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# Ternary, Tunable Polyelectrolyte Complex Fluids Driven by Complex Coacervation

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S Supporting Information

ABSTRACT: Complex coacervation was achieved by combining poly(allylamine) (PAH) or branched poly(ethylenimine) (PEI) with poly(acrylic acid) (PAA) and poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA). We systematically investigated the effects of stoichiometry, salt concentration, and pH. Ternary coacervates formed over a broader range of stoichiometries compared to the base PAA/PDMAEMA system. An enhanced resistance to salt, that is, resistance to dissolution of the complex with added salt, was observed for ternary coacervates. PEI-containing systems showed a considerable difference in salt resistance at pH 6−8 due to the dramatic change in charge density. This change was interpreted in the context of a theoretical treatment that relies on the Voorn−Overbeek model for free energy. Coacervate stability and viscoelastic behavior were affected by stoichiometry, salt, and pH. Ternary coacervates maintain the characteristics and tunability of typical binary coacervates, but the choice of the third component is important, as it significantly affects the response and material properties.
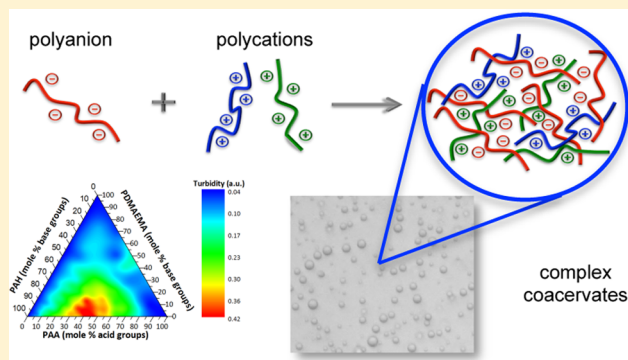
1. INTRODUCTION

A variety of materials with diverse structures and properties are formed as a result of electrostatic interactions between oppositely charged macromolecules. Examples include membranes,[1] spherical or wormlike micelles,[1,2] fibers,[3] and hydrogels.[4] Under defined conditions,[5] complexation of oppositely charged polyelectrolytes in aqueous media can lead to liquid−liquid phase equilibria. This polyelectrolyte complex formation is referred to as *complex coacervation* (whereas the term coacervation more generally refers to liquid−liquid phase separation in systems of macromolecules, charged or uncharged) and is driven by a combination of entropic and enthalpic effects.[6−8] Dispersed liquid droplets are observed when coacervation occurs. Coalescence of the droplets leads to a polymer-rich (coacervate) phase, which coexists at equilibrium with a dilute, polymer-lean (aqueous) phase.

Because of their interesting interfacial and bulk material properties, complex coacervates find applications in many fields, including the pharmaceutical and food industries. In particular, complex coacervates exhibit very low interfacial energy in aqueous solution,[9,10] a property that enables them to engulf a variety of materials.[11] Work on biomacromolecule-based complex coacervates has revealed that they have great potential as wet adhesives.[12,13] There has also been increasing interest in understanding the rheological properties of coacervates[14,15] in order to improve and optimize applications. For this purpose, a series of biopolymers[16,17] and/or synthetic polymer systems[18]

have been considered previously. Complex coacervates typically exhibit a viscoelastic response,[19] and rheological studies have shown that the viscoelasticity and viscosity of coacervates are affected by a number of parameters, including salt concentration, mixing stoichiometry, and chain length.[20,21]

In this report we extend the current understanding of complex coacervation to include more complicated, ternary polymer systems. We present experimental data and a theoretical treatment that allows us to rationalize our experimental observations. In an early report, Trinh et al.[22] described the formation of binary and ternary polyelectrolyte complexes (PECs) from synthetic polyelectrolytes, while similar macromolecules were used for the formation of multilayer films.[23] However, in these examples, only water-insoluble PECs were formed regardless of the mixing conditions. Ternary complexes constructed with plasmid DNA (pDNA), poly(ethylenimine) (PEI), and various polyanions have also been prepared and successfully used as gene delivery systems with high transfection efficiency and low toxicity.[24−26] Wang et al.[27] prepared alginate/poly(L-arginine)/chitosan ternary complexes by a combined mixing and absorption method that can be used as microcapsules, while the miscibility of three similar polysaccharide-based compo-

nents was explored in the presence of different amounts of supporting simple salt.[28]

The above examples reveal the variety of ternary complexes that have been described and the interesting applications and potential that such materials exhibit. However, to the best of our knowledge, complete studies of complex coacervation in three-component systems that include analysis of the ternary-coacervate properties are not available, but are necessary to achieve a comprehensive understanding of complexation in multicomponent systems and to facilitate their use in applications such as those described above.

A well-studied coacervate system consisting of poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA) and poly-(acrylic acid) (PAA) was used as the base system for this report.[29] A second polycation, poly(allylamine) (PAH) or branched poly(ethylenimine) (PEI), was introduced, thereby creating two separate ternary systems (see example in Scheme 1). The conditions that lead to complex coacervation were

Scheme 1. Schematic Illustration of Ternary Complex Coacervate Formation
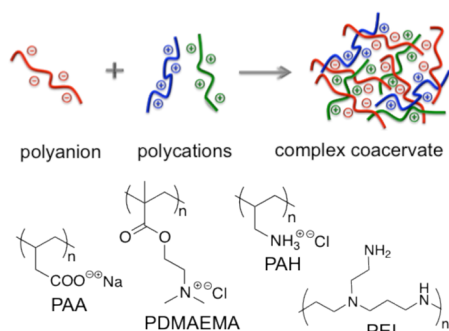
experimentally determined for both systems. Rheology was used to gain insights into the dynamic properties of these materials. Of particular interest were the effects of salt concentration, polyelectrolyte ratio, and pH. A comparison between the ternary and the binary coacervate systems, with regard to effects of external conditions on the formation and the properties of the coacervates, was performed where possible. The existence of a maximum salt concentration and its dependence on the pH value were rationalized in the context of a Voorn−Overbeek model, which was extended here to describe ternary systems.

