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Effect of Polymer Chemistry on the Linear Viscoelasticity of Complex Coacervates

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Abstract

Complex coacervates can form through the electrostatic complexation of oppositely charged polymers. The material properties of the resulting coacervates can change based on the polymer chemistry and the complex interplay between electrostatic interactions and water structure, controlled by salt. We examined the effect of varying the polymer backbone chemistry using methacryloyl- and acryloyl-based complex coacervates over a range of polymer chain lengths and salt conditions. We simultaneously quantified the coacervate phase behavior and the linear viscoelasticity of the resulting coacervates to understand the interplay between polymer chain length, backbone chemistry, polymer concentration, and salt concentration. Time-salt superposition analysis was used to facilitate a broader characterization and comparison of the stress relaxation behavior between different coacervate samples. Samples with mismatched polymer chain lengths highlighted the ways in which the shortest polymer chain can dominate

the resulting coacervate properties. A comparison between coacervates formed from methacryloyl vs. acryloyl polymers demonstrated that the presence of a backbone methyl group affects the phase behavior, and thus the rheology in such a way that coacervates formed from methacryloyl polymers have similar phase behavior to those of acryloyl polymers with $\sim 10\times$ longer polymer chains.

Introduction

Polyelectrolyte complexation is an associative phase separation resulting from electrostatic and entropically-driven interactions between oppositely charged polymers or macro-ions.¹⁻¹³ Depending on the choice of salt, pH, macro-ion identity, charge density, polymer chain length, charge stoichiometry, and/or the presence of other intermolecular interactions, polyelectrolyte complexes can result in solid precipitates and/or a liquid-liquid phase separation known as complex coacervates.^{9,14-27} In particular, the dense, macro-ion-rich coacervate phase has a long history of use in applications such as food²⁸⁻³⁰ and personal care products,³¹ is finding increasing traction in medical applications,^{3,32} and is found in naturally-occurring underwater adhesives.³³⁻³⁷ Coacervation has also been implicated in the formation of liquid-liquid phase separated compartments and granules observed in living cells.^{4,38-40}

The phase behavior of polymeric complex coacervates is an area of active study from both an experimental and a computational/theoretical perspective. General trends are starting to emerge regarding how ionic strength, salt valence, polymer chain length, charge stoichiometry, charge density, and the patterning of charges affect coacervate phase behavior.^{15,41-52} However, there have been very few systematic studies investigating how changing other aspects of the polymer, such as the hydrophobicity,⁵²⁻⁵⁵ or chemistry of the backbone impact coacervate outcomes. Furthermore, efforts to establish correlations between these various parameters and the dynamics or rheological properties of coacervates have been even more limited.

A key series of papers by Spruijt *et al.* were the first to provide extensive characterization of the phase behavior and rheology of polymeric coacervates formed from anionic poly(acrylic acid) (PAA) and cationic poly(*N,N*-dimethylaminoethyl methacryloyl) (PDMAEMA) over a range of different polymer chain lengths.^{41,56,57} These reports demonstrated the ability to fit a series of phase diagrams to a theoretical model,⁴¹ and established a new framework for understanding the linear viscoelastic response of coacervate materials using time-salt superposition and a

'sticky' Rouse model,^{56,57} based on the slowed dynamics resulting from the presence of ion pair interactions between oppositely charged polymers.

While the initial work by Spruijt *et al.* was done using two polymers with relatively different chemical structures,⁵⁶ subsequent efforts in the field have looked to use model systems to better control and more systematically change the properties of the polymer. Reports examining the polarity and/or hydrophobicity of the polymer have done so in a variety of ways; by modifying the charged group directly,⁵³ the side-chain,⁵² or through the addition of comonomers.²³ Intuition, along with theoretical models that capture polymer 'hydrophobicity' through an effective χ parameter, would suggest that increasing the hydrophobicity (or decreasing the polarity) should broaden the range of conditions over which phase separation is observed by favoring self-association of the polymers, and generally this has been the observed result. However, the two reports that combine some aspect of phase diagram determination with rheological characterization observed distinctly different outcomes.

Sadman *et al.* used a quartz crystal microbalance with dissipation (QCM-D) to characterize the water uptake, or swelling, of polyelectrolyte complexes of poly(styrene sulfonate) with a poly(4-vinylpyridine) that was made increasingly hydrophobic via quaternization with methyl, ethyl, and propyl substituents.⁵³ Their results were consistent with the hypothesis that more hydrophobic polymers would favor self-association over interactions with water, leading to lower water uptake and therefore less swelling. Subsequent analysis of the rheological response of the material, also via QCM-D, suggested that changing the hydrophobicity of the material merely shifted the range of swelling-moduli that could be accessed for a given concentration of salt. This result was further supported by considering the swelling effect of different salts, showing that the apparent differences in material properties achieved by using different salts was merely a consequence of the amount of water carried into the complex by the salt ions.

Separately, work by Huang *et al.*, investigated the effect of polymer hydrophobicity via the incorporation of either acrylamide (hydrophilic) or butyl acrylamide (hydrophobic) comonomers.^{23,58} The authors characterized the phase behavior of their coacervates in terms of a salt resistance (*i.e.*, the concentration of salt above which phase separation was no longer observed), and the mass fraction of polymer present in their coacervates. Interestingly, they saw no significant difference in either the phase behavior, or the viscoelasticity (described in terms of a characteristic relaxation time). In contrast, work by Tabandeh and Leon using polypeptides showed that increasing the hydrophobicity of the comonomer would increase the salt resistance, though no rheology was performed.⁵⁸

The results by both Sadman *et al.*⁵³ and Huang *et al.*²³ suggest a direct correlation between trends in the coacervate phase behavior and the material properties. However, the methods applied to date have used indirect measures of the phase behavior, such as relative swelling and salt resistance, rather than direct quantification of the polymer and salt concentration in the coacervate phase. Furthermore, the different outcomes reported by Huang *et al.*²³ and Tabandeh and Leon⁵⁸ suggest that subtle effects, such as how water structures around a given chemical group can have dramatically different outcomes in terms of the resulting phase behavior.

The goal of this study is to establish a clear understanding of how increasing the hydrophobicity of the polymer backbone affects both the coacervate phase behavior and the resulting material properties. We compared coacervates formed from low polydispersity methacryloyl and acryloyl polyelectrolytes of different chain lengths. This approach allowed us to demonstrate how small changes in polymer chemistry, such as the presence or absence of a single methyl group on the polymer backbone, dramatically affect the resulting coacervate phase behavior and rheology. Furthermore, our choice of structurally-similar polymers allowed for isolation of chain length effects in coacervates formed from length-mismatched polymers.

These chain length effects were modeled using recent coacervate theories, the results of which were used to quantify the effect of the change in polymer chemistry on the polymer χ -parameter. We find that this methyl group affects the interaction between the polymer and *both* the water and salt species.

Experimental Methods

Figure 1. The chemical structures of the two polyelectrolyte systems used in this study. Poly(3-sulfopropyl methacryloyl) (PSPMA) and poly([2-(methacryloyloxy)ethyl]trimethylammonium) (PTMAEMA) were chosen as a set of well-matched methacryloyl-based polymers for comparison with poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS) and poly([2-(acryloyloxy)ethyl]trimethylammonium) (PTMAEA) to examine the effect of changing the polymer backbone chemistry. Counterions have been omitted for clarity.

