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Phase Behavior and Coacervation of Aqueous Poly(acrylic acid)−Poly(allylamine) Solutions

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ABSTRACT: Phase separation and coacervate complex formation of poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) were investigated as model pair of oppositely charged, weak polyelectrolytes in aqueous solution. Both fully or partially neutralized PAA (sodium polyacrylate) and PAH were employed. Important factors affecting the complexation were systematically varied including the polyacid/polybase mixing ratio (10−90 wt %), ionic strength as salt concentration (0−4700 mM), polymer concentration (0.02−2.0 wt %), pH (5 and 7), and temperature (30−75 °C). Sample turbidity was utilized as an indicator of polyelectrolyte complex formation. Phase separation in the solution was also observed by optical microscopy in the distinguishable forms of either precipitate or coacervate. In the absence of salt, polyelectrolyte complexation always resulted in the formation of a precipitate. In the presence of sodium chloride, complex formation does not take place (neither precipitate nor coacervate) when either polyelectrolyte is present in large excess. Increasing salt concentration causes a change from solid precipitate to fluid coacervate phase, and finally a one-phase polyelectrolyte solution is obtained. Temperature affected the precipitate-to-solution transition only in the case of samples with low concentrations of either PAA or PAH. The data generated led to the construction of phase diagrams that illustrate how the various parameters control the demixing and the precipitate−coacervate−solution phase transitions. We find such phase diagrams for simple, flexible synthetic macromolecular systems to be rare in the polymer science literature. Ternary phase diagrams were prepared, which showed the influence of relative polymer and salt concentration on the phase behavior of the aqueous PAA/PAH system. We believe data such as these will both improve both the reliable applications of polymer coacervates and the development of new macromolecular assemblies based on charge complexation.

1. Introduction

Complex coacervation (formation of a fluid complex between oppositely charged polymers) is a well-known and widely used, but little understood, phenomenon in physical polymer science. This paper aims to establish better the physical chemistry of complex coacervation via experiments on well-defined polymer systems.

Polyelectrolyte complexes (PEC) may result on mixing oppositely charged polyelectrolytes in aqueous solution. The electrostatic interaction is a driving force to form a complex between the polyanion and the polycation. The occurrence of PECs often goes along with a phase separation, resulting either in two liquid phases or in a precipitate. The polymer-rich liquid phase which may appear as droplets is called a coacervate and dispersed in the polymer-deficient aqueous phase. The physics of such charge complexation is leading to new routes for the self-assembly of interesting macromolecular structures.[1,2]

The phenomenon of polyelectrolyte complexes has been first observed and described by Bungenberg de Jong[3] in connection with studies on the phase behavior of water-soluble natural polymers such as gelatin (weakly positively charged) and arabic gum (weakly negatively charged). The complex formation is dependent on many factors, among others molecular weights, polymer concentration, ratio of the two interacting polyelectrolytes, ionic strength and pH of the solution, and temperature. This was already recognized in the early work, and also the implications of coacervate formation in biology, as well as for for the utility of coacervation in many technologies.[3] In this context, it is worth noting recent studies on complex biological macromolecular self-assembled systems such as amphiphile and protein/DNA complexes[4−6] and PECs for microencapsulation or as membranes to control drug, enzyme, or flavor delivery in fields such as medicine, pharmaceuticals, food, cosmetics, papers, and textiles.[7,9−11]

Theoretical treatment of complex coacervation of solutions of biocolloids and of synthetic polyelectrolytes followed shortly[12] after Bungenberg de Jong's pioneering studies.[3] These early approaches and extensions coupled Flory−Huggins theory with an additive Debye−Hückel electrostatic term, implicitly assuming that the macroions could be treated as well-separated, high-valence, pointlike particles. Such an assumption is clearly invalid in the coacervate phase, which is locally of high polymer concentration beyond c^*. A more modern approach, based on the random phase approximation (RPA), accounts for the extended nature of polyelectrolytes and the coupling between conformational and electrostatic degrees of freedom in a mixed