2. EXPERIMENTAL SECTION

2.1. Materials. In this study PAA was used as the polyanion and PDMAEMA and either PAH or branched PEI as the polycations. Solutions of PAA (40 wt % in water) and PAH (20 wt % in water) and branched PEI with molecular weights of 30 000, 17 000, and 25 000 g/mol, respectively, were purchased from Sigma-Aldrich. PDMAEMA (20 wt % in *tert*-butanol) with a molecular weight of 50 000 g/mol was purchased from Polysciences Inc. Dispersities (Đ) of the used polymers were 1.1, 1.18, 1.25, and 1.9. for PAA, PAH, PDMAEMA, and PEI, respectively. PDMAEMA was dried and redissolved in water. All other polymers were used as received without any further purification. The molecular characteristics of all the polymers used are reported as provided by the supplier. For branched PEI (bearing three different amine groups) the acid/base group mol % ratios were calculated assuming an average monomer molecular weight of 43 g/mol. Polycations and polyanions had comparable degrees of polymerization.

2.2. Mixture Preparation. Coacervation was achieved by mixing aqueous solutions of the polycations and polyanion with concentrated NaCl solutions. First, separate stock solutions of 1 wt % of each polyelectrolyte were prepared. The pH was then adjusted as desired by adding small amounts of either NaOH or HCl to these stock solutions. Final mixtures (1.5 mL final volume) of different polyanion/polycation mixing ratios were then prepared by sequential addition of calculated amounts of both polycations (first and second) and polyanion (third) stock solutions into NaCl solutions with precalculated concentrations to achieve a final salt concentration from 0 to 4000 mM NaCl in the experimental mixtures. The polyelectrolyte complexes were prepared in microcentrifuge vials and were vigorously vortexed for 5 s after the addition of each component. The order of mixing was kept the same for all experiments. All complex coacervates were prepared immediately before turbidity measurements and studied at room temperature (25 °C). All water was dispensed from a Milli-Q water purification system at a resistivity of 18.2 MΩ·cm.

2.3. Turbidity and ζ-Potential Measurements. A plate reader equipped with a UV spectrophotometer (Tecan, Infinite M200) was employed at a wavelength of 550 nm for the turbidity measurements. None of the polymers absorb light at this wavelength; thus, turbidity is due to light scattering from suspended coacervate droplets. The turbidity (T) is defined by $T = \ln(I/I_0)$, with I_0 = incident light intensity and I = intensity of light passed through the sample volume. Turbidity is measured in adsorption units (a.u.). The particle sizes and ζ-potentials of ternary complexes were measured using a Zetasizer Nano ZS (Malvern Instruments, Ltd., Worcestershire, UK). Coacervates were prepared with a 0.1 wt % total polymer concentration for spectrophotometry. The ζ-potential measurements were made with coacervate solutions of 0.005 wt % total polymer concentration. These concentration differences change the amount, but not the nature, of the complex formed.

2.4. Optical Microscopy. An optical microscope (Leica DMI6000) using Leica AFC image acquisition software was used to obtain physical images of the PECs before and after centrifugation. The coacervate mixture (as previously described) was placed on a glass slide to image the droplets. After centrifugation, the supernatant equilibrium aqueous phase was carefully removed by using a micropipet while the coacervate (viscous liquid) was left at the bottom of the vial.

2.5. Rheological Measurements. Rheological measurements were performed on a Bohlin Gemini HR nanorheometer (Malvern) fitted with a parallel plate geometry (8 mm in diameter). Ternary coacervate samples were loaded onto the plate and allowed to equilibrate for 10 min. Strain-sweep tests were carried out to determine the linear viscoelastic range (0−10%). Oscillatory tests were used to determine the storage modulus (G') and loss modulus (G'') while varying the frequency from 0.1 to 100 rad/s with a constant strain of 10%, which was found to be in the linear regime. A steady shear rate sweep was performed to measure viscosity. Data presented in all figures are the average of at least three measurements.

3. RESULTS AND DISCUSSION

Complex coacervation nucleates in the form of polyelectrolyte-rich fluid droplets, here around 1−10 μm in size (Figure 1A), which display a unique combination of physical properties. Coalescence of the droplets forms the dense, polymer-rich coacervate phase, which equilibrates with a very dilute, polymer-deficient phase as seen in Figure 1B. Physical distinction between a coacervate and a precipitate complex is usually made using centrifugation, where the former appears as a relatively clear viscous liquid and the latter as a white solid. In our experiments, mixing of polyelectrolytes resulted in the formation of coacervate phases that appeared as viscous liquids (Figure 1B). In order to be certain we are in fact reporting on coacervation, microscopy was used in every case. With this technique, we were able to detect the existence of small coacervate droplets prior to coalescence (Figure 1A) and
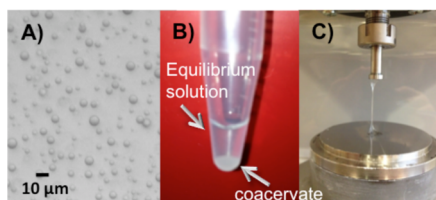
Figure 1. (A) Optical micrograph of complex coacervate droplets formed from PAA/PDMAEMA/PAH (polymer ratio 41/12/47 mol % acid/base groups, salt concentration 1000 mM NaCl, pH 7.0). (B) Photograph of polyelectrolyte complex after centrifugal separation showing the formation of a coacervate phase from the coalescence of droplets. (C) Photograph of the adhesive and liquid nature of a ternary coacervate sample stretched between the two plates of the rheometer.

confirm the fluid nature of the complex. Vortexing the polyelectrolyte mixtures after centrifugation also helped distinguish complex coacervates from precipitates, since precipitates redisperse into the solution, whereas coacervates remain coalesced as a separate, viscous fluid phase.

Note that the effects of stoichiometry, salt, and pH on coacervate formation as well as the rheological properties of coacervates (e.g., viscoelasticity, viscosity) have been described previously for binary systems of polyelectrolytes.[5,30−32] In the following sections, we describe how changes in these external conditions affect the formation and properties (e.g., rheological) of ternary coacervate complexes. We identify the existence of a maximum salt concentration for which coacervation is observed, which we interpret by calculating the binodal curves using the Voorn−Overbeek model;[49] the

effect of pH value is explained by calculating the charge density explicitly using the appropriate pK_a values.