Monomer Synthesis

The anionic monomer 3-sulfopropyl methacryloyl potassium salt was obtained from Sigma-Aldrich and used without further purification. The cationic monomer [2-(methacryloyloxy)ethyl]trimethylammonium iodide was synthesized via alkylation of 2-(dimethylamino)ethyl methacryloyl with iodomethane in tetrahydrofuran (THF, ACS grade) (**Figure S1a**). 2-(dimethylamino)ethyl methacryloyl (15 g, 95.4 mmol, 1 equiv., Sigma Aldrich) was dissolved in THF (150 mL, dry) and the solution was cooled on an ice bath. Iodomethane (27.1 g, 190.1 mmol, 2 equiv., Sigma Aldrich) was added drop-wise to the solution, and the resulting mixture was stirred under $N_{2(g)}$ for 1.5 h in the ice bath. The mixture was allowed to thaw to room temperature and was stirred for 23 h. The product [2-

(methacryloyloxy)ethyl]trimethylammonium iodide precipitated out of solution and was filtered and washed extensively with dry diethyl ether. 28.4 g (99% yield) of a white solid was recovered, and stored at 4°C. ¹H NMR (500 MHz, D₂O, d): 6.18 (s, 1H, CH₂=), 5.79 (s, 1H, CH₂=) 4.66 (t, J 2.23 Hz, 2H, C(O)O-CH₂), 3.82 (t, J 5 4.45 Hz, 2H, CH₂-N⁺), 3.26 (s, 9H, -N⁺-(CH₃)₃), 1.95 (s, 3H, C-CH₃) ¹³C NMR (500 MHz, D₂O, d): 168.43 (C=O), 135.17 (C=CH₂), 127.70 (CH₂=C), 64.65 (C(O)O-CH₂), 58.62 (CH₂-N⁺), 53.83 (N⁺-CH₃)₃, 17.30 (CH₃) (**Figure S2**).

The cationic monomer [2-(acryloyloxy)ethyl]trimethylammonium iodide was also synthesized via alkylation of 2-(dimethylamino)ethyl acryloyl with iodomethane (**Figure S1b**) using a variation on literature procedures.^{59,60} 2-(dimethylamino)ethyl acryloyl (8 g, 56.0 mmol, 1 equiv., Sigma Aldrich) was dissolved in dry toluene (30 mL), and the solution was degassed. The solution was cooled using an ice bath prior to slow, drop-wise addition of iodomethane (9.1 g, 64.3 mmol, 1.15 equiv., Sigma Aldrich), and the resulting mixture was stirred under Ar(g) for 1.5 h in the ice bath. The mixture was allowed to warm to room temperature and was stirred for >12 h. The product [2-(acryloyloxy)ethyl]trimethylammonium iodide precipitated out of solution as a crystalline white solid and was filtered and washed extensively with dry hexanes. 15.7 g (98% yield) of a white solid was recovered, and stored at 4°C.

Polymer Synthesis

Poly(3-sulfopropyl methacryloyl) (PSPMA) and poly([2-(methacryloyloxy)ethyl]trimethylammonium iodide) (PTMAEMA) were synthesized through reversible addition-fragmentation chain transfer (RAFT) polymerization (**Figure 2**). Polymers were synthesized in water using 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid as the chain transfer agent (CTA) and 4,4'-azobis(4-cyanovaleric acid) (ACVA) as the thermal initiator. Polymers were synthesized in 5 g batches, with monomer-to-CTA molar ratios adjusted to achieve a target degree of polymerization of 50, 250 and 500 at 100% monomer conversion.

The molar ratio of CTA-to-ACVA was 5:1 for all polymerizations. In a representative example, TMAEMA monomer (5 g, 16.7×10^{-3} mol, 100 equiv.), CTA (46.7 mg, 16.7×10^{-5} mol, 1 equiv.) and ACVA (9.4 mg, 3.4×10^{-5} mol, 0.2 equiv.) were weighed into a 50 mL round bottom flask and dissolved in water (16.7 mL). The resulting pink solution was deoxygenated by bubbling with $N_{2(g)}$. The degassed solution was immersed in an oil bath preheated to 70°C, and after a certain amount of time, the reaction was quenched by immersing the round bottom flask in $N_{2(l)}$ while simultaneously exposing the solution to air. For the polymers with a target degree of polymerization of 500, the solutions were degassed for 1 h and heated at 70°C for 48 h. For the polymers with a target degree of polymerization of 50 and 250, the solutions were degassed for 30 min and heated at 70°C for 16 h. After quenching, the solution was allowed to thaw to room temperature and the polymer was precipitated into acetone, recovered by centrifugation and washed repeatedly with acetone to eliminate water and residual monomer.

Figure 2. The reaction schemes for (a) poly([2-(methacryloyloxy)ethyl]trimethylammonium iodide) (PTMAEMA) and (b) poly(3-sulfopropyl methacryloyl potassium) (PSPMA).

Poly([2-(acryloyloxy)ethyl]trimethylammonium iodide) (PTMAEA) was synthesized through photoiniferter-RAFT, a reversible-deactivation radical polymerization (RDRP) (Figure 3a).^{61,62} Polymers were synthesized in water using 2-(ethylthiocarbonothioylthio)-2-methylpropionic acid.

Polymers were synthesized in >4 g batches, with a monomer-to-CTA molar ratio adjusted to achieve a target degree of polymerization of 250, 1000, and 2000 at 100% monomer conversion. In a representative example, the trithiocarbonate compound (83 mg, 39.5×10^{-5} mol, 1 equiv.) was dissolved in a minimal amount of DMF in a 50 mL Schlenk flask and the TMAEMA monomer (4.7 g, 16.5×10^{-3} mol, 50 equiv.) was added. Water was added to achieve a 2 M concentration with respect to the monomer. The resulting yellow solution was deoxygenated by bubbling with Ar_(g) for 30 min. The degassed solution was irradiated for 12 h at a wavelength of 365 nm and an intensity of ~ 7 mW/cm². The solutions were dialyzed and lyophilized to yield the purified polymer.

Figure 3. The reaction schemes for (a) poly([2-(acryloyloxy)ethyl]trimethylammonium iodide) (PTMAEA) and (b) poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS).

Poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS) was synthesized through RAFT polymerization in water using 2-(ethylthiocarbonothioylthio)-2-methylpropanoic acid as CTA and 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) as an initiator (**Figure 3b**). Polymers were synthesized in >4 g batches, with monomer-to-CTA molar ratios adjusted to achieve a target degree of polymerization of 250, 1000, and 2000 at 100% monomer conversion. The monomer 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) was purchased from Sigma-Aldrich. The molar ratio of CTA-to-initiator was 10:1 for all

polymerizations. In a representative example, AMPS monomer (4.7 g, 22.6×10^{-3} mol, 50 equiv.), CTA (114 mg, 54.3×10^{-5} mol, 1 equiv.) and VA-044 (5.4 mg, 5.4×10^{-5} mol, 0.1 equiv.) were weighed into a 50 mL round bottom flask and dissolved in water to bring the concentration to 2 M (11.3 mL) with respect to monomer. The resulting yellow solution was deoxygenated by bubbling with $\text{Ar}_{(\text{g})}$ for 30 min. The degassed solution was irradiated at 365 nm ($\sim 7 \text{ mW/cm}^2$) for 8 h. The polymer was dialyzed and lyophilized to yield the purified polymer.