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polyelectrolyte solution.[13−15] These theories are, however, restricted to weakly charged polyelectrolytes at modest salt concentrations where local details of ion size and hydration are relatively unimportant. Similar approaches have been used to study pH effects on charged colloids.[16] Most recently, "field-theoretic" simulations have been used to examine the conditions under which oppositely charged polymers produce complex coacervates.[18,19]

The effects of pH and ionic strength on coacervate complex formation have been particularly well studied for the gelatin/acacia[20] and other biopolymer polyelectrolyte pairs[21−24] as well as synthetic polyelectrolyte pairs.[25−30] One result common to all studies is that high salt concentration (ionic strength) inhibits complex coacervation; in other words, coacervates dissolve upon salt addition. The salt effect is explained by the screening effect of the added salt on the ionic groups of the polymers. However, for systems containing strong polyelectrolytes, adding of excess salt may also cause secondary aggregation.[29]

One important aspect of coacervate formation, relevant to modern polymer science, is its relationship to the construction of polyelectrolyte multilayer films by sequential adsorbed layers of weak polyelectrolytes.[31] It has been established that polymer and salt concentration as well as pH affect the structure and composition of the multilayer composite sheet,[32−35] similarly as discussed for bulk coacervate formation, and common features have been specifically addressed.[36] Layer-by-layer growth is an arrested, nonequilibrium form of PEC.

In the context of polyacid/polybase polyelectrolyte complexes, the use of acid−base interactions to overcome the free energy barrier to the mixing of rods and coils, originating primarily from the reduced entropy of mixing in the presence of rods, has to be mentioned, too. The mixing of solutions of rigid-rod macromolecules with random coil macromolecules of opposite charge gives molecular polymer−polymer composites upon precipitation or film casting from solution. Introducing a negative χ-parameter into the original Flory theory[37] was used to model the experiments.[38]

Although much information on coacervate complex formation is found in the literature, a comprehensive picture and more general quantitative understanding are still missing. The reported data thus far have been obtained with several different, and usually structurally complex, polyelectrolyte pairs. Therefore, the motivation of the present study is to generate a complete and complementary set of data on coacervate phenomena by investigating the phase behavior of a structurally simple, weak polyelectrolyte model pair under various experimental conditions.

Poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH), which have identical polymer backbones and differ only in the ionic side group, were chosen as model polymers for PEC formation. The chosen PAA and PAH macromolecules were of comparable overall chain length. The complex formation was studied as a function of varying concentration and mixing ratio of the oppositely charged polymers, the ionic strength and pH of the aqueous solution, and the temperature.

2. Experimental Section

2.1. Materials. Poly(acrylic acid) (PAA) was purchased from Polyscience as 25 wt % solution in water. The catalog specification of molecular weight $M_w = 50\,000$ kg/mol, $M_w/M_n = 2.9$, gives a weight-average degree of polymerization $P_w = 696$. Poly(allylamine hydrochloride) (PAH; molecular weight $M_w = 70\,000$ kg/mol; $P_w \sim 750$) was purchased from Sigma-Aldrich. The polymers were used as received without further purification.

2.2. Preparation of the Mixtures. 2 wt % stock solutions of the polyelectrolytes were prepared with deionized Milli-Q water. The pH was adjusted with either NaOH or HCl. For the fully neutralized aqueous PAA (poly(acrylic acid sodium salt; PAANa) stock solution, the pH was adjusted with 0.1 NaOH from initial pH ~ 2.3 to alkaline pH ~ 8.5. The aqueous PAH stock solution (fully neutralized) had pH ~ 3.0. For the partially neutralized systems, the stock solutions of each PAA and PAH were adjusted to pH 7.0.