3.1. Effect of Polyelectrolyte Mixing Ratio on Complex Formation. The effect of polyelectrolyte mixing ratio on complex formation was studied with the use of turbidity. This technique has been used extensively,[5,29,32] mainly due to its simplicity. The measured turbidity of two ternary systems, as a function of polyelectrolyte mixing ratios, is depicted in Figure 2 (A1 and B1). The three axes in the ternary diagrams represent the amount of polyelectrolyte charged sites (in mol %) used for mixing, while the extent of complex formation (as indicated by turbidity) is described with the use of different colors. Blue and red describe minimum and maximum complex formation, respectively. We consider a turbidity signal of >0.1 to indicate the presence of complex formation.

Careful examination of both ternary graphs shows that polyelectrolyte complexes form over a wide range of compositional ratios. For the PAA/PDMAEMA/PAH system, complexes formed over the range of 5−90 PAA mol % acid groups with 1−90 PAH mol % and 1−80 PDMAEMA mol % base groups (Figure 2A). Utilizing PEI instead of PAH shifts this range to 10−85 mol % PAA with 1−90 PEI mol % and 1−80 PDMAEMA mol % (Figure 2B). The variation in these limits between the two systems is related to the different charge densities of the polyelectrolytes, as will be discussed in more detail later.

It is also interesting to note that the range of compositions over which polyelectrolyte complexes formed in these ternary systems were significantly broader than would be suggested by
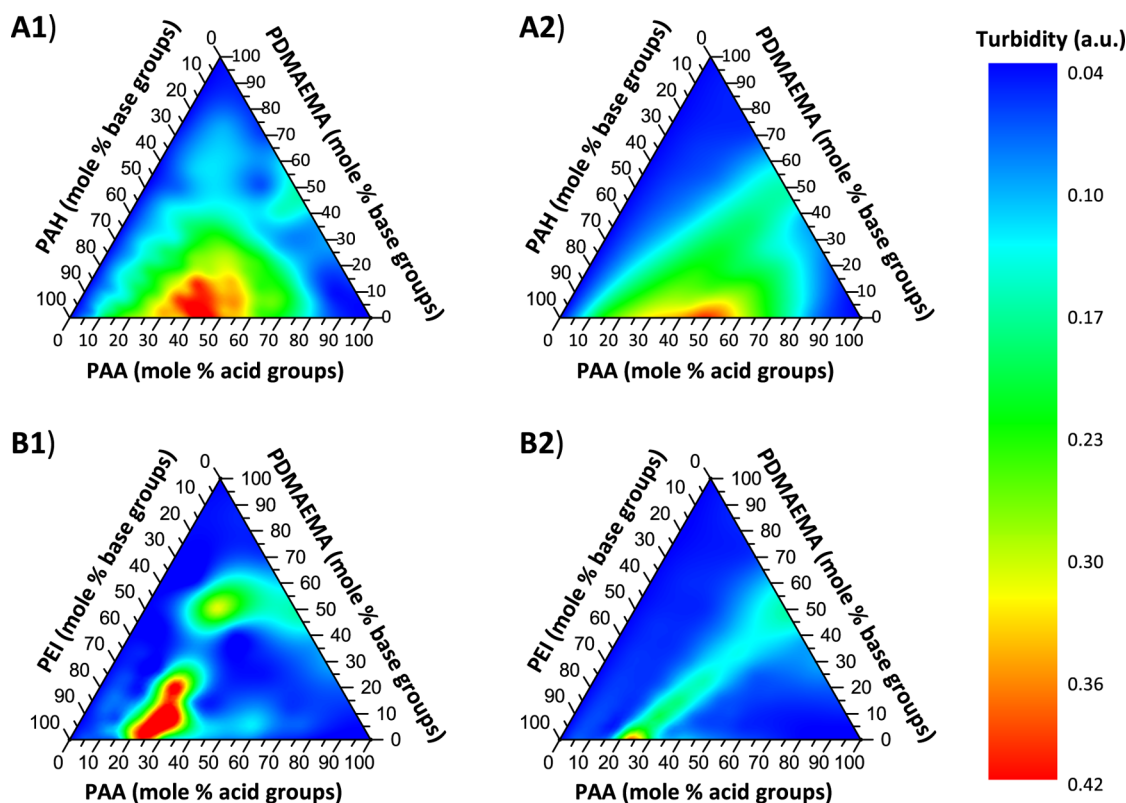

Figure 2. Plots of turbidity as a function of composition for (A) PAA/PDMAEMA/PAH and (B) PAA/PDMAEMA/PEI mixtures as a function of acid/base groups (mol %). Samples were prepared with a total polymer concentration of 0.1 wt %, pH 7.0, and no added salt. Experimental data are shown in (A1) and (B1). (A2) and (B2) show predicted turbidity diagrams calculated by linear interpolation of the individual binary turbidity data.

Table 1. ζ-Potential of Ternary Coacervates Prepared with Different Mixing Ratios[a]

ratio (mol %)	sample	ζ-potential (mV)	ratio (mol %)	sample	ζ-potential (mV)
29/15/56	PAA/PDMAEMA/PEI	55.3	33/17/50	PAA/PDMAEMA/PAH	75.0
37/10/53	PAA/PDMAEMA/PEI	−37.9	41/12/47	PAA/PDMAEMA/PAH	51.5
43/06/51	PAA/PDMAEMA/PEI	−48.2	50/07/43	PAA/PDMAEMA/PAH	−53.4
49/02/49	PAA/PDMAEMA/PEI	−53.2	54/06/41	PAA/PDMAEMA/PAH	−72.6

[a]Samples were prepared at pH 7.0, no added salt, and a total polymer concentration of 0.005 wt %. The data are average of at least three measurements.

the various individual binary systems (data on the axes are from binary systems). This is indicated in Figure 2 (graphs A2 and B2) where linear interpolation of measured turbidity data was used to predict the ternary turbidity diagrams of the individual binary systems (i.e., PAA/PAH, PAA/PEI, PAA/PDMAEMA). Furthermore, the predicted turbidity diagrams based on linear interpolation were unable to capture the extent of complex formation, suggesting that synergistic rather than merely additive interactions are present between the various polymers.