Materials Characterization

PSPMA and PAMPS polymers were characterized by gel permeation chromatography (GPC) using an 80/20 v/v mixture of 0.1 M sodium nitrate (Sigma-Aldrich) and acetonitrile (ACS Grade, Fisher) as the mobile phase (**Figure S5**). Samples were dissolved in the eluent at 2.2 mg/mL. Assuming 100% mass recovery, analysis of molecular weight was based off multi-angle light scattering (Wyatt Technology, DAWN HELEOS II) (**Tables S1 and S3**). PTMAEMA and PTMAEA polymers were characterized by GPC, eluting in trifluoroethanol (TFE, ACS grade) with 0.02 M sodium trifluoroacetate (ACS grade) at 40°C, was performed on an Agilent 1200 series system equipped with a degasser, an isocratic pump operated at 1 mL/min, an auto-sampler, a Polymer Standards Service (PSS) PFG guard column (8 x 50 mm), three PSS PFG analytical linear M columns (8 x 300 mm, particle size 7 μm), and a refractive index detector. (**Figure S4**) Molecular weights and molecular weight distributions were estimated relative to poly(methyl methacrylate (PMMA) standards (**Tables S2 and S4**).

Coacervate Preparation

Polymer stock solutions of 0.125 M (on a monomer basis) were prepared gravimetrically with Milli-Q water (resistivity of 18.2 M Ω cm, Millipore, USA) and filtered through a 0.22 μm filter (poly(ethersulfone), Thermo Scientific). A stock solution of 4 M potassium bromide (KBr, ACS Grade, Fisher) was prepared. To make coacervates, water, followed by KBr solution, 0.125 M polycation and polyanion were added into a 50 mL Falcon tube (Fisher). The sample was

vortexed after each addition to ensure that the sample was well mixed. The resulting samples were then centrifuged at 4,000 rpm (Thermo Scientific Legend X1R) for five minutes to facilitate coalescence of the dense phase and stored overnight at room temperature (~25°C) to allow for sample equilibration.

Turbidimetry and Optical Microscopy

Turbidity was used to qualitatively measure the extent of phase separation as a function of charge stoichiometry and salt concentration. Turbidity measurements were made using a microplate reader (Bio Tek Synergy H1). Turbidity was measured at a wavelength of 562 nm and a temperature of 25 °C. None of the polymers absorb light at this wavelength. Turbidity is defined as $-\ln(I/I_0)$, with I_0 = incident light intensity and I = intensity of light passed through the sample volume, and is measured in absorption units (a.u.).

Samples for turbidimetry were prepared using the same protocol as above, but at a 200 μ L scale and were not centrifuged. After preparation, 50 μ L of sample was pipetted in triplicate into a 384-well plate (Fisher) for analysis. Triplicate measurements were made for each well, and all experiments were repeated three times. Error bars on turbidity plots represent the calculated standard deviation of the data. All samples were referenced to Milli-Q water.

Phase Diagram Determination

The volume of supernatant phase was determined by pouring all the supernatant into a graduated cylinder. The volume of coacervate phase was determined by subtracting supernatant volume from the total sample volume (50 mL). Assuming all polymer was incorporated into the coacervate phase, the polymer concentration in coacervate phase was determined by dividing total polymer added by the volume of coacervate phase (see **Tables S6, S7** for an analysis of the potential error associated with this assumption). The concentration of salt in the supernatant was measured using a Tetracon 325 conductivity meter and referenced to a calibration curve over the range of 0.1 M to 0.6 M KBr (**Figure S7**).

Rheology

Linear viscoelasticity experiments were performed using a Malvern Kinexus Pro stress-controlled instrument, run in strain-controlled oscillatory mode. A 20 mm diameter stainless steel parallel plate fixture with a solvent trap was utilized for all rheological experiments for more gel like material. For methacryloyl samples above or equal to 0.3 M salt concentration and all the acryloyl samples, a 50 mm 2° cone-and-plate fixture was used. In this study the experimental temperature was consistently 25°C. The gap resolution is 0.1 μm . The temperature of the plates can be controlled to within 0.01°C resolution and has a range of -40°C to 200°C. The torque range of the rheometer in oscillatory mode is 0.05 $\mu\text{N}\cdot\text{m}$ to 200 $\text{mN}\cdot\text{m}$ with a 0.1 $\text{nN}\cdot\text{m}$ resolution. In this study a frequency range of 1 rad/s to 100 rad/s was used. The strain amplitude used in the measurements was at 0.1 % and 1 %. This strain was confirmed to be in the linear viscoelasticity region for all samples by strain-sweep experiments.

For methacryloyl samples below 0.3 M salt concentration, the 20 mm parallel plate fixture was mounted to the rheometer, allowed to equilibrate at 25°C, and then the gap was zeroed. The gap then set to 70 mm for loading. The supernatant was poured from the centrifuge tubes and the coacervate was removed from the tubes and transferred to the bottom plate of the rheometer using a stainless steel spatula. Care was taken to prevent bubble formation. The reservoir around the plate was filled with the supernatant removed from the centrifuge tube. This reservoir does not contact the sample but is enclosed with the sample under the solvent trap. The top plate was then lowered to a gap of 0.81 mm and the sample was trimmed using a nylon edge and a razor blade. Once the sample was trimmed, the gap was lowered to 0.80 mm and the solvent trap was put into place. The experimental procedure was identical for samples that are more liquid like requiring the cone-and-plate fixture, except the trimming gap was 0.077 mm and the working gap was 0.07 mm. The system was then allowed to equilibrate at 25°C for around 3 minutes before measurements commenced. Two frequency sweeps were run in series

and then compared to ensure that drying did not significantly change the material during the experimental time period. The rheological data were analyzed using the IRIS software package (IRIS Development LLC).

Theoretical Methods

Transfer Matrix Model for Polyelectrolyte Complex Coacervation

Polyelectrolyte complex coacervation was modeled using the Transfer Matrix (TM) model developed by Lytle and Sing.^{49,50,63} The TM model considers a test polycation or polyanion in the coacervate phase. Previous work has shown that the correlations among monomers in the coacervate phase are dominated by their nearest neighbors.⁶⁴ We describe the local environment along the contour of the chain by considering the nearest oppositely charged species for each monomer. If the oppositely charged species is within an arbitrary cutoff distance r_c , that ion is said to be ‘adsorbed’ onto that monomer. By accounting for all of the ‘adsorbed’ ions, we can develop a partition function for the chain, from which the interaction free energy of the chain can be calculated.

Starting at the first monomer of the test polyelectrolyte chain, we walk along the chain assigning an adsorption state to each monomer. For a salt-ion adsorbed monomer, the state S is assigned. In the case where a monomer from an oppositely charged chain is adsorbed onto the test chain, we distinguish between the first monomer from the adsorbing chain and the subsequent adsorbed monomers with P' and P respectively. It is possible to have a monomer on the chain without a nearby oppositely charged species; in this case the state 0 is assigned.

