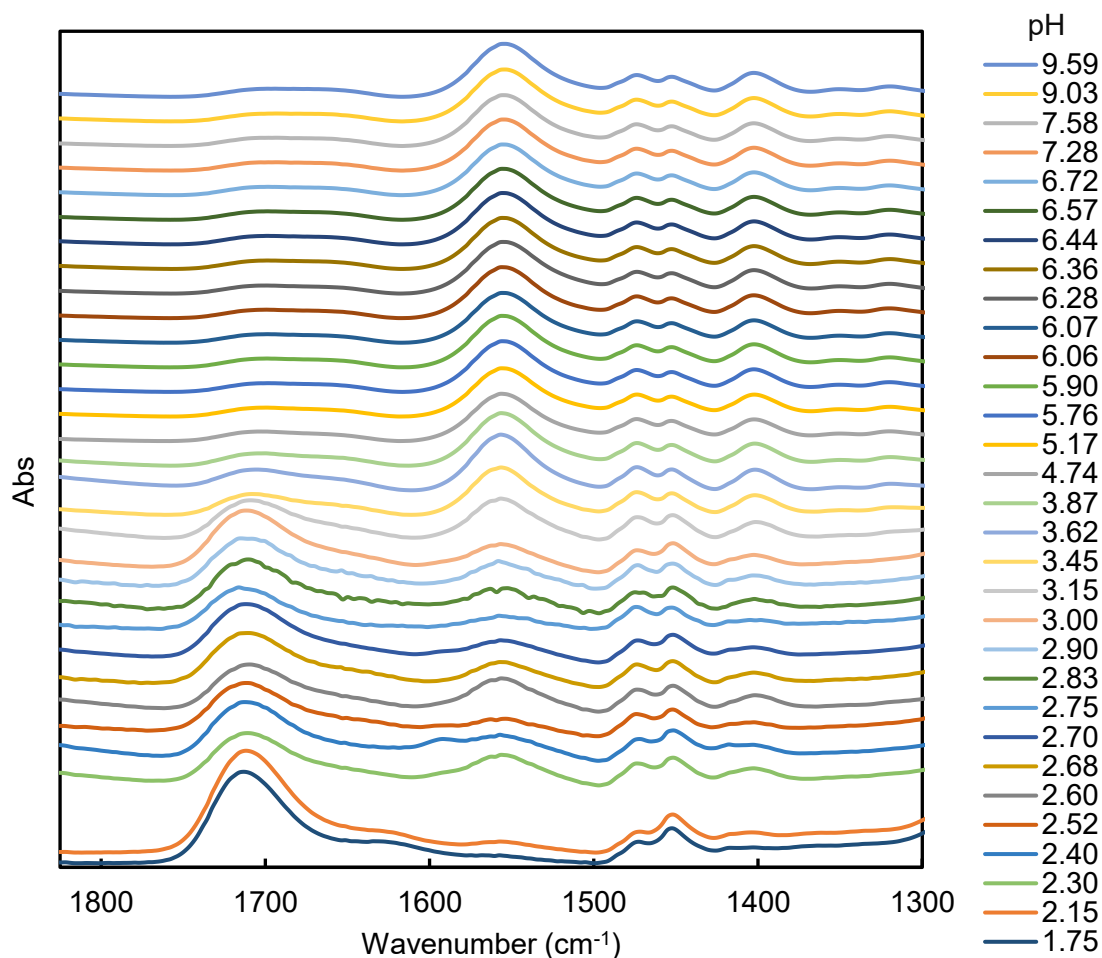


Figure 8. ATR-FTIR of PDADMA/PAA tablets soaked for 24 h in 0.05 M NaCl. pH was measured in situ immediately prior to IR measurement. Spectra are represented with the bottom-most spectra as the lowest pH, increasing in order upwards.

ATR-FTIR spectra of PDADMA/PAA in 0.05 M NaCl can be found in Figure 8, while the spectra of this PEC in 0.3 M NaCl are given in Figure S7 with α_2 in Supporting Information Tables S1 and S2. Tablets immersed in 0.05 M NaCl and 0.30 M NaCl have pK_{app} values of 2.69 and 3.77 compared with solution pK_{app} values of 6.08 and 5.62, respectively. The respective pK_a shifts of 3.39 and 1.85 units fall within expectations based on the literature and display nearly identical curve shapes.²⁴⁻²⁵ Figure 6 includes both α_1 and α_2 titration curves for comparison. With the titration curve for PDADMA/PAA in 0.05 M NaCl giving α_2 , ΔH_{PEC} in 0.05 M NaCl can now be calculated.