Polyelectrolyte complexes (PECs) systems of different polyion mixing ratios (from 1/9 to 9/1 w/w) and various total polyelectrolyte concentrations (0.02−0.20 wt %) were prepared by sequentially pipetting the corresponding amount of the 2% (w/w) stock solutions into Milli-Q water to give a final volume of 1.5 mL in a microcentrifuge vial.

In practice, first the adjusted amount of Milli-Q water was placed into the vial, then the calculated amount of the PAA stock solution, and finally the calculated amount of the PAH stock solution. The mixture was vigorously shaken after each step.

In case of PEC systems with added salt, first the amount of salt required for a given salt concentration was measured into Milli-Q water from a 5 M aqueous NaCl stock solution, followed by sequential addition of the given amounts of PAA and PAH stock solution. Again, after each step of adding solution, the system was well-mixed. The total volume of the PEC systems in each sample was controlled to 1.5 mL.

2.3. Analysis of Polyelectrolyte Complex Systems. All polyelectrolyte complex systems were equilibrated for 1 day before analytical investigation. For each system and condition, the experiments and measurements were repeated at least three times. A classification of the phase characteristics as either precipitate, coacervate, or solution was done by light microscopic investigation.

Turbidity measurements were carried out by employing a UV spectrophotometer at a wavelength of 500 nm. PAA and PAH do not absorb light at this wavelength. 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.

In order to determine the degree of phase separation, i.e., the relative phase volumes and compositions, the given sample was centrifuged for 15 min at 10 000*g* using a microcentrifuge. The supernatant equilibrium sol phase was carefully removed by using a micropipet. The sediment phase remaining at the bottom was obtained as a white solid in case of the precipitate and as a whitish viscous liquid or transparent gel in the case of the coacervate, or it was a clear solution without any precipitates or coacervates.

As to the centrifugation to densify the coacervate phase, it is referred to the preparation of PEC nanoparticles where this method was initially used with certain benefits with respect to monomodality of the particle size.[39]

The polymer concentration in the sol phase was determined by means of colloidal titration.[40,41] The method is based on the 1:1 stoichiometric interaction between cationic (P^+) and anionic (P^-) polyelectrolyte and the binding of excess anionic polyelectrolyte to the cationic dye indicator (I^+), *o*-toluidine blue (*o*-Tb):[41]

$$P^+ + P^- \rightleftarrows P^+P^- \quad (K_1)$$

$$I^+ + P^- \rightleftarrows I^+P^- \quad (K_2)$$

The end point is detected by a color change from blue (free dye in the solution) to pink (*o*-Tb bound to anionic polyelectrolyte). For this reaction, the complex formation between cationic and anionic polyelectrolyte must occur faster than the complex formation with the indicator, i.e., $K_1 \gg K_2$. Whereas the cationic polyelectrolyte can be titrated directly with polyanion, anion polyectrolyte concentration is obtained by back-titration after adding an excess of a known quantity of cation polyelectrolyte.[40]

The suitability of the colloidal titration method for the PAA/PAH PEC system was established by control experiments employing known concentrations of PAA or PAH. This also