If we consider the volume immediately near the test chain, a chemical equilibrium exists across an open boundary, set by the chemical potentials of the oppositely charged salt ions (μ_s) and polyelectrolyte chains (μ_p). We assign an energetic penalty ε , which reflects the electrostatic penalty for unpaired monomers. We use the ‘transfer matrix’ method to develop a

partition function for the test chain, which bookkeeps the adsorption state contributions to the partition function for each monomer and its preceding monomer:⁴⁹

$$\mathbf{M}(s_i, s_{i-1}) = \begin{bmatrix} SS & SP & SP' & S0 \\ PS & PP & PP' & P0 \\ P'S & P'P & P'P' & P'0 \\ 0S & 0P & 0P' & 00 \end{bmatrix} = \begin{bmatrix} e^{\mu_s} & e^{\mu_s} & e^{\mu_s} & e^{\mu_s} \\ 0 & 1 & 2 & 0 \\ e^{\mu_p} & e^{\mu_p} & e^{\mu_p} & e^{\mu_p} \\ e^{-\varepsilon} & e^{-\varepsilon} & e^{-\varepsilon} & e^{-\varepsilon} \end{bmatrix} \quad (1)$$

The partition function can be expressed in terms of the transfer matrix as follows:

$$\Xi = \psi^T \mathbf{M}^N \psi \quad (2)$$

where the vectors ψ are comprised of ones. For sufficiently large values of N , the partition function can be approximated by the largest eigenvalue of the matrix $\mathbf{M}$, denoted by ξ . We assume the salt and polymer chemical potentials have the forms: $\mu_s = \mu_s^0 + \ln \gamma + \ln \phi_s = \ln A_0 \phi_s$ and $\mu_p = \mu_p^0 + \ln \gamma + \ln \phi_p = \ln B_0 \phi_p$. The factors A_0 and B_0 are fitted parameters that consolidate both the reference chemical potential and activity coefficient γ contributions. These values are constants assuming that γ is concentration-independent, consistent with Henry's law, and reflect the strength of pairwise interactions between the test polymer and its neighboring salt and polymer monomers. As these parameters are associated with the pairwise interactions between specific monomers, they provide a coarse-grained way to account for any number of short-range interactions (e.g. hydrogen bonding, cation-pi interactions, etc.). The interaction free energy is directly related to the partition function, which is approximated using the largest eigenvalue of $\mathbf{M}$:

$$\mathcal{F}_{int} = -k_B T \ln \Xi = -k_B T N \ln \xi; \quad (3)$$

To this interaction free energy term, we add an entropic mixing contribution term for each species, a phenomenological contribution to capture the excluded volume interactions, as well as a Flory Huggins interaction term, χ_{ij} , where the subscripts i and j indicate interactions between pairs of species (polymers, p , salt s , and water, w). Using the expressions for the

chemical potentials described earlier, we arrive at the following expression for the free energy of the system⁴⁹:

$$\begin{aligned} \frac{\mathcal{F}}{Vk_B T} = & -\frac{\phi_p}{2} \ln \left[A_0 \phi_s + B_0 \phi_p + e^{-\varepsilon} + 1 + \right. \\ & \left. \sqrt{(1 + A_0 \phi_s + B_0 \phi_p + e^{-\varepsilon})^2 - 4(A_0 \phi_s + B_0 \phi_p + e^{-\varepsilon})} \right] + \frac{\frac{\phi_p}{2} \ln(\frac{\phi_p}{2})}{N_{p+}} + \frac{\frac{\phi_p}{2} \ln(\frac{\phi_p}{2})}{N_{p-}} + \phi_s \ln \left(\frac{\phi_s}{2} \right) + \\ & \phi_w \ln \phi_w + \kappa (\Lambda \phi_p + \phi_s)^3 + \frac{1}{2} \sum_{i,j=p,s,w} \chi_{ij} \phi_i \phi_j \end{aligned} \quad (4)$$

In this model, the parameters A_0, B_0, κ , and Λ are left as fitting parameters. κ sets the magnitude of a phenomenological third order term that accounts for the free energy penalty of densely packing particles with a finite excluded volume, with a parameter Λ that can account for differences in the excluded volume of the salt versus polymer species. For simplicity, we set $\lambda = 1$, and all $\chi = 0$ except for χ_{pw} and χ_{ps} . The values of $A_0, B_0, \kappa, \Lambda, \chi_{pw}$, and χ_{ps} are determined by comparing the coexistence curves from the model to those determined experimentally. The values of these parameters are listed in Table 1, and are consistent with the orders of magnitude of these values found in previous papers.⁴⁹ In this work, we will use χ_{pw} and χ_{ps} to reflect changes in the backbone chemistry.

Table 1. Listing of parameters

Polymer Chemistry	A_0	B_0	κ	Λ	χ_{pw}	χ_{ps}
Methacryloyl	60.0	25.0	30.0	1.0	3.00	3.00
Acryloyl	60.0	25.0	30.0	1.0	1.25	2.00

Phase Diagrams using Convex Hull Construction

Phase coexistence curves were constructed using free energy convexification as shown by Mao *et al.*⁶⁵ In the free energy expression, the polycation and polyanion are treated as a single

species, similarly the cations and anions are treated as a single species. A discretized free energy manifold is constructed from the 3-component system as a function of polymer ϕ_p and salt ϕ_s concentration. The convex envelope of this manifold is constructed using the Python wrapper for the Qhull Algorithm. This convex envelope, for a 3-component system, is made up of 2-simplices with 3 vertices each, the vertices of the simplices are projected onto the composition space. The sides of these projected simplices are distorted where two or more phases are in coexistence.⁶⁵ In this case, the points connected by a long segment are distinct phase compositions in coexistence.⁶⁵ We constructed the coexistence curve by determining the projected simplex vertices that are connected by segments approximately ~ 5 times larger than the grid spacing.

Results and Discussion

The goal of this study was to understand how variations in polymer chemistry affect the thermodynamic phase behavior and resulting material dynamics of complex coacervates. To date, such fundamental characterization data has been sparse, with only a few reports, or combinations of reports, providing matched thermodynamic and viscoelastic information.^{15,41,56,64,66,67} One of the biggest limitations in developing a larger understanding of the impacts of polymer chemistry on complex coacervation has been the absence of data for chemically similar model systems. Previous reports have considered the effect of increasing the hydrophobicity of the charged group itself,⁵³ the polarity of the side chain,⁵¹ or the incorporation of hydrophobic co-monomers,⁵¹ on either the phase behavior or the rheology, but were not able to directly link changes in composition (*i.e.*, a quantitative analysis of the phase diagram) to material properties. Here, we selected structurally similar, low polydispersity methacryloyl and acryloyl polyelectrolyte systems to enable the straightforward comparison of variations in the polymer backbone chemistry on the resulting phase behavior and viscoelastic properties.

