Lower Critical Solution Temperature in Polyelectrolyte Complex Coacervates

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Supporting Information

ABSTRACT: A model linear oppositely charged polyelectrolyte complex exhibits phase separation upon heating consistent with lower critical solution temperature (LCST) behavior. The LCST coexistence curves narrow with increasing monovalent salt concentration ($C_s$) that reduces the polymer concentration ($C_p$) in the polymer-rich phase. The polymer-rich phase exhibits less hydration with increasing temperature, while an increase in $C_s$ increases the hydration extent. The apparent critical temperature, taken as the minimum in the phase diagram, occurs only for a narrow range of $C_s$. Mean field theory suggests an increasing Bjerrum length with temperature can lead to an electrostatic-driven LCST; however, the temperature dependence of the Flory–Huggins interaction parameter and solvation effects must also be considered.

A ssociative phase separation of oppositely charged macromolecules (coacervation) leads to a dense polymer-rich liquid phase in coexistence with a dilute polymer phase. The miscibility envelope is typically shown as salt concentration ($C_s$) versus total polymer concentration ($C_p$). The Voorn–Overbeek (VO) extension of the Flory–Huggins (FH) theory qualitatively predicts several aspects of the coacervation phase behavior.1 Theoretical2–7 and simulation8–10 models that go beyond the VO theory are able to describe the molecular mass dependence11,12 of the $C_s$–$C_p$ phase boundary, effects of the ratio of oppositely charged polyelectrolytes,13 and an unequal salt partitioning between coexisting phases12 on the $C_s$–$C_p$ plane.

Coacervation reflects a complex interplay between charge and van der Waals interactions, and solvation (hydration) in addition to the microscopic details of the association equilibria between oppositely charged polymers that couple to the entropic release of counterions. The complexion between oppositely charged polymer segments may lead to polar segments.13–16 The topological distribution of such ion pairs, chemical identity, stoichiometry, and host–guest length are additional important details.13–16 It is known that aqueous soluble polar polymers, such as polyethylene glycol, display temperature-dependent hydration that naturally leads to lower critical solution temperature behavior. Mean field models for polymer solvation have found agreement with data for polyethylene glycol.18,19 Such specific interactions and polymer solvation go beyond solvent continuum descriptions.20,21 For instance, the standard FH theory does not predict a lower
added KBr salt. Charge stoichiometric (1:1) mixtures of KPSS-PDADMAB with an initial polymer concentration \( C_{pi} \) of 0.3 mol/L exhibit stable one-phase (1-\( \Phi \)) at \( C_{KBr} \geq 2.15 \) mol/L at 20 °C (ref 26), with \( C_{KBr} \) defined by the concentration of polyelectrolyte counterions and added KBr. For \( C_{KBr} \leq 2.10 \) mol/L, the mixture exhibits two phases (2-\( \Phi \)). The separate polyelectrolytes are stable and soluble in aqueous KBr solutions, so phase separation occurs due to charge complexation.

Figure 1a shows the laser light transmittance % (power transmitted/incident power) as a function of \( C_{KBr} \) for \( C_{pi} = 2.15 \) mol/L and \( C_{pi} = 0.3 \) mol/L. The transmittance plateaus between 5 and 10 °C, indicating a stable single phase, Figure 1b. However, the transmittance smoothly decreases before the sharp drop at the cloud point temperature \( T_{cp} \). At \( T > T_{cp} \), the cloudy sample, Figure 1c, exhibits micron-sized spherical droplets, as shown by confocal microscopy, Figure 1d. The meniscus between the coexisting phases forms over time with rate depending upon the salt composition and temperature. The kinetics of this process can be accelerated by centrifugation to form clear phases. This clear dense phase exhibits a viscoelastic liquid response \( (G'' > G') \) with terminal relaxation exhibiting storage and loss moduli scaling as \( G' \sim \omega^{0.7} \) and \( G'' \sim \omega^{0.5} \), respectively, from frequency \( (\omega) \) sweep small-amplitude rheology. These results show that macroscopic phase separation occurs upon heating. Further heating within the two-phase region again causes the transparent coacervate to become turbid, rather than an instantaneous movement of the meniscus. This is due to the formation of micron-sized polymer-poor droplets, as measured by ultrasmall angle neutron scattering and confocal microscopy (Supporting Information).

Figure 2a summarizes the effect of \( C_{KBr} \) on \( T_{cp} \) at fixed \( C_{pi} = 0.3 \) mol/L. Mixtures prepared at \( C_{KBr} = 1.75 \) mol/L and \( C_{pi} = 0.3 \) mol/L remain phase separated down to 0 °C. \( T_{cp} \) initially increases slowly, then sharply increases at higher \( C_{KBr} \) values. The cloud point shifts by 50 °C, with a 0.25 mol/L change in salt concentration. Figure 2b shows a 20 °C increase in \( T_{cp} \) for varied \( C_{pi} \) from 0.01 to 0.3 mol/L at fixed \( C_{KBr} = 2.15 \) mol/L. All samples exhibit two phases above the \( T_{cp} \).