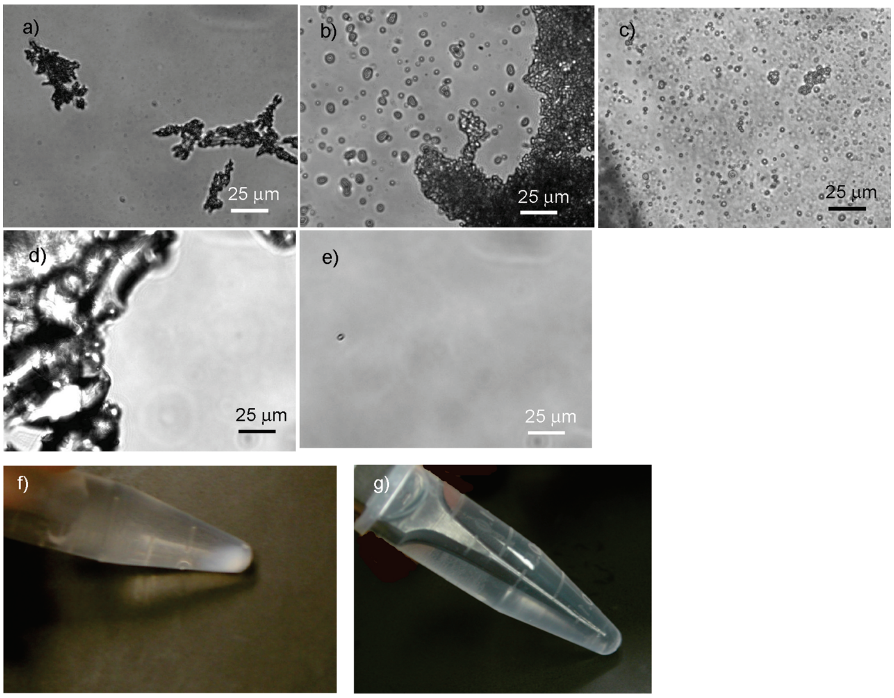

Figure 1. Optical micrographs (a−e) and observation of PEC phase after centrifugal separation (f, g) illustrating the complex formation of mixtures prepared from PAH and fully neutralized PAA (PAANa); 1:1 stoichiometry of acid/base units; 0.05 wt % total polyelectrolyte concentration. (a, b, f) Example of precipitate phase of a salt-free system. (c) Coacervate phase with no gel (400 mM salt concentration). (d, g) Coacervate gel phase and gel settled at the bottom of the vial (800 mM salt concentration). (e) Solution phase (3 M salt concentration).

allowed the assurance of the proper end-point detection by visual observation of the color change from blue (free *o*Tb dye) to light pink (PAA bound dye). However, in accordance with the literature,[42] this method was found not to be suited for high NaCl salt concentration.

3. Results and Discussion

Important experimental variables affecting the polyelectrolyte complex (PEC) formation have been determined. First, the influence of the PAA/PAH mixing ratio for constant total polymer concentration (0.02 and 0.05 wt %) on PEC formation was studied for fully and partially neutralized PAA. This was followed by investigating the effect of added sodium chloride (ionic strength) on the precipitate/coacervate phase behavior, again for different contents and degree of neutralization of PAA. These studies allowed establishing ternary phase diagrams. Finally, the influence of total polymer concentration (0.02−0.20 wt %), pH, and temperature was investigated for 1:1 acid/base unit stoichiometry and various salt concentrations.

3.1. Observation of Precipitate, Coacervate, and Sol Phase. Figure 1 shows optical micrographs of each complex formation. For the aqueous mixture of fully neutralized PAA (PAANa) and PAH not containing additional salt, a precipitate is formed as aggregated solid structure (Figure 1a,b). The precipitate accumulates with time after mixing (Figure 1b) similar to that reported for time-dependent PEC phase formation of whey proteins and arabic gum.[17] When the system contains a sufficiently high salt concentration, the PEC changes from precipitate to coacervate, as visually detected by an emulsion-like appearance (Figure 1c). These observations are in agreement with coacervate phase appearance described in the literature for other polyanion/polycation pairs.[26] For higher salt concentration, the coacervate phase may look like a gel (Figure 1d), and further increase of the salt concentration finally leads to a single solution phase without any PEC (Figure 1e).

Examples of the appearance of the PECs after centrifugation are given in Figure 1f,g. In the case of the precipitate, small particles are sedimented to the bottom of vial (Figure 1f) and can be redispersed by shaking the system. For the coacervate, a highly viscous liquid phase is obtained at the bottom of vial (Figure 1g) that cannot be redispersed.

3.2. Effect of Polyanion/Polycation Stoichiometry and Salt Concentration on Polyelectrolyte Complex Formation. As already mentioned above in connection with the general features and appearance of polyelectrolyte complex (PEC) formation, salt-free mixtures of partly or fully neutralized PAA (PAANa) and PAH always resulted in the formation of a precipitate. Since the presence of ions in the solution may screen the charges of the polyelectrolyte and is expected to influence the formation of PECs, the complex formation was studied for NaCl salt concentrations ranging from 20 to about 5000 mM under a variety of experimental conditions.