Comparing Coacervate Phase Behavior

We used a combination of experimental methods to determine the binodal phase boundary of each of our polymer systems (**Figures 4** and **S8-S10**, as well as **Table S5**). While a variety of techniques can be used to determine the concentration of polymer in the coacervate and supernatant phases (*e.g.*, UV-vis spectroscopy, NMR, thermogravimetric analysis, etc.), recent work has highlighted the need to simultaneously quantify the concentration of salt present in the two phases.^{2,15,66,68} Thus, we used the large-scale samples prepared for rheological analysis (50 mL total ~1 mL coacervate) and used conductivity to directly measure the concentration of salt present in the supernatant phase. There are two reasons that we used large-scale samples. First, a sufficiently large enough volume of supernatant (~20 mL) was required for conductivity measurements. Second, it was critical to prepare a sample with a sufficiently large volume of coacervate (relative to the total) to detect potential salt partitioning between the coacervate and supernatant phases. These data, combined with measured values for the volume of the coacervate and supernatant phases were used to construct phase diagrams (**Figure 4**). Additionally, turbidimetry was used to map out the location of the phase boundary at low polymer concentrations and high salt by identifying the concentration of salt at which phase separation was no longer observed (**Figures S8-S10**).

Figure 4. Experimentally determined salt-polymer phase diagrams for the complex coacervation of **(a)** methacryloyl polymers showing the effect of increasing polymer chain length for length-matched polymers with $N_{\text{anion}}-N_{\text{cation}}$ of approximately 50-50, 250-250, and 500-500, as well as two length mismatched systems with $N_{\text{anion}}-N_{\text{cation}}$ of 50-500 and 500-50. Tie lines connect data for corresponding coacervate (closed symbols) and supernatant (open symbols) sample, but are only indicated for the 500-500 system for clarity. The inset optical micrograph shows coacervate droplets for $N_{\text{anion}}-N_{\text{cation}} \sim 50-50$ at 0.3 M KBr. **(b)** A comparison between length-matched methacryloyl (red) and acryloyl (blue) polymers with $N_{\text{anion}}-N_{\text{cation}}$ of approximately 250-250. The inset optical micrograph shows coacervate droplets for acryloyl polymers with $N_{\text{anion}}-N_{\text{cation}} \sim 250-250$ at 0.3 M KBr. All samples were prepared at a 1:1 stoichiometric charge ratio. Polymer concentration is on a total monomer basis. Additional details and individual phase diagrams with tie lines can be found in the SI **Table S5** and **Figures S8-S10**. Error bars are based on the uncertainty of measurement and propagation of error.

Figure 5. Theoretical predictions for salt-polymer phase diagrams, in analogy to **Figure 4**. **(a)** Salt-polymer phase diagrams calculated for the complex coacervation of methacryloyl polymers showing the effect of increasing polymer chain length for length-matched polymers with $N_{\text{anion}}-N_{\text{cation}}$ of 50-50, 250-250, 500-500, and 50-500. Tie lines connect data for corresponding coacervate and supernatant samples, exhibiting qualitative agreement with **Figure 4**. We note that, as the identities of the polyelectrolytes (*i.e.*, polycation versus polyanion) in the theoretical model are arbitrary, the 50-500 prediction is identical to the 500-50 prediction. **(b)** Calculated phase diagrams that introduce χ -parameters to capture the difference between length-matched methacryloyl (grey) and acryloyl (blue) polymers with $N_{\text{anion}}-N_{\text{cation}}$ of 250-250.

Figure 4 shows the binodal curves for methacryloyl and acryloyl coacervates as a function of salt concentration vs. polymer concentration at a 1:1 charge stoichiometry. The shape of the two-phase region is similar to both previous experimental reports and theoretical predictions (**Figure 5**).^{2,10,15,41,44,45,47-49,52,66,68-72} Furthermore, we observed the expected exclusion of salt from the polymer-rich coacervate phase, as indicated by the negative slope of the tie

lines.^{44,45,49,50,71} We also observed a similar loss of salt partitioning with increasing as-prepared salt concentration (*i.e.*, the partition coefficient describing the concentration of salt in the coacervate phase relative to the supernatant approaches 1, **Figure S11**), as reported previously.^{49,66} However, it should be noted that we neglected the presence of polymer in the supernatant phase in our calculations. Previous efforts by our group and others demonstrated that the concentration of polymer in the supernatant phase is very low, typically on the order of 1-10 mM on a monomer basis.^{20,41,44} While this assumption does not markedly affect the shape of the phase diagram or the trends in our data (**Figures 4** and **S8-S10**), it could introduce error on the order of 10-20% with respect to the polymer concentration in the coacervate phase because of the large volume of supernatant present in our samples (a detailed estimation of this error is available in the SI **Tables S4, S5**).

With increasing polymer length, the binodal curve shifted to higher salt and polymer concentrations for both the methacryloyl (**Figures 4a** and **S8**) and acryloyl systems (**Figure S10**), in agreement with previous experimental reports and theoretical predictions.^{2,41,56} While the uncertainty and error associated with our data do not show high levels of statistical difference between the various binodal curves, there is a trend of increasing probability regarding the statistical difference of the datasets with increasing polymer chain length. This trend of increasing salt stability with increasing polymer chain length has been attributed to the increased number of interacting polyelectrolyte charges per chain, polymer chain entropy, and the decreased importance of weakened localization of opposite charges at polymer chain ends.^{41,45,51}

In addition to general trends of increasing chain length, we also considered the phase behavior of polymers with mismatched chain lengths. As shown in **Figures 4a** and **S9c,d**, the phase diagrams for $N_{\text{anion}}-N_{\text{cation}}$ 50-500 and 500-50 are nearly identical, indicating that the polycation and polyanion play an equal role in the complexation process. Furthermore, these

data also match the binodal curve for the 50-50 system, indicating that the phase behavior is dominated by the shortest polymer chain. This result makes sense in the context of the entropic arguments used to explain polymer chain lengths in general, and is further supported by previous reports indicating that the effect of polymer chain length was attenuated above $N \sim 200$.^{21,41}

Theory predicts a similar trend, shown in **Figure 5a** for parameters chosen to compare with phase diagrams shown in **Figure 4a**. Here, the effect of different $N_{\text{anion}}-N_{\text{cation}}$ values is not sensitive to the specific choice of TM model parameters, but indeed shows that the 50-50 binodal curve demarcates a smaller two-phase region than for 250-250 or 500-500. Similarly, the 50-500 is close to the result for 50-50, reflecting the importance of the short chains in changing the coacervate phase diagram. We do note that our simple model neglects end-effects that would be important for the 50-50, likely decreasing the two-phase region further and improving agreement with experimental results. This could be accounted for in known modifications to the TM theory.^{45,73}

To understand the effect of polymer backbone chemistry, we compared the phase behavior of our methacryloyl polymers with those from an equivalent acryloyl system. Importantly, all of the polymers used in this study were synthesized via controlled polymerization in order to minimize the effects of polydispersity (**Tables S1, S2**).⁶¹ Again, the data suggested an increase in the size of the two-phase region with increasing acryloyl polymer chain length when comparing the $N \sim 250$ data to those from the longer polymers (**Figure S10**). Upon first inspection, there appeared to be a reversal in this trend for the $N \sim 1000$ and 2000 datasets. However, a statistical comparison showed a very low probability that the two datasets were different. This attenuation of length effects would be expected due to the longer length of the acryloyl polymers ($N \sim 250, 1000, 2000$) compared to the methacryloyl polymers ($N \sim 50, 250, 500$).