Figure 2a can be rationalized with respect to the \( C_{pi} - C_{KBr} \) diagrams at a fixed temperature. The 1-\( \Phi \) is reached with an increase in salt concentration. Similarly, Figure 2b at a fixed temperature, an increase in polymer concentration at fixed \( C_{pi} \) leads to 1-\( \Phi \). However, the trends of temperature are the key finding. Figure 2b has the appearance of a lower critical solution temperature. \( T_{cp} \) corresponds to the point where the kinetics of phase separation becomes observable, rather than an exact thermodynamic phase boundary. A kinetics study demonstrates that these observations are not strongly dependent upon the heating rate from 0.05 to 0.4 °C/min. These cloud point data were further examined as coexistence curves by analysis of the liquid–liquid phase separated solutions.

The coexistence curves in Figure 3 on the \( T - C_{PSS} \) plane for \( C_{PSS} = 0.15 \) mol/L \( (C_{pi} = 0.3 \) mol/L) at three salt concentrations are consistent with lower critical solution behavior. As expected, above the apparent critical temperature, taken as the minimum, the solution phase separates into polymer-poor and polymer-rich phases. At \( C_{KBr} = 1.75 \) mol/L (Figure 3a), the apparent critical temperature was not experimentally accessible. The ratio of the \( C_{PSS} \) between the coacervate and supernatant is \( \approx 60 \) at 5 °C for Figure 3a. The concentration of PSS generally increases in the dense phase as temperature increases. This implies the extent of solvation (hydration) decreases as temperature is increased. The shape of the phase boundaries depends on the salt concentration. The PSS concentration in the supernatant increases with
increasing C_{KBr} while it decreases in the dense phase. This increased hydration of the coacervate is consistent with the observations of Schlenoff et al. The coexistence curves appear more symmetric at the highest C_{KBr} in Figure 3c.

The initial polymer concentration does influence the equilibrium phase concentrations. Figure 3b includes initial C_{PSS} = 0.3 mol/L (C_{PSS} = 0.6 mol/L) that phase separates into a slightly higher coacervate concentration. Since the coacervate concentration is dependent on the initial polymer concentration, one cannot treat the system as pseudobinary and draw a tie line. This is not surprising, since the polycation concentration and salt concentrations are also required for the multicomponent analysis. We note that a nonequal salt partitioning on the C_s−C_p plane may be responsible for these observations. As shown in the Supporting Information, at fixed temperature and salt concentration an initial polymer concentration increased by a factor of 12 changes the coacervate C_{PSS} by 37%.

What molecular parameters are responsible for phase separation upon heating? By considering one concentration within the 2-Φ region of the C_s−C_p diagram, the phase envelope must shift down to recover 1-Φ upon cooling. The cloud point temperatures (Figure 2a) and critical temperature (Figure 3) increase with increasing added salt. The key temperature-dependent parameters are \( \chi \), the Bjerrum length, \( l_b = \sqrt{k_B T} \), and the Debye screening length \( \kappa^{-1} \) for monovalent salt, \( \kappa^{-1} = \frac{4 \pi l_b C_s}{\kappa} \), where \( \kappa \) is the elementary charge, \( k_B \) is Boltzmann’s constant, and \( \epsilon_0 \) and \( \epsilon \) are the permittivity of free space and dielectric constant of the medium, respectively. Therefore, changing temperature has implications in the binary interaction parameter and treatment of ionic interactions, when considering mean field theory.

Thermodynamic models for polyelectrolyte complexation were recently reviewed. Salehi and Larson considers a mean field treatment of ion condensation upon polyelectrolytes, ion pairing of charged segments, and charge regulation as reversible equilibria that lead to laws of mass action. They find that a decrease in the ion pairing equilibrium constant \( K_p \) lowers the C_s−C_p phase envelope (Figure 4 in ref 4). Therefore, the temperature-dependence of the ion pairing equilibrium constant can lead to upper critical or lower critical solution temperature behavior depending upon the Gibbs free energy of formation. However, as they point out, counterion condensation and ion pairing are inter-related, since they have common ions. Independent measurements of these equilibrium constants are challenging since enthalpic contributions may arise from intermediate steps, such as solvation.

Fu and Schlenoff estimated the enthalpy of complexation by isothermal titration calorimetry as exothermic (\( \Delta H_p = -0.30 \text{ kJ/mol at 0.1 mol/L KBr} \)) for KPSS and PDADMAB at fixed salt concentration. If the standard free enthalpy \( \frac{d \ln (K_p)}{dT} = \frac{\Delta H_p}{RT^2} \) (1) for ion pair formation were negative (exothermic), then \( K_p \) would decrease with increasing temperature via the Van’t Hoff equation.

When combined with the predictions by Salehi and Larson, lowering temperature raises the ion pairing equilibrium constant (eq 1), causing the phase envelope to shift upward, maintaining the 2-Φ region. This predicts that the LCST may not be due to the temperature dependence of the coupled ion pairing and counterion condensation.