In this context, the effect of added NaOH required for partial or complete neutralization of PAA, and/or pH adjustment of aqueous PAA or PAH solutions, on the overall salt concentration in the PEC system shall be shortly addressed. At first glance, it may appear that the sodium ions of added sodium hydroxide could affect the total salt concentration as based on added NaCl salt. However, a calculation shows that the amount of NaOH (1.1 μM) used for carboxylic group neutralization or pH adjustment is about 3 orders of magnitude smaller than the added salt (20−3000 mM). The quantity of sodium ions originating from NaOH is negligible compared to the added NaCl.

3.2.1. Mixtures of Fully Neutralized PAANa and PAH

3.2.1.1. Salt-Free Mixtures of Aqueous PAANa and PAH Solutions. First, the turbidity of PEC systems obtained by mixing fully neutralized PAA (PAANa) and PAH solutions were measured for various polyanion/polycation weight fractions, i.e., different acid/base unit ratios. The measured turbidity of the systems as a function of wt % PAA (or mol % AA acid repeat units) in the mixture is shown in Figure 2a.

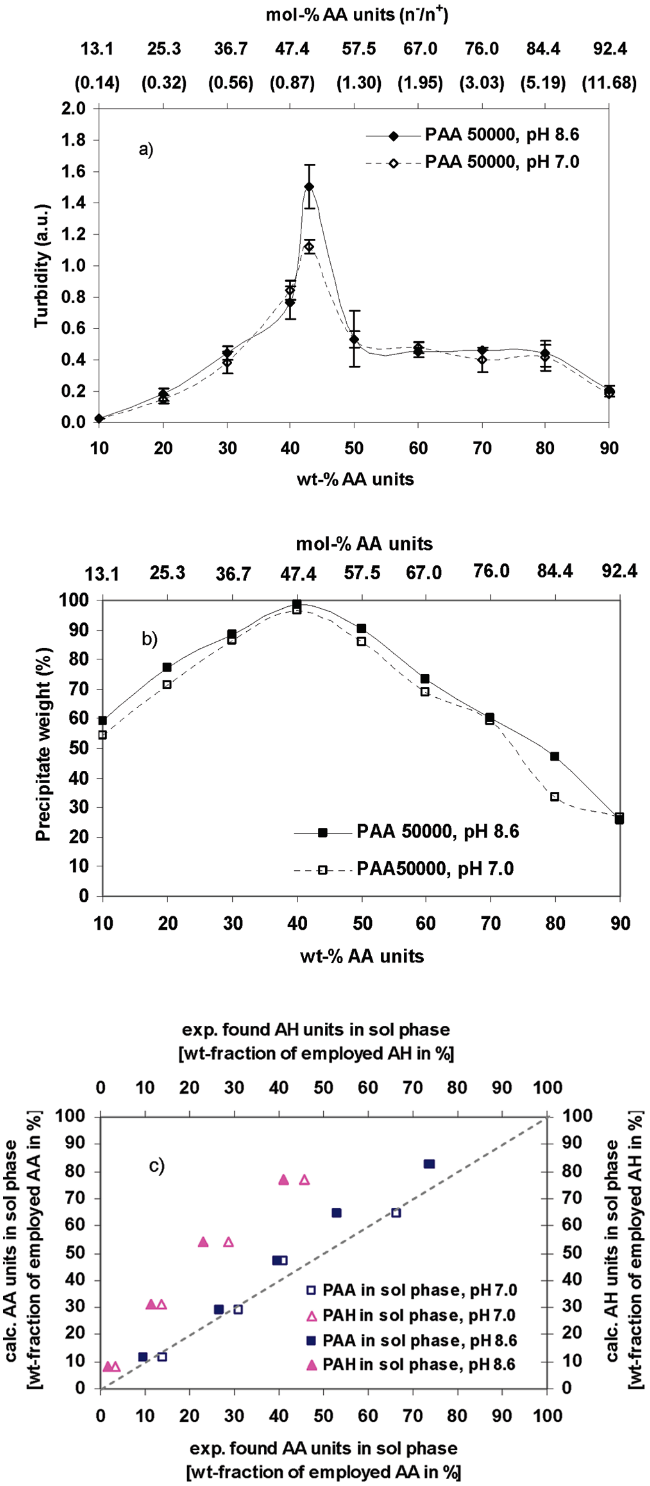

Figure 2. Turbidity (a) and percentage of precipitate weight (b) as a function of PAA content (wt % and mol % AA units; n^-/n^+ = AA/AH units mixing ratio) in the employed polyelectrolyte mixture (0.05 wt % total polyelectrolyte concentration); (■, ◆, ▲) fully neutralized PAA employed (PAANa solution of pH = 8.6); (□, ◇, △) partially neutralized system (PAA solution of pH = 7); (c) calculated and experimentally found AA and AH units in the sol phase related to the amount of employed PAA and PAH, respectively.

Although this turbidity is not a precisely quantitative measure of the phase separation because the measured change in light transmission depends on the size and composition of the dispersed phase (precipitate or coacervate), these turbidity data reflect the extent of phase separation and its relation to the relative polyanion/polycation composition of the system. In that respect, it is meaningful that the highest turbidity is measured for the 1:1 stoichiometry since in this case the maximum PEC formation is possible.