Interestingly, a comparison of the phase diagrams for polymers of the same chain length (**Figure 4b**) demonstrates that the two-phase region for the methacryloyl polymers is significantly larger than that of the acryloyl system. This increase in salt stability is consistent with an increase in hydrophobicity, and is also in agreement with reports for systems where the hydrophobicity^{22,52,53} and/or the polarity⁵² of the polymer is changed across all of the monomer units, and has been attributed to changes in the solvation shell, or the number of bound water molecules, per ion pair. Interestingly, studies examining the effect of incorporating hydrophobic co-monomers have described both increases in stability⁵³ and the absence of any significant change in the phase behavior,²³ suggesting the importance of chemical subtleties on materials design.

To quantify the effect of hydrophobicity, we changed *only* the values of χ_{pw} and χ_{ps} that are associated with the short-ranged interactions between polymer species and water and salt respectively. Parameters associated with the charges are not changed, as we do not expect the addition or subtraction of a methyl group to make a significant contribution to long-range Coulomb interactions. The value of χ_{pw} shifts the location of the binodal, which we plot in **Figure 5b** in a manner consistent with experimental results (**Figure 4b**). The value of χ_{pw} decreases from 3.0 to 1.25 as we go from the methacryloyl to acryloyl polymer, indicating a significant decrease in the hydrophobicity.

In addition to the polymer-solvent interactions described by χ_{pw} , polymer-salt interactions (χ_{ps}) have a significant effect on the slope of the tie lines, which also decreases from the methacryloyl to the acryloyl in **Figure 4b**. To reproduce this observed experimental result in the theoretical predictions (**Figure 5b**), we must decrease the value of χ_{ps} indicating a lessening of the energetic penalty for salt to be located in the polymer-rich phase. We speculate that this change in χ_{ps} could reflect differences in the effective dielectric constant in the two different coacervate phases, with the salt ions in the denser coacervate phase (methacryloyl)

experiencing a higher Born self-energy (*i.e.*, an energetic penalty) than in the less-dense (acryloyl) coacervate.⁷⁴⁻⁷⁶

The fact that our acryloyl system of polymers is actually a mixture of a cationic acrylate and an anionic acrylamide also raises the possibility for interpolymer interactions via hydrogen bonding between the carbonyl group of the acrylate and the amide nitrogen of the acrylamide.²⁰ While our experiments have not eliminated the possibility of such an interaction, previous studies on polypeptide materials suggest that such hydrogen bonding would be stabilizing to the coacervate,²⁰⁻²² and would not affect the trends observed in our data. Similarly, one could argue that the two methyl groups on the PAMPS side chain would allow for increased hydrophobic interactions. While these moieties are located on the side chain rather than the polymer backbone, the total number of carbons in PAMPS and PSPMA is actually the same, with PTMAEA having one fewer carbon than PTMAEMA. Thus, we would again anticipate that these molecular features could only serve to stabilize the coacervate, and would not alter the trends reported here.

Comparing Coacervate Linear Viscoelasticity

Quantifying the phase behavior of our various coacervate samples as a function of salt concentration, polymer chemistry, and chain length allowed us to characterize the linear viscoelasticity of our materials and decouple these various effects. For each of our polymer systems we observed an increase in modulus with decreasing salt concentration, corresponding to the expected stiffening of the samples (**Figures 6a-c, 7a-c, 8a,b**).^{14,15,24,42,43,56,77} Additionally, we observed a crossover between the storage and loss moduli (G' and G'') for the lowest salt concentrations tested for our longest polymers (*i.e.*, methacryloyl polymers with $N_{\text{anion}}-N_{\text{cation}} \sim 250-250$ and $500-500$ (**Figure 6b,c**), and acryloyl polymers with $N_{\text{anion}}-N_{\text{cation}} \sim 2000-2000$ (**Figure 7c**)).

Figure 6. Frequency sweep data for coacervates formed at different salt concentrations from length-matched methacryloyl polymers with degree of polymerization $N_{\text{anion}}-N_{\text{cation}}$ of (a) 50-50, (b) 250-250, (c) 500-500, and (d-f) the corresponding time-salt superposition plots. The data from the lowest salt concentration for each sample was used as the reference for the superposition.

While the trends from the individual frequency sweep experiments provide insight into the effects of salt concentration on individual coacervate samples, the use of time-salt superposition allows for comparison of trends between different polymer lengths and different chemistries across a wider range of frequency space (**Figures 6d-f, 7d-f, 8c,d**).^{1,15,41,56} This superposition can be achieved by shifting the individual curves both horizontally (adjusting for relaxation behavior) and vertically (adjusting for polymer concentration) (**Figures 9 and S13-S15**). Trends in the shift factors will be discussed later.

Generally speaking, the form of the various time-salt superposition curves was similar to that of a polymer melt. At low frequencies, the data suggest an approach to the terminal regime, with the slope for the loss modulus (G'') equaling the expected value of 1, and the slope for the storage modulus (G') approaching 2. At the low frequency end of some of the higher salt concentration samples, we observed some flattening of the storage modulus, which most likely

reflects instrumental limitations for measuring very soft samples. This deviation in the expected behavior is matched with deviations in the smoothness of the superposition, particularly for samples with the lowest modulus (*i.e.*, shortest polymer chains and highest salt concentrations). However, plotting the superposed data in a Cole-Cole plot (**Figure S12**) suggests that the superposition is reasonable, despite these deviations. At higher frequencies, we observed the appearance of a crossover between G'' and G' , which provides information about a characteristic timescale for the relaxation of the material. However, we were only able to observe this crossover for the longer-chain polymer samples. While it is possible that such a crossover exists for our shorter polymer samples, we were not able to access it in our experiments.

Figure 7. Frequency sweep data for coacervates formed at different salt concentrations from length-matched acryloyl polymers with degree of polymerization $N_{\text{anion}}-N_{\text{cation}}$ of (a) 250-250, (b) 1000-1000, (c) 2000-2000, and (d-f) the corresponding time-salt superposition plots. The data from the lowest salt concentration for each sample was used as the reference for the superposition.

The time-salt superposition data in **Figures 6-8** are presented using the lowest salt concentration sample for each polymer as the reference condition. However, the various graphs can be shifted to use any of the individual samples as the reference, thus providing information on the frequency-dependent linear viscoelastic response of the selected reference condition. For example, we can re-graph the data for the methacryloyl systems in **Figure 6d-f** using the same as-prepared salt concentration of 0.3 M KBr for all three sets of polymers to eliminate the “apparent” effect of salt on the viscoelastic response (**Figure S16**). As would be expected for polymer melts or solutions, we observed a shift in the crossover point, or a characteristic relaxation time, to higher frequencies (or even beyond the range of the measured data) with decreasing chain length. While longer chain polymers would be expected to relax more slowly, the phase diagram highlights that differences in both the polymer concentration and the actual salt concentration present in the various coacervate samples may also be affecting the relaxation behavior. For samples prepared at an initial total salt concentration of 0.3 M KBr, the resulting polymer concentration in the coacervate phase was found to be 1.87 M, 2.04 M and 2.04 M for $N \sim 50$, 250, and 500 (**Figures 4a** and **S8**). Thus, it is important to consider the quantitative phase behavior of coacervate materials when attempting to understand and predict trends in the viscoelastic response.