Dipolar attractions by ion pairs was considered by Adhikari et al. with strength proportional to \( l_b^{-2} \) that enhance the effective hydrophobicity when combined with \( \chi \). They predict that lowering temperature raises the C_s−C_p envelope (Figure 3 in ref 7) by considering an inverse temperature dependence on \( \chi \) and dipolar attractions. While this does not recover the behavior in Figure 3, the inverse-temperature dependence of \( \chi \) counteracts the added effective hydrophobicity from dipolar attractions. However, the dielectric constant was fixed in that calculation. The temperature dependence of the dielectric constant of water increases the Bjerrum length from 0.696 to 0.738 nm between 0 and 50 °C. This suggests increased immiscibility with increasing temperature via dipolar attractions. A related study by Kudlay et al. predicts an increasing \( \chi \) increases the coacervate concentration. It would be desirable to independently measure the temperature dependence of \( \chi \) to properly evaluate these predictions.

The liquid state theory by Zhang et al. predicts that an increase in the Bjerrum length raises the C_s−C_p phase envelope (Figure 3 in ref 6), consistent with the current experimental

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Figure 3. Coexistence curves for KPSS/PDADMAB mixture at C_{KBr} = (a) 1.75, (b) 2.0, and (c) 2.05 mol/L showing the two-phase (2-Φ) and one-phase (1-Φ) region. The initial PSS concentration was 0.15 mol/L (circles) and 0.3 mol/L (squares) that phase separates into the supernatant (open symbols) and concentrated phases (filled symbols) at the given temperature. Measured T_{co} at C_{PSS} = 0.15 mol/L (A) and 0.3 mol/L (▼) are indicated in (b) and (c). Error bars represent one standard deviation estimated from the uncertainty in the sampling micropipette volumes. While error bars are shown, they may be smaller than the symbols used.
trends. Similarly, Riggleman et al. predict an increasing Bjerrum length or reduced excluded volume parameter increases the driving force for phase separation. These theoretical studies show that phase separation of electrostatic origin is intimately tied to the Bjerrum length.

Phase separation upon heating must also include the correct temperature dependence of the nonelectrostatic aspects of the model. One may reconsider the temperature dependence of \( x \), such as \( x = A / T + B + C \ln T \), or separation into enthalpic and entropic terms. This may be an oversimplification due to the multicomponent polyelectrolyte solutions, since the system may not be described by one interaction parameter. The observations of the coacervate exhibiting less hydration with increasing temperature is suggestive of less polymer and ion solvation. An increase in salt concentration increases the extent of hydration. Further characterization of the solvation properties is required.

In general, one needs to know the temperature dependence of \( x \) to compare to predictions for both lower and upper critical solution behavior.

**EXPERIMENTAL SECTION**

Polymers with mass-average relative molecular mass \((M_w)\) of KPSS \((M_w \approx 200 \text{ kg/mol})\) and PDADMB \((M_w \approx 150 \text{ kg/mol})\) were prepared at predetermined polymer and KBr concentrations. The component solutions are then mixed at room temperature, followed by vortex mixing and stored at \( 5^\circ \text{C} \) in sealed vials for 24 h. The mixtures are cooled to \( 3^\circ \text{C} \) and vortex mixed repeatedly to observe uniform solutions. All samples were stored at \( 5^\circ \text{C} \) before performing further measurements.

Ultraviolet–visible (UV–vis) absorption spectroscopy was used to quantify the PSS polymer concentrations as functions of temperature and salt concentrations. A phase separated mixture is equilibrated and centrifuged in a temperature-controlled environment. This accelerates the coalescence of dilute droplets and leads to the coexisting supernatant and transparent coacervate at \( T > T_{cp} \). Next, known volumes of supernatant and coacervate are sampled and diluted using a 2.5 mol/L KBr solution at room temperature to quantify the PSS polymer concentrations as functions of temperature and salt concentrations. A phase separated mixture is equilibrated and centrifuged in a temperature-controlled environment. This accelerates the coalescence of dilute droplets and leads to the coexisting supernatant and transparent coacervate at \( T > T_{cp} \). Next, known volumes of supernatant and coacervate are sampled and diluted using a 2.5 mol/L KBr solution at room temperature to quantify the PSS concentration based on the absorbance calibration at the absorbance peak at 261 nm. The measured values of \( C_{PSS} \) are used to construct the coexistence curves.

Sample transmission was measured by a solid-state 532 nm laser or visible (UV–vis) absorption spectroscopy was used to quantify the PSS polymer concentrations as functions of temperature and salt concentrations. A phase separated mixture is equilibrated and centrifuged in a temperature-controlled environment. This accelerates the coalescence of dilute droplets and leads to the coexisting supernatant and transparent coacervate at \( T > T_{cp} \). Next, known volumes of supernatant and coacervate are sampled and diluted using a 2.5 mol/L KBr solution at room temperature to quantify the PSS concentration based on the absorbance calibration at the absorbance peak at 261 nm. The measured values of \( C_{PSS} \) are used to construct the coexistence curves.

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