Since the amount of the precipitate was too small to be properly isolated and weighed after centrifugation, the weight of the precipitate was calculated from the polyelectrolyte concentration in the supernatant sol phase. The amount of precipitate is directly obtained from the difference between the polyelectrolyte concentration in the sol phase as determined by the colloid titration and the total amount of polyelectrolyte employed in the experiment. Deviations from the theoretically expected amount of PEC precipitate consisting of 1:1 stoichiometry would indicate nonstoichiometric compositions.

The amount of precipitate increases when going from stoichiometrically unbalanced systems (mol % AA units $< 50\%$ or $> 50\%$) toward the 1:1 stoichiometric carboxylate acid/ammonium base unit ratio (50 mol % AA; Figure 2b), with a maximum value for the 1:1 stoichiometry (43 wt % PAA). At a first glance, and qualitatively, this finding is as expected, given the observed change in turbidity (Figure 2a).

However, the plotting of the experimentally determined PAA (molar surplus of AA units in employed PAA/PAH mixture ($>$ 43 wt % PAA)) or PAH (molar surplus of AH in the employed PAA/PAH mixture ($<$ 43 wt % PAA)) vs the calculated residual amount of PAA or PAH in the sol phase (Figure 2c) shows that for PAA/PAH mixtures with AA/AH mole ratio < 0.5 (PAA $<$ 43 wt %) the PAH experimentally found in the sol phase is less than calculated for 1:1 acid/base unit composition of the precipitate; i.e., the precipitate always contains more PAH than calculated for 1:1 acid/base unit stoichiometry of the precipitate. On the other hand, the data imply that, in case of PAA $<$ 43 wt %, the precipitate contains all the employed PAA, and in the case of PAA $>$ 43 wt %, the composition of the PEC precipitate is close to 1:1 acid/base unit stoichiometry. This is seen from the comparison of the experimental data with the diagonal (dotted line in Figure 2c) which describes the 1:1 stoichiometry of the precipitate.