To better decouple the effects of polymer chain length and concentration, we can use the phase diagrams (**Figures 4, S8-S10**) to identify conditions with similar salt or polymer concentrations in the coacervate phase. We looked to compare the response of coacervates at a similar actual salt concentration in the coacervate. For the methacryloyl polymers, we could compare data from coacervates with an actual salt concentration 0.137 M, 0.120 M, and 0.125 M for $N \sim 50$, 250, and 500, respectively. However, these two conditions corresponded to the same as-prepared salt concentration of 0.3 M, meaning that the shifts would be the same (**Figure S16**). This result is not unexpected given the similarity in the trends in salt partitioning

(**Figure S11**) and the lack of statistical difference between the binodal curves. Further comparison of the data referenced instead to the same polymer concentration of 1.6 M shows the same shift in the crossover point between G' and G'' to higher frequency with decreasing chain length (**Figure S17a**). This result confirms the importance of polymer chain length on rheology. Our results suggest that similar trends in the relaxation behavior occur for coacervates made from different length acryloyl polymers, though we were only able to observe the crossover in modulus for the longest chain sample (**Figure S17b**).

Similarly, we can compare the superposition data for the shortest 50-50 methacryloyl polymers (**Figure 6d**) with the data for the mismatched 50-500 and 500-50 systems, all of which are at effectively the same polymer concentrations (**Figures 8c,d** and **S18**). As with the phase behavior, the viscoelastic response of these three samples is similar, suggesting that the shortest polymer dominates the response. A more quantitative comparison shows that the moduli of the mismatched samples are actually approximately $10\times$ larger than that of the 50-50 samples (**Figure S18a**), as might be expected for materials containing a longer-chain polymer. Additionally, a comparison between the two mismatched samples shows that the moduli for the $N_{\text{anion}}-N_{\text{cation}} \sim 50-500$ sample is slightly larger than for the $N_{\text{anion}}-N_{\text{cation}} \sim 500-50$ case (**Figure S18b**). This result is similar to results from a previous study looking at the effects of mismatched polymer chain lengths, where the polycation dominated the rheological response.⁵⁶ However, this previous observation could have been a consequence of using polymers with two different backbones. We hypothesize that the slight variation in our work is due to small differences in the degree of polymerization of the different samples. While we use the terminology of “50” and “500” for simplicity, the average chain length of the different samples was determined to be $N_{\text{anion}}-N_{\text{cation}} \sim 45-470$ and $N_{\text{anion}} - N_{\text{cation}} = 450-47$ (**Tables S1, S2**). Thus, while the overall viscoelasticity is dominated by the shortest polymer, variations in the length of the longer polymer do have an effect.

Figure 8. Frequency sweep data for coacervates formed at different salt concentrations from length-mismatched methacryloyl polymers with degree of polymerization $N_{\text{anion}}-N_{\text{cation}}$ of **(a)** 50-500, **(b)** 500-50, and **(c-d)** the corresponding time-salt superposition plots. The data from the lowest salt concentration for each sample was used as the reference for the superposition.

Let us now consider the differences in the rheological response of coacervates formed using the two different polymer chemistries with the same chain length ($N \sim 250$). For these samples, the overall shape of the curves generated from time-salt superposition is quite different, with a crossover between G' and G'' observed for the methacryloyl sample, and only terminal regime behavior observed for the acryloyl (**Figures 6e** and **7d**). In thinking about the effect of polymer chemistry on this rheological response, it is somewhat natural to immediately try and consider how the molecular level details of the polymer affect the behavior. For example, the methyl group present on the methacryloyl backbone could change the flexibility of the polymer, and thus the persistence length, affecting potentially both the inherent structure of the coacervate and possibly the number of correlated electrostatic interactions that occur between complexing chains.^{47,78} The backbone methyl group could also increase the hydrophobicity of the polymer,

leading to chain clustering and/or alter the structure of water around the polymer chain, both of which could slow relaxation dynamics.^{53,79} However, it is important to separate these detailed molecular-level effects from larger-scale differences in concentration etc.⁸⁰

Based on the phase diagrams, we can compare samples at a coacervate salt concentration of 0.067 M and 0.060 M for the methacryloyl and acryloyl samples, respectively (**Figure 4b**). Shifting the time-salt superposition curves for these samples to this same reference condition, the data for the methacryloyl polymer sample suggests a rheological response consistent with a much higher polymer concentration sample and/or longer polymer chains (*i.e.*, the crossover point is at a significantly lower frequency, **Figure 9a**). However, this comparison at the same salt concentration does not allow for the deconvolution of these two possible effects. Instead, we can consider the same two datasets, now shifted relative to samples at different salt concentrations (0.353 M vs. 0.140 M for the methacryloyl and acryloyl samples, respectively), but the same polymer concentration in the coacervate (**Figure 9b**). This analysis suggests that the rheological response of the two materials is actually very similar when considered at constant polymer concentration. In addition, the actual magnitude of the elastic and viscous moduli is similar between methacryloyl and acryloyl ($N \sim 250$) at the same polymer concentration (**Figure 9b inset**).⁵⁶ Thus, we hypothesize that the differences in the viscoelastic response of methacryloyl and acryloyl polymers can largely be understood in terms of how those differences in chemistry affect the resulting polymer concentration in the coacervate, in agreement with previous reports,^{23,53} and consistent with the differences in χ_{pw} determined from the TM theory.

We can also extend the concept of comparing the viscoelastic response of our acryloyl and methacryloyl polymers to include the effect of polymer chain length. Although we do not have samples that allow for a direct comparison of the effects of chain length while taking differences in polymer concentration into account (*i.e.*, data over the same range of frequencies), we can consider the overall shape of the frequency sweep curves. The most easily recognizable feature

in our frequency sweep data is the presence of a crossover between the moduli. We observe this only for the longest chain acryloyl (**Figure 7f**). The emergence of this feature in the data for the $N \sim 2000$ acryloyl system correlates with our $N \sim 250$ data for the methacryloyls, suggesting that for acryloyl coacervates to have similar properties to methacryloyls, the acryloyl polymers have to be approximately ten times longer than the methacryloyl polymers.

Figure 9. Plots of the time-salt superposition master curves for coacervates formed from length-matched methacryloyl (blue) and acryloyl (red) polymers with $N \sim 250$ as a function of frequency. **(a)** The data between the two vertical lines are the frequency sweep data that was collected directly at a coacervate salt concentration of approximately 0.06 M, and were used as the reference condition. **(b)** The data between the two vertical lines are the frequency sweep data that was collected directly at a coacervate polymer concentration of 1.6 M, and were used as the reference condition. Curves are shifted vertically for clarity, while the inset figure in **(b)** shows the data on the same vertical scale.