In addition to the range of compositions over which complexation can occur, the area of maximum complex formation is different for the two ternary systems. In electrostatic systems, the extent of complexation is linked to the relative amounts of oppositely charged groups (i.e., COO^- and NH_3^+) present in the solution.[5,32] A maximum is observed when a 50/50 mol % acid/base ratio (charge neutralization ratio) is used. Turbidity data in Figure 2A1 show that maximum complex formation, in the PAA/PDMAEMA/PAH system, was indeed observed when a mol % acid/base ratio close to 50/50 was used. However, in the case of the PAA/PDAMEMA/PEI system (Figure 2B1), maximal complex formation was observed near a 30/70 mol % acid/base ratio, which is different from the ratio of the previous cases.

The explanation for this behavior comes from the nature of PEI and the assumption that all acid or base groups are charged when calculating the molar ratios. As mentioned earlier, the amount of complex formed depends on the number of charged sites that are available to interact. Branched PEI has three types of charged groups (primary, secondary, and tertiary amines), each with three different pK_a values.[33,34] The different degrees of ionization of each of these groups at pH 7.0 means that only a fraction of the amine groups are in a charged state and available for complexation. If the possible inaccessibility of some charged groups due to steric hindrance effects is also taken into account, the shift of the charge neutralization ratio to lower values can easily be understood. Similar behavior has been described for binary coacervate systems where branched PEI was used as the polycation.[21]

Interestingly, the type of complex formed (i.e., precipitate or coacervate) varies significantly between ternary and binary systems as a function of polyelectrolyte ratio. Polyelectrolyte complexes can either be solid-like or liquid-like, indicating whether they are in a kinetically trapped state or at equilibrium, respectively. Earlier studies of PAA/PAH and PAA/PDMAE-MA binary systems have described the formation of precipitate complexes when polymers were mixed at charged-matched stoichiometries in the absence of salt.[5,29,32] In the ternary systems described here, the formation of coacervates without salt was not limited to cases in which a 50:50 mol % acid/base ratio was used and is a much broader phenomenon compared to that observed in the binary examples provided above. Coacervates were formed in PAA/PDMAEMA/PAH and PAA/PDMAEMA/PEI systems with polyanion contents as

low as 7 mol % acid groups (see images in Supporting Information, Figure S2).

A thermodynamic−kinetic description of polyelectrolyte complex formation could help to better understand the reasons for this behavior. Earlier work has shown that a reasonable description of polyelectrolyte complex formation involves two steps.[38] The first step is the formation of initial water-soluble complexes among small numbers of macromolecules, which is entropically driven (from the release of counterions) and is the dominant driving force of complexation. The second step involves the rearrangement of the initial complexes into a coacervate phase, resulting from exchange reactions between polymer chains within the complex or with other free chains in the solution.[35,36] The driving force for this step is an increase in configurational entropy, which is much smaller than the driving force of the first step. The rate of polyelectrolyte exchange reactions has been related to the nature of the polyelectrolytes.[37,38] Charge density, hydrophobicity, or significant chain length variations can all affect polyelectrolyte exchange rate, as can external parameters such as the ionic strength or pH of the mixture. It appears that the existence of a third polymer in the mixture,[38] combined with the experimental conditions and the nature of the polymers, favors this type of exchange reaction, thereby preventing irreversible precipitation.

The ζ-potential measurements bring detailed insight into the causes of dispersion, aggregation, or flocculation between charged molecules and have therefore been used to study ternary polyelectrolyte complexes. A series of complex coacervate mixtures prepared with different mixing ratios at low polymer concentrations were measured, and the results are summarized in Table 1. Coacervates here have either negatively or positively charged surfaces. The sign of the ζ-potential corresponds directly with the composition of charged acid/base groups of the coacervate. In the presence of excess charged amine or carboxylic acid groups the charge is positive or negative, respectively. The ζ-potentials approached zero at stoichiometric ratios where maximal turbidity readings were observed (around 30/70 mol % acid/base ratio), corresponding to conditions of overall charge neutrality. As discussed earlier for the case of maximum turbidity, these ratios are different from the expected 50:50 mixing ratio due to difference between the number of amine groups used to calculate the mixing ratio and the ones that are actually in a charged state at the pH of measurement. As the mixing ratio between acid/base groups was moved further away from this range, the charge balance also moved away from the stoichiometric mass ratio, leading to a decrease in complex formation (as seen by turbidity) and an increase in surface charge (as seen by ζ-potential).

It is worth noting here the difference in the polymer concentration between samples prepared for turbidity and ζ-potential measurements. In the latter, very low concentrations (0.005 wt %) were used in order to make an accurate measurement. A change like this will affect the absolute value of

ζ-potential, which relates to the droplet stability (i.e., higher concentration gives smaller ζ-potential value and therefore more droplet coalescence). At the same time changes in surface charge (i.e., sign, decrease or increase of the value) are not related to the concentration and can be used to discuss the effect of external parameters such as ratio, pH, or salt.

Having studied the molecular properties of coacervates, we also sought to determine the effect of composition on the bulk rheological properties. In order to investigate the effect of mixing stoichiometry on the structure and rheological behavior of ternary coacervates, we prepared complexes with various mixing ratios at different salt concentrations. Frequency scans demonstrated that all ternary complex coacervates exhibit a typical viscoelastic behavior (Figure 3) also observed in coacervates made of other polyelectrolytes,[21] including the binary PAA/PDMAEMA system.[20]
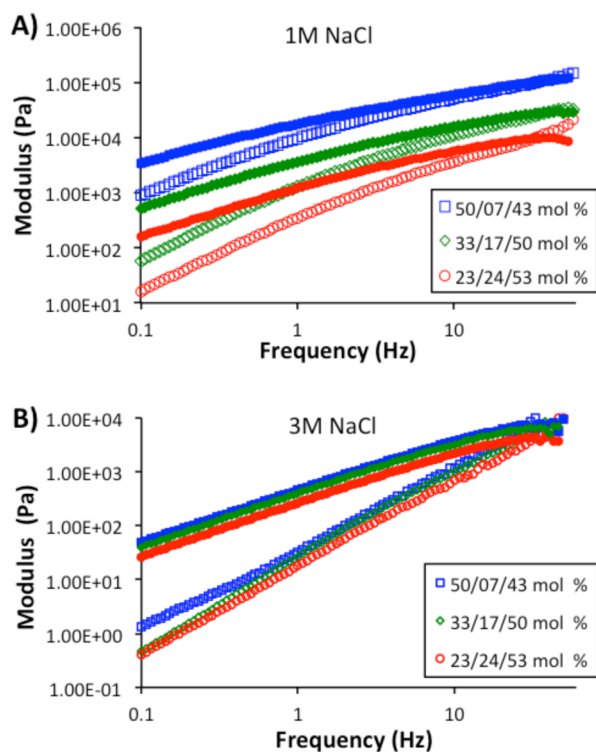

Figure 3. Frequency sweeps showing loss (G'', filled data point) and storage moduli (G', open data points) of PAA/PDMAEMA/PAH at a pH 7.0 at a total polymer concentration 0.8 wt % and an applied stress of 10 Pa for coacervates prepared with different polymer ratios at (A) 1 M NaCl and (B) 3 M NaCl.