Table 2. Ionizations and enthalpies, J mol⁻¹, in PAA_{aq} and PEC^a in 0.05 M NaCl.

pH	α_1	α_2	ΔH_{PEC} expt ^b	^a $\alpha_2 \Delta H_{Pair}$	$\Delta \alpha H_i$	ΔH_{PEC} calc ^{a,b}
10	1	1	2320	2320	0	2320
9	0.97	1	2230	2320	-90	2230
8	0.92	1	2060	2320	-260	2090

7	0.77	1	1640	2320	-680	1670
6	0.51	0.99	1110	2300	-1190	934
5	0.24	0.97	417	2250	-1830	177
4	0.09	0.92	-211	2130	-2340	-223

^afrom the highest point in Figure 5 $\Delta H_{\text{Pair}} = 2.32 \text{ kJ mol}^{-1}$

^b ΔH_{PEC} calculated from Eq 3 using an averaged $\Delta H_i = -2.84 \text{ kJ mol}^{-1}$

Though Equation 4 predicts α_1 at pH 10 is 0.99, literature reports suggest that α_1 is 1 at this pH²⁴⁻²⁵ thus $\Delta\alpha$ is 0. A ΔH_{PEC} value of 2.32 kJ mol^{-1} , estimated from Figure 5, was used along with the values in Table 2 to calculate ΔH_i , which was found to average -2.84 kJ/mol .

ΔH_{Pair} and ΔH_i are almost equal and opposite. Equation 3 shows that these work against each other to lower ΔH_{PEC} as pH decreases. Because α_2 is near unity for all pH > 4, only when $\Delta\alpha$ is maximized can ΔH_i switch ΔH_{PEC} to exothermic. It is surprising that such a small degree of exothermicity can prevent dissolution, but it suggests the ability to observe a CSC depends sensitively on whether ΔH_{PEC} is exothermic or endothermic.

Nonstoichiometry within PECs has a significant influence on mechanical properties such as the modulus and glass transition temperature.⁵⁹ It is not known how the pK_{app} shifts in response to nonstoichiometry. In the current work, only (nearly) stoichiometric PECs of PDADMA/PSS and PDADMA/PAA were prepared and validated using radiolabeling techniques with errors as low as 0.1% (Figures S8 and S9, Table S3). For the PDADMA/PAA tablets used to determine the CSC and α_2 , the stoichiometry was 1.026:1 (i.e. 2.6 % excess PDADMA). PDADMA was complexed with PAANA at pH >7, which corresponds to full ionization within the PEC (Figure 6).

Consequences for the CSC

The CSC represents a convenient comparison for the relative “strengths” of coacervation/complexation of synthetic and bio polyelectrolytes. If the pairing macromolecules bear the same combination of charges (e.g. always lysine and glutamic acid), and NaCl near pH 7 is used, the CSC may provide a reliable comparison. The contribution of an endothermic ΔH_{PEC} to enforcing Path 1→3→4 in Scheme 2 should be appreciated.

There is more diversity of charged functional groups available for synthetic polyelectrolytes. Aromatic sulfonate, i.e. PSS, has been a staple of all aspects of research into synthetic polyelectrolytes, but is not one of the typical charges in biopolymers (although the aliphatic *sulfate* group is found, for example on heparin). Studies of PECs in non-biological systems also offer greater flexibility in choice of pH and salt environment. There are some caveats: individual polyelectrolytes must be soluble in the salts used to break up PECs. For example, hydrophobic anions associate more strongly with Pol^+ , giving more endothermic ΔH_{PEC} values. An example is PDADMA in SCN^- , I^- , and ClO_4^- , ions at the hydrophobic end of the Hofmeister series.¹² Though these ions dope a PDADMA/PSS PEC strongly, PDADMA is not soluble in a solution of the ions and no CSC may be observed.