This means that the expected 1:1 acid/base unit stoichiometry of PEC precipitate is only obtained for systems with molar surplus of AA in the employed PAA/PAH mixture (PAA $>$ 43 wt %), whereas the unexpectedly high amount of precipitate obtained for AA/AH mole ratio < 0.5 (PAA $<$ 43 wt %) is due to coprecipitation of a higher amount of PAH as needed for the formation of a 1:1 acid/base unit stoichiometric PEC precipitate. Since the same phenomenon is observed for both the systems prepared with fully neutralized PAA (PAANa; pH $\sim$ 8.5 of employed aqueous PAA solution) of partially neutralized PAA (pH = 7.0 of employed aqueous PAA solution), but the relative change of pH with varying the PAA/PAH mixing ratio is significantly different for both systems (see Figure 8), it is not feasible to associate the observed PEC composition directly with pH-dependent differences in the degree of dissociation of the polyelectrolytes. It may be that site-specific interaction phenomena between the carboxylate and ammonium groups of PAA and PAH that are linked to local chain and functional group hydration have to be taken into account (compare ref 26).

Irrespective of the origin of the observed deviations from 1:1 stoichiometry of the PEC precipitate, the amount of precipitate obviously depends on the degree of neutralization of the employed PAA as reflected from the comparison of the data (Figure 2b) obtained with fully neutralized PAA (aqueous PAANa solution of pH = 8.6) and partially

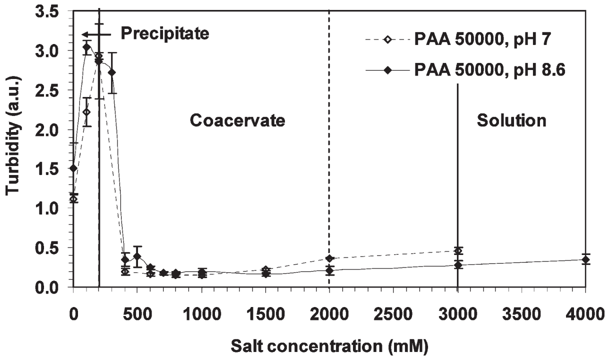

Figure 3. Turbidity indicating complex formation of polyelectrolyte mixture (1:1 stoichiometry of acid/base units; 0.05 wt % total polymer concentration) as a function of salt concentration; solid line (◆): fully neutralized PAA employed (PAANa solution of pH = 8.6); dashed line (◇): partially neutralized system (PAA solution of pH = 7). Vertical line at 200 mM salt concentration: precipitate−coacervate phase transition for both systems; vertical dashed and solid lines: coacervate phase−solution transition for partially and fully neutralized systems, respectively.

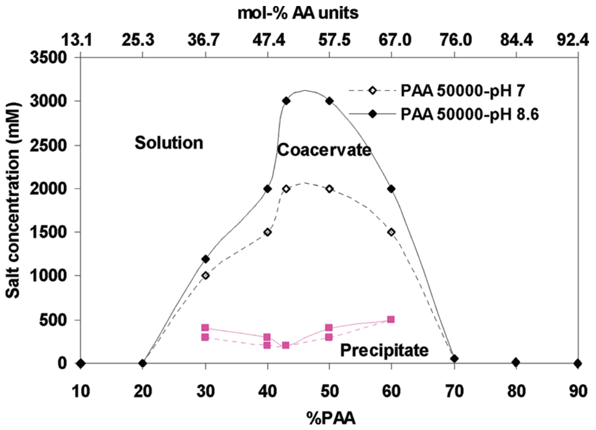

Figure 4. Phase diagram of PAA/PAH mixtures (0.05 wt % total polymer concentration) as a function of PAA content (in wt %) and added salt; solid line (◆, ■): fully neutralized PAA employed (PAANa solution of pH = 8.6); dashed line (◇, □): partially neutralized system (PAA solution of pH = 7). The bell-shaped curves separate the coacervate phase regimes from the polyelectrolyte solutions. The curves within the bell-shaped curves distinguish the precipitate from the coacervate phase regime.

(not fully neutralized) aqueous PAA solution (pH = 7). There is a clear trend of the formation of less precipitate when PAA is employed that contains nonionic −COOH groups, meaning that the amount of PAH participating in PEC formation is directly linked to the number of ionic $-COO^-$ groups of the polyanion. The smaller amount of precipitate is also reflected in the somewhat weaker turbidity (Figure 2a). This will be further addressed in section 3.2.2.