Particularly in the case of PAA/PDMAEMA, previous studies showed that the linear viscoelastic properties of these coacervates are not affected by the overall stoichiometry (molar ratio of polyanion and polycation chains), as the excess polyanion or polycation chains end up in the dilute phase and the coacervate phase maintains a constant "preferred" composition (50:50 mol % charge ratio).[39] However, this system was studied only at one salt concentration (0.7 M). We extended our study to a range of stoichiometries and salt concentrations (Figure 3B). We found that at high salt concentrations (3 M NaCl) both storage and loss moduli of ternary PAA/PDMAEMA/PAH and PAA/PDMAEMA/PEI coacervates are effectively unaffected by changes in the stoichiometry (curves overlap as seen in Figure 3B), in

agreement with what was described earlier for binary PAA/PDMAEMA coacervates. Interestingly, at lower salt concentrations (e.g., 1 M NaCl, shown in Figure 3A) polymer-mixing ratio appears to have a significant effect on the rheological properties of the coacervates. In fact, a change of 2 orders of magnitude in the coacervate phase moduli (storage and loss) was observed when the system stoichiometry was changed. The highest moduli values were observed when a charge-balanced system was used. The weak dependence of the viscoelastic coacervate properties to changes in composition, when high salt concentrations are used, can be related to the small variations in polymer concentration of the coacervate phase, leading to similar rheological response of the materials. At the same time, the increase in the salt concentration induces a decrease in the polymer concentration of the coacervate phase, leading to more "liquid-like" coacervates with different rheological properties.

3.2. Effect of Salt Concentration on Complex Formation. Formation of electrostatic complexes can fundamentally be affected by changes in parameters such as pH or ionic strength. To test the effect of ionic strength on the formation of ternary coacervates, we prepared a series of mixtures at different salt concentrations (Figure 4). An
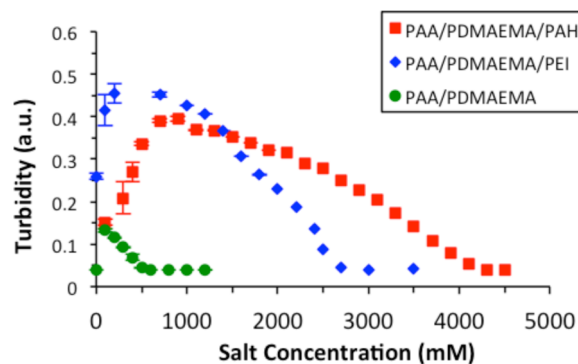

Figure 4. Turbidity as a function of salt concentration for both binary and ternary systems (pH 7.0, polymer ratios 41/12/47, 37/10/53, and 40/60 acid/base group mol %, total polymer concentration 0.1 wt %).

examination by turbidity showed that the addition of small amounts of salt leads to an increase in coacervation in all systems (binary and ternary), while the use of higher salt concentrations leads to a decrease in complex formation. Initial increases in turbidity at low salt concentration are thought to be the result of enhanced coacervation by promoting polymer solubility.[40] However, higher salt concentrations destabilize complex formation by decreasing the entropic driving force for complexation.[6,7] The behavior of both ternary systems (red squares and blue diamonds) is similar to what has been reported for various binary systems,[5,29,32] including the PAA/PDMAEMA system (green circles).

At sufficiently high salt concentrations, turbidity disappears completely and phase separation does not occur. The coacervate−solution boundary is defined by the salt concentration, above which the formation of a polyelectrolyte complex coacervate cannot be measured with the use of turbidity and microscopy anymore, and a single solution phase is always physically observed. This boundary appears to vary significantly between the binary and ternary systems presented in Figure 4, with the former occurring at much lower salt concentrations. The addition of a third polyelectrolyte (i.e., PEI or PAH) to the base PAA/PDMAEMA system leads to liquid complexes

(coacervates) with an enhanced resistance to salt. The variation in the salt concentration of the coacervate–solution boundary between the two ternary systems can be related to the different the nature of the polymers, including differences in the charge density due to branching and the different pK_a values of the polyelectrolytes (as will be discussed in a later section).

Besides its effect on coacervate yield, salt also changes the rheological properties of the complexes. An increase in salt concentration from 0 to 3 M NaCl decreased the loss modulus of coacervates by 2 orders of magnitude (Figure 5A). Similar behavior was observed in both ternary systems for all mixing ratios. As discussed earlier, increasing the concentration of salt
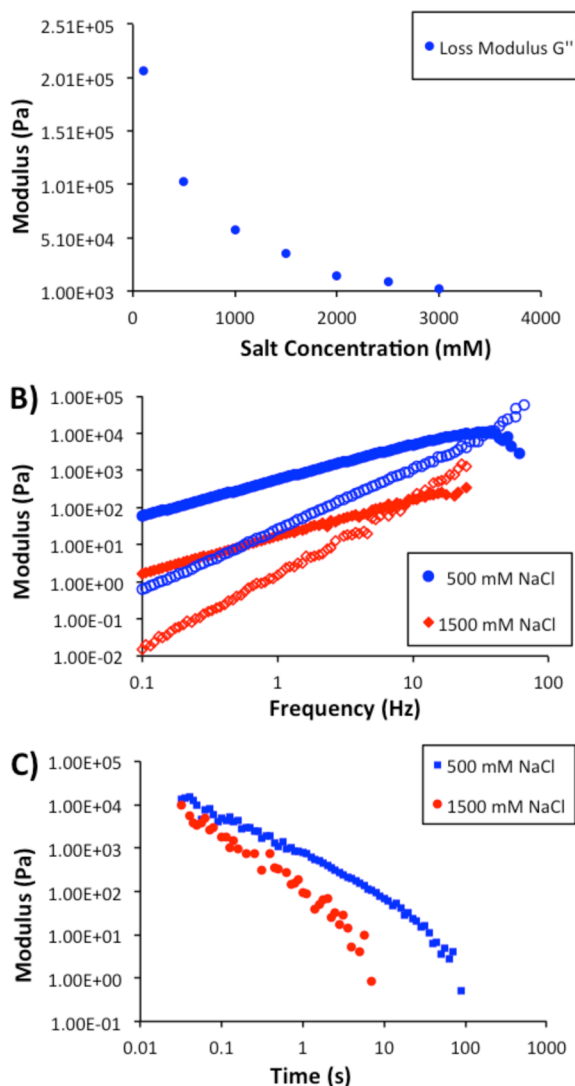