The stability of PECs against salt depends on the volume (not linear) charge density.¹² A PEC relies on accommodating salt ions in response to increasing solution

ionic strength. PECs with higher charge density are able to accomplish this to higher ionic strength without breaking apart.¹² Researchers are often interested in “tuning the (linear) charge density” of a polyelectrolyte, which makes weak acid/bases and control of pH an obvious experimental variable. As shown above, the linear charge density, in terms of α , of PAA in PEC is close to 1 for most pH values. What is actually being “tuned” is the shift in ionization, $\Delta\alpha$, which is a driving force *for* complexation (see column 6 Table 2).

The salt resistance, given by the critical salt concentration, is a key feature in the phase space of coacervates made from charged bio(polymers). It is not always observed for reasons that are uniquely polymeric. Phase separation is preceded by gradual unpairing of charge pairs with added salt, but the fraction f of salt ions actually breaking Pol^+Pol^- pairs decreases towards the CSC.

The CSC is promoted by endothermic pairing or complexation of Pol^+ and Pol^- , which is an indication of ion specificity (or preference for counterion *versus* co-ion roles). Estimating the value of f as a function of ion content is a challenging but required step for modeling PEC response to salt: f depends on how much volume is created by breaking a charge pair – the more volume the lower f .

Judging from the comparison of ΔH_{PEC} and the CSC in Figure 5 there may be a fine line between soluble and insoluble PECs. It may require less than 1 kJ of ΔH_{PEC} to make the difference between solubility and insolubility. This may have significant consequences in disease conditions characterized by the aggregation of biopolymers.⁶⁰ For example, a change in amino acid or post-translational modification could mean the difference between a reversibly-associating pair of proteins or folding and an irreversibly-aggregated system.

Conclusions

When weak polyelectrolytes are complexed their degree of ionization changes. It has been demonstrated that enthalpies due to changes of ionization are important contributors to ΔH_{pec} and to the phase space of PECs. Because PEC pairing enthalpies are usually small, even slight changes in ionization are significant. Whereas polycarboxylates experience *lower* pK_{app} on complexation, the pK_{app} of polyamines *increases* when they complex.⁵⁷ Thus, complexation promotes the ionized form of both polycarboxylates and polyamines. For the present example, ionization is exothermic, which works against achieving full unpairing of PECs. The maximum measurable change in ionization occurs at around $\text{pH} = 4$, below which PDADMA/PAA cannot be fully dissolved. Shifts in pK_{app} when PAA is incorporated into the PEC studied here are as large as 3.6 pH units. The mechanisms and magnitudes of this pK_{app} shift have much in common with those induced in amino acids within folded proteins, where such pK_{app} shifts are thought to be essential in enzyme activity. While naturally-occurring coacervates, such as those formed into membrane-less organelles in cells, may not have the sophisticated structure of folded proteins, pK_{app} shifts essential to accelerating reactions may still be realized by proximity to oppositely-charged repeat units close enough to form charge pairs as seen here. Catalysis need not require structure.

For the PEC investigated here, both the enthalpy of ionization and the entropy of complexation are coupled to the ions. If the driving force for coacervates includes interactions that are relatively insensitive to ionic strength, such as hydrogen bonding,⁴³ the CSC may not be achievable, or at least measurable. Hydrogen bonding between polymers can be challenged by small or macro- molecules with hydrogen bonding properties, or by increasing the temperature.

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Notes

The authors declare no competing financial interests.

Data and materials availability

All data are available in the manuscript or supplementary materials.

Supplementary materials

Turbidimetry of PDADMA/PSS complexes via UV-Vis; enthalpies of PDADMA/PSS complexation; DLS of PDADMA/PAA solution in 0.6 M NaCl; DLS of PDADMA/PAA solution in 2.0 M NaCl; associations and enthalpies scheme with doping; ITC thermograms of PDADMA/PAA at various pH; extended Henderson–Hasselbalch plots of PAA; ATR-FTIR of PDADMA/PAA tablets; ionization of PAA inside the PEC at 0.05 M NaCl; ionization of PAA inside the PEC at 0.30 M NaCl; $^{22}\text{Na}^+$ calibration for radiolabeling; $^{35}\text{SO}_4^{2-}$ calibration for radiolabeling; radiolabeling PEC Tablets data.

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TOC Graphic