The time-salt superposition analysis takes advantage of both a horizontal shift (a_s) to account for differences in the relaxation behavior of the material, and a vertical shift (b_s) to account for differences in concentration. Plots of these shift factors as a function of the as-prepared salt concentration, the measured salt concentration in the coacervate phase, and the polymer concentration in the coacervate are shown in **Figures 10** and **S13-S15**. Generally speaking, the trends in the vertical shift factor b_s are significantly smaller than those of the horizontal shift factor a_s . Additionally, we observed a range of different behaviors for b_s as a function of polymer or salt concentration, the exploration of which is beyond the scope of the current work.

Figure 10. Plots of the horizontal shift factor a_s for coacervates formed from (a) length-matched methacryloyl polymers, (b) length mismatched methacryloyl polymers, and (c) length-matched acryloyl polymers with respect to the square root of the measured salt concentration in the coacervate phase. (d) The

corresponding plot of the horizontal shift factor a_s for coacervates formed from length-matched methacryloyl and acryloyl polymers with respect to the polymer concentration in the coacervate phase. The lines are a fit to the data. Fitting parameters are listed in **Tables S6,S7**. Error bars are based on the uncertainty of measurement and propagation of error.

The plots of the horizontal shift factor a_s as a function of the measured salt concentration (**Figures S13c, S14c, S15c**) show a more pronounced non-linear trend than the as-prepared salt concentrations (**Figures S13a, S14a, S15a**), as would be expected based on the changes in the slope of the tie-lines in the phase diagrams (**Figures 4 and S8-S10**). However, previous work by Spruijt *et al.*, along with additional work by Colby and coworkers suggested that the trends in the horizontal shift factor should follow a square root dependence with respect to the salt concentration in the coacervate phase based on combining ‘sticky’ Rouse chain dynamics with Debye-Hückel electrostatics.^{41,51,56} Indeed, **Figure 10a-c** show that plotting a_s as a function of the square root of the salt concentration present in the coacervate phase results in a linear trend.

The ‘sticky’ Rouse model describes the stress relaxation of the polymer chains in the coacervate in terms of an effective friction, related to the dissociation rate of the relevant ion pairs. Thus, we can qualitatively relate trends in the magnitude of the slope of the line in the horizontal shift factor vs. square root of salt concentration plot to an effective activation energy barrier height.^{56,57,81} Comparing the data in **Figure 10a-c**, our analysis indicates that the activation energy barrier for ion pair rearrangement is significantly lower for the acryloyl polymers as compared to the methacryloyl polymers. This observation is in agreement with our other general observations regarding the slower relaxation behavior and higher modulus of the methacryloyl polymers, and is consistent with observed differences in the glass transition temperature (T_g) of poly(propyl acrylate) ($T_g = 231^\circ\text{F}$) and poly(propyl methacrylate) ($T_g = 316^\circ\text{F}$) as neutral analogues of our polyelectrolyte systems.⁸² Interestingly, this energy barrier difference also exists despite the fact that the chemical identity of the ions in the two polymer

sets is identical (*i.e.*, sulfonate and quaternary amine). This implies the difference in backbone chemistry must affect the number of correlated interactions along the chain that form a ‘sticky’ point⁵¹ and/or the local environment around the chain.^{53,79}

It should be noted that there are some apparent variations in the observed activation energy barrier as a function of polymer chain length. In particular, we observed a larger slope for the length matched 50-50 methacryloyl samples, as compared to the matched systems with longer chains or the two length-mismatched methacryloyl samples. The reason for these variations is unclear, and should be investigated across a wider range of polymer systems. It is also noteworthy that the trends in horizontal shift factor as a function of polymer concentration are very similar across all of our samples, suggesting the possibility of polymer concentration as a more universal descriptor for coacervate rheology (**Figure 10d**).

The similarity in the trends for horizontal shift factor as a function of polymer concentration are also interesting in the context of a recent report by Lutkenhaus and coworkers, who were able to collapse dynamic mechanical analysis (DMA) data on solid complexes of poly(acrylic acid) and poly(allylamine) prepared at different relative humidity environments onto a universal curve by scaling the data based on the water content of the films, normalized by or controlling for the number of intrinsic ion pairs (*i.e.*, polymer-polymer ion pairing interactions).^{83,84} While we did not explicitly track the water content in our coacervate samples, it would be a reasonable assumption to assume that water content scales inversely with polymer concentration. Future work is needed to determine if this same scaling relationship determined for solid polyelectrolyte complexes also holds for liquid coacervates.

Conclusions

Our systematic analysis of the effect of polymer chain length and polymer backbone chemistry on complex coacervation highlights the importance of simultaneous characterization of both coacervate rheology and phase behavior. The use of time-salt superposition referenced

to the actual salt and polymer concentrations present in the coacervate phase allowed for a variety of comparisons between samples of different polymer chain lengths or chemistries – even those prepared at different total salt concentrations. We observed that polymer concentration in the coacervate dominates the rheological response of the material, though chain length effects do play an important secondary role. Interestingly, we observed that the identity (cation vs. anion) of our polymers had no significant effect on the phase behavior or the viscoelasticity of coacervates formed from length-mismatched polymers. Furthermore, the phase behavior and rheology of these mismatched coacervates was dominated by the shortest chain. Theoretical calculations show that short chains more strongly affect phase behavior due to the increased translational entropy per charge, which is only non-negligible in short chains.

In comparing the effect of polymer backbone chemistry, we observed dramatic differences in the size of the two-phase region for coacervates formed from acryloyl vs. methacryloyl polymers. For polymers of the same chain length, the two-phase region was significantly larger for the methacryloyl polymers, as compared to the acryloyl. We used theory to quantify these differences, showing that both polymer-water and polymer-salt interactions were affected by the backbone chemistry. These results support the hypothesis that the methacryloyl polymers are more hydrophobic, although the mixed use of an acrylate and an acrylamide polymer complicate the comparison. However, these differences did not translate as significantly to the linear viscoelastic response if differences in polymer concentration were taken into account. Lastly, we compared trends in the horizontal shift factors used for the time-salt superposition as a function of salt concentration to extract information related to activation energy barrier for chain rearrangement. Consistent with a more stable coacervate, we observed a higher activation energy barrier for our methacryloyl polymers, which followed trends in T_g for analogous neutral polymers.

This work provides a foundation for future explorations of the effect of polymer chemistry on coacervate phase behavior and material properties. While changes in hydrophobicity were shown to have a dramatic effect in our current materials, it is an open question whether similar chemical perturbations would give the same result if applied to a side chain or to the charged group itself. We anticipate that the effect of different polymer chemistries can be condensed into three possible contributions: (i) steric effects, (ii) changes in the polymer stiffness, and (iii) changes in the structuring of water.

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Supporting Information

Details on the synthesis and characterization of monomers; polymer characterization; calibration curves for salt concentration as a function of solution conductivity; propagation of error analysis; tabulated binodal curve data including uncertainty and potential systematic errors; individual and comparison binodal curve plots with error bars; plots of salt partitioning trends; Cole-Cole plots; individual and comparison plots of horizontal and vertical shift factors as a function of salt and polymer concentration; tabulated parameters describing fits to shift factor data; additional rheology comparison graphs.

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