Figure 5. (A) Rheological analysis of the loss modulus for PAA/PDMAEMA/PAH, as a function of salt concentration (pH 7.0, polymer ratio 50/07/43 acid/base group mol %, total polymer concentration 0.8 wt %, stress 10 Pa, angular frequency used 1 s^{-1}). (B) Frequency sweeps showing loss (G'', filled data point) and storage moduli (G', open data points) for PAA/PDMAEMA/PEI coacervates prepared at two different salt concentrations (pH 7.0, 37/10/53 acid/base group mol %, total polymer concentration 0.8 wt %, stress 10 Pa). (C) Stress relaxation modulus of PAA/PDMAEMA/PAH (pH 7.0, polymer ratio 41/12/47 acid/base group mol %, total polymer concentration 0.8 wt %, stress 10 Pa) coacervates at different salt concentrations.

leads to changes in the polymer density of the coacervate phase. Specifically, polymer concentration in the equilibrium solution is increased at the expense of the concentration in the coacervate phase,[29] and a looser polyelectrolyte network (i.e., coacervate phase) is formed. Therefore, coacervates prepared at higher salt concentrations exhibit different rheological behavior (i.e., lower viscosities).

The viscous (G'') and elastic (G') moduli of PAA/PDMAEMA/PEI coacervates, formed with various salt concentrations, are presented in Figure 5B as a function of frequency. All coacervates exhibited a viscoelastic behavior indicated by a crossover point observed between the viscous and elastic moduli. The viscoelastic behavior of the ternary coacervates was found to be dependent on the salt concentration (seen from the shift of the crossover point of the curves in Figure 5A and Figure S2). This behavior is in line with results on binary complexes of oppositely charged biological[15] or synthetic macromolecules[20] and other soft materials.[41] Consistent with increases in the critical salt concentration, changes in ternary coacervate viscoelasticity were observed at much higher salt concentration compared to binary systems.

Figure 5C shows the relaxation modulus of PAA/PDMAEMA/PAH ternary complex coacervates as a function of time at different salt concentrations, below the critical salt concentration. A careful look at the curves showed a continuous decrease of the modulus at all time scales and until the terminal relaxation time was reached. While the shape of the curves did not seem to be affected by changes in salt concentration, the terminal relaxation time increased with increasing salt concentration. This observed relaxation behavior of ternary coacervates is consistent with the reported behavior of PAA/PDMAEMA binary coacervate systems, where relaxation of stress occurs via "sticky" Zimm and Rouse modes of the chains hindered by the ionic bonds of a chain with oppositely charged neighboring chains.[19] Since salt affects the bond energy of all ionic bonds, relaxation modes are shifted when salt is added.

A noticeable difference between binary and ternary systems (using polymers of similar length) was that for a similar shift of the curves to lower relaxation times, a much higher salt concentration is required in the case of ternary coacervates. This is in agreement with the observed increase in the critical salt concentration in ternary systems. An explanation for this behavior could be found in the different complexation ability of the polyelectrolytes used due to various charge densities or structure.

The effect of salt type on ternary coacervate moduli was also investigated. Coacervates prepared with different concentrations of a multivalent salt (i.e., Na_2SO_4) showed the same viscoelastic behavior as NaCl, with both moduli decreasing as the salt concentration is increased (Figure S4). It appears that difference in the ionic strength between the two salts does not significantly change the rheological behavior of these coacervates.

The rheological measurements described above were all performed on the coacervate phase after centrifugation and careful removal of the dilute aqueous phase. Earlier, we discussed how complex coacervates are initially observed in the form of polyelectrolyte-rich fluid droplets (Figure 1A). To study a possible effect of salt on coacervate droplets, we used ζ-potential measurements. In the example presented in Table 2 (other examples available in the Supporting Information, Table S1) we can see that the ζ-potential values of ternary PAA/

Table 2. ζ-Potential of PAA/PDMAEMA/PEI Ternary Coacervates Prepared at Different Salt Concentrations[a]

NaCl concentration (mM)	ζ-potential (mV)
0	55.3
500	43.1
1000	32.7
1500	27.7

[a]Samples were prepared at pH 7.0, a polymer ratio 29/15/56 acid/base group mol %, and a total polymer concentration 0.005 wt %. The data are the average of at least three measurements.

PDMAEMA/PEI coacervates prepared with different salt concentrations decrease with increasing salt concentration. Higher salt concentrations lead to an increased screening effect on the charges and therefore lower ζ-potential values since fewer charged groups exist on the surface.

3.3. Effect of pH on Complex Formation. Complex coacervates are mainly formed in the presence of moderate amounts of salt or when two oppositely charged macro-molecules with low charge densities are mixed together.[42−44] This indicates how important both ionic strength and charge densities are for the formation and properties of coacervates. The effect of pH on electrostatic self-assembly of weak or strong polyelectrolytes has also been highlighted in layer-by-layer studies.[45,46] Generally, in polyelectrolyte multilayer construction pH has an effect on the thickness of the multilayer. For example, in the case of the PAA/PAH system a significant change in multilayer growth was observed when the charge density of the weak polyelectrolytes decreased from a fully charged state to 70−90% charge units due to change in pH.[47]

In the previous section, we described the impact of salt and charge screening on coacervation. Here, we will discuss how the nature of the polyelectrolyte charge can have a similar effect. At this point, it is important to note that all polyelectrolytes used in this study are weak polyelectrolytes, meaning that the number of charged moieties on each polymer chain depends on the pH of the system. A weak polycation is fully charged at high pH and uncharged at low pH, while a polyanion has the opposite behavior. The exact pH where a weak polyelectrolyte is half charged (i.e., its pK_a value) is related to the nature of the polymer.

In the set of comparative experiments presented in Figure 7A, ternary coacervates were prepared by using polyelectrolyte solutions at different pH conditions. As shown, turbidity values obtained at the lower pH were higher for all salt concentrations. Furthermore, the coacervate−solution boundary was shifted to lower salt concentrations for higher pH. Similar trends were present in both ternary systems. These findings can be explained based on the changes in polyelectrolyte charge density as a function of pH. At the range of pH values studied, the charge density of the polyanion is barely influenced, since the pK_a of PAA is approximately 4.2.[32] Contrarily, at the same range of pH, the ability of both PDMAEMA to interact with oppositely charged polymers is significantly affected as we approach its pK_a value (around 8.5).[29] PEI is affected more than any other polymer in the system from the change in pH due to its highly branched nature. As mentioned earlier, PEI has three types of charged groups with various pK_a values at 5.0, 7.5−8.5, and 10.5. Thus, as the pH of the mixture is increased from 6 to 8, fewer ionic groups ($-NH_3^+$) are available to interact. Fewer charged groups means fewer attractive electro-
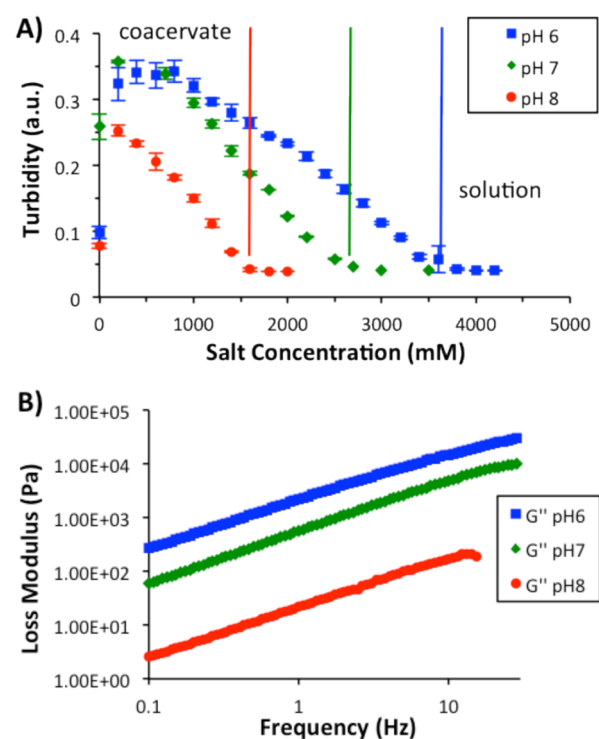

Figure 7. (A) Turbidity of PAA/PDMAEMA/PEI mixtures at different pH, as a function of salt concentration (polymer ratio 36/10/54 acid/base group mol %, total polymer concentration 0.1 wt %) and (B) frequency sweeps showing loss modulus (G'') of PAA/PDMAEMA/PEI coacervates prepared at different pH (polymer ratio 37/10/53 acid/base group mol %, salt concentration 500 mM NaCl, stress 10 Pa).

static interactions, and formation of significantly less complex, as verified by the reduced turbidity observed in Figure 7A. Furthermore, reduced electrostatic attraction leads to a looser polymer network (coacervate) that requires less salt to disassemble, explaining the shift in the critical salt concentration.

Rheological measurements showed that both the loss and storage moduli of the ternary coacervates are highly pH dependent. In Figure 7B, the loss modulus of PAA/PDMAEMA/PEI coacervates prepared at different pH is presented as a function of frequency. The highest modulus was observed at the lowest pH value where the polycation is fully charged, as seen in earlier studies, too.[15] Increasing the pH decreases the number of charged groups and lowers the loss modulus, which describes the viscous character of the coacervates. As discussed earlier, at pH 6, the highest number of oppositely charged sites ($-NH_3^+$ and $-COO^-$) are available to interact leading to higher electrostatic attraction and denser polymer networks with higher viscosity.

To study the effect of pH on ternary coacervates, we also used ζ-potential measurements. Table 3 shows how a change in pH from 6 to 8 influences the surface charge of the coacervate droplets in the solution. An increase in pH causes a decrease in charge density, leading to weaker attractive interactions and a change in the sign of ζ-potential at pH 8. For the sample used in these experiments, at pH 6.0, where all of the ionic groups are fully ionized, there is an excess of $-NH_3^+$ groups, corresponding to the positive ζ-potential. However, as the pH of the sample increases, fewer and fewer $-NH_3^+$ groups are charged ($pK_a = 8.5$), leading to a situation where a crossover

Table 3. ζ-Potential of Ternary Coacervates Prepared at Different pH Conditions[a]

sample	pH	ζ-potential (mV)
PAA/PDMAEMA/PEI	6.0	61.4
PAA/PDMAEMA/PEI	7.0	55.3
PAA/PDMAEMA/PEI	8.0	−12.9

[a]Samples were prepared at 29/15/56 acid/base groups mol % mixing ratio for PAA/PDAMAEMA/PEI, no salt, total polymer concentration 0.005 wt %; data presented in table are average of at least three measurements.

occurs (i.e., the sample is net neutral), and eventually we observe an excess of −COO⁻ groups at pH 8.

3.4. Theoretical Considerations. The traditional Voorn−Overbeek model for binary polyelectrolytes can be extended to ternary systems to rationalize the variation of maximum salt concentration observed experimentally for all three systems considered here. It represents one of the simplest models that can be used to describe the polyelectrolyte coacervation[49] and has been successfully applied in the past to interpret experimental data for binary systems.[29] Within this model, the correlation free energy of a polyelectrolyte solution in the presence of salts or counterions can be written as a sum of the mixing entropy term and the Debye−Hückel correlation free energy:

$$f = \sum_{i=1}^{c} \frac{\phi_i}{N_i} \ln(\phi_i) + \psi \ln(\psi) + \left(1 - \sum_{i=1}^{c} \phi_i - \psi\right)$$
$$\times \ln\left(1 - \sum_{i=1}^{c} \phi_i - \psi\right) - \alpha\left(\sum_{i=1}^{c} \sigma_i \phi_i + \psi\right)^{3/2}$$

Theoretically, for a given pH value, the maximum salt concentration is identified by the salt concentration at the binodal curve corresponding to the polymer concentration (0.1 wt %; since polymer density is comparable to that of water, 0.1% can also be used as the volume fraction). The binodal curves are calculated by varying both salt concentration ψ and total polymer concentration ϕ to balance the chemical potential. The binodal curves calculated at different pH values for the binary PAA/PDMAEMA system are shown in Figure 8A. Since the average charge density σ decreases with pH value, the window of two-phase regime becomes smaller as pH increases. The values of salt concentration corresponding to 0.1 wt % of polymer volume fraction as a function of pH are plotted in Figure 8B and are compared to experimental results obtained using turbidity measurements (Figure 7A). To obtain the results in Figure 8B, we used the parameter values tabulated in Table S3 and used the average chain length N_{ave} and the average charge density σ_{ave}. The only adjustable parameter in our calculations is the maximum charge density. We reduced the maximum charge density of PAH by 2% and that of PEI by 23%, so that σ_{max}(PAH) and σ_{max}(PEI) decrease to 0.31 and 0.34, respectively. This charge regulation is necessary because the salt ions are preferentially attracted to the chains with higher value of charge density and screen the electrostatic interactions.[50]

Given the fact that the Voorn−Overbeek model is only a first approximation to the true behavior of coacervates and given that only one adjustable parameter (maximum charge density) is used to generate the predictions shown in Figure 8B, the level of agreement between experimental results and theoretical predictions is satisfactory. Further analysis of the predictions of
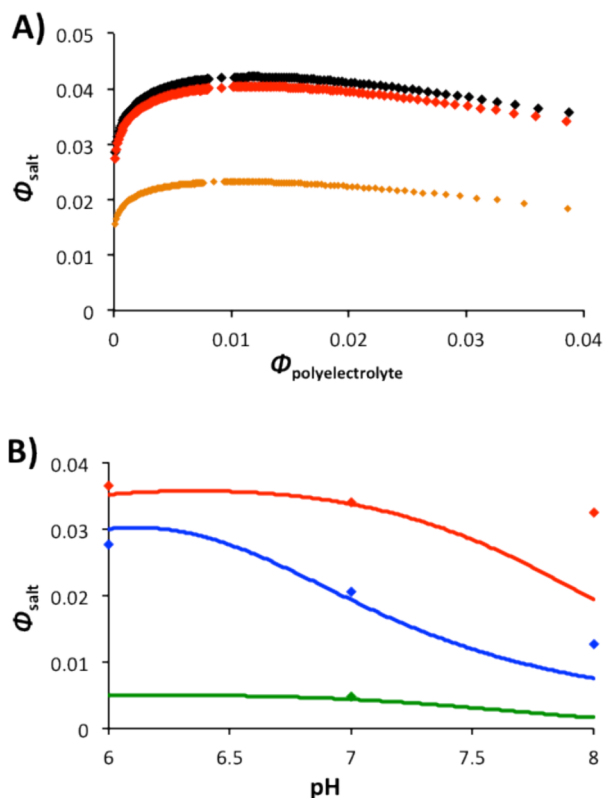

Figure 8. (A) Binodal curves for ternary system PAA/PDMAEMA/PAH at different pH values (black: pH 6; red: pH 7; orange: pH 8) and (B) dependence of the maximum salt concentration on the pH value for the three systems. Curves are theoretical predictions. Diamonds are experimental data points from turbidity measurements (red: PAA/PDMAEA/PAH; blue: PAA/PDMAEMA/PEI; green: PAA/PDMAEMA; experimental conditions are the same as in Figures 4 and 7A).

the model shows that, for a given charge density, the binodal curve shifts toward the high-salt concentration side with increasing N and saturates according to a $N^{-1/2}$ scaling law toward a finite limit; the limiting salt concentration only depends on the charge density. So for sufficiently long chains, the maximum salt concentration is mainly determined by the effective charge density of the polyelectrolytes. As the effective charge density increases, the maximum salt concentration also increases. This is the main reason why the critical salt concentration increased in the two ternary systems studied here as compared to the binary system: the third components, PAH and PEI, both have much higher charge densities (see Table S3).

4. CONCLUSIONS

In this work, we investigated the assembly of three synthetic polyelectrolytes and showed that complex coacervates form when a second polycation (PAH or branched PEI) is added to the binary PAA/PDMAEMA system. Ternary coacervates maintain the characteristics and tunability of typical binary coacervates. However, the third component can significantly change their response to changes in external stimuli or their properties due to synergistic interactions between the three polymers, rather than simple additive contributions. For example, ternary coacervates can form over a broader range of ratios compared to the binary systems and exhibit an enhanced resistance to salt. This behavior can be beneficial in

situations where environmental parameters such as the salt concentration cannot be controlled. These variations were not limited between binary and ternary systems but were also observed between the two ternary systems studied, indicating the importance of the choice of polymers.

Our studies demonstrated that salt and pH also affect the structure and the rheological properties (e.g., relaxation time, viscoelasticity) of ternary coacervates, mainly due to changes in the charge density. Noticeably, a much higher salt concentration is required to produce an equivalent shift of the relaxation curves to lower relaxation times for ternary coacervates compared to binary due to their highly packed polymer network. Furthermore, increases in the critical salt concentration for complex formation were described using the Voorn–Overbeek model. The two added components, PAH and PEI, both have much higher charge densities, leading to an increase in the resistance of the coacervate phase to higher concentrations of salt. Similar trends were observed as a function of pH and also correlate with changes in the charge density. This suggests that increasing the charge density improves the stability of the coacervate phase with respect to salt addition and pH.

As multicomponent complexes composed of polyelectrolytes, biomacromolecules, or other charged species (e.g., DNA and RNA) become increasingly important in materials and biomaterials research, the ability to understand the complexation process of multicomponent mixtures and control the structure and properties of the resulting material will provide further means to enhance their functionality.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Effect of mixing ratio on binary coacervates, optical microscopy images of ternary coacervates at various ratios, frequency sweeps of ternary coacervates with different salt concentrations and salts and pH, ζ-potentials of ternary coacervates with different salt and pH, theoretical considerations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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