3.2.1.2. Effect of Added Salt on PEC Formation. The PEC formation between PAANa (fully neutralized PAA) and PAH was studied as a function of ionic strength (as expressed by the concentration of added NaCl) for 1:1 stoichiometry of acid/base repeat units (0.05 wt % total polyelectrolyte concentration). The change in turbidity as related to the salt concentration is shown in Figure 3. The classification of the formed PEC as precipitate or coacervate and the discrimination of these PEC phases from a solution (all sol phase) is based on inspection of the phases by optical microscopy and observation of the sediment after centrifugation (see Figure 1).

At zero salt concentration, a PEC precipitate is formed. Adding small amounts of salt causes a shrinkage of the PEC, finally leading to PEC flocculation and resulting in an increased turbidity.[29,30] Upon further increasing the salt concentration, due to the salt screening effect on the polyelectrolyte charges, the precipitate phase changes to the coacervate phase. The phase transition goes along with a decrease in turbidity, finally giving a highly transparent system. The coacervate phase can be separated as viscous gel at the bottom of vial by centrifugation.

Above a critical salt concentration, the coacervate is no longer stable but redissolved, and the whole system turns into a single solution phase. This results from the fact that the free energy change ΔF between the situation that polymer is in a polyelectrolyte complex (PEC) and the situation that all polymer is in solution is positive above a critical salt concentratin for all values of the complex composition,[16] and complexation is completely inhibited. In this context it shall also be referred to the erosion an decomposition of polyelectrolyte multilayers where the trends observed with multilayers directly follow from the properties of PECs in solution:[36] the multiple polymer/polymer ionic pair contacts completely dissociate as a result of competition with added low molecular weight ions, and the polyelectrolyte multilayer dissolves similarly as a PEC in solution.

As for the 1:1 acid/base unit stoichiometry, the turbidity curves as a function of salt concentration were established similarly for different PAANa/PAH mixing ratios. The determined critical salt concentrations required for the transition of precipitate to coacervate, and coacervate to solution, were used for establishing the phase change characteristics as a function of both the PAANa content and the salt concentration (Figure 4).

PECs are not formed for large surplus of either PAH (corresponding to low PAANa content) or PAANa. Only if the unbalance of the acid/base units is below a critical value—about 24 mol % and 75 mol % PAANa as lower and upper limit, respectively—a precipitate or coacervate is observed. This is plausible considering that a critical number of intramolecular interactions between anionic carboxylate and cationic ammonium groups of the oppositely charged polyelectrolytes must be given in order to generate a stable, macroscopic gel phase (precipitate or coacervate); i.e., a quasi-percolation threshold must be exceeded.

The fact that PEC formation does not occur for the extremely nonstoichiometric polyacid/polybase ratio (PAANa concentration < 24 mol % or > 75 mol %)—even for zero salt concentration where a precipitate was obtained for all other polyacid/polybase ratios—could also be treated as a redissolution of a collapsed PEC upon addition of further PAANa (concentration regime > 75 mol % AA) or further PAH (concentration regime < 24 mol % AA).

This view is in analogy to the treatment of two structural transitions which biological and synthetic polyelectrolytes undergo upon addition of multivalent salt or molecules.[43,44] The oppositely charged polyelectrolyte (PAH polycation or PAA polyanion) is simply considered as multivalent counterion. In this picture, charge screening of the collapsed PEC is achieved by the polyelectrolyte in excess: a redissolution transition in initially multivalent induced precipitated polyelectrolytes takes place with further addition of multivalent particles (here: polyions), as predicted for flexible linear polyelectrolyte chains in multivalent salt solutions.[43,44]

In the potentially two-phase regime between about 20 and 70 wt % PAANa, precipitate and coacervate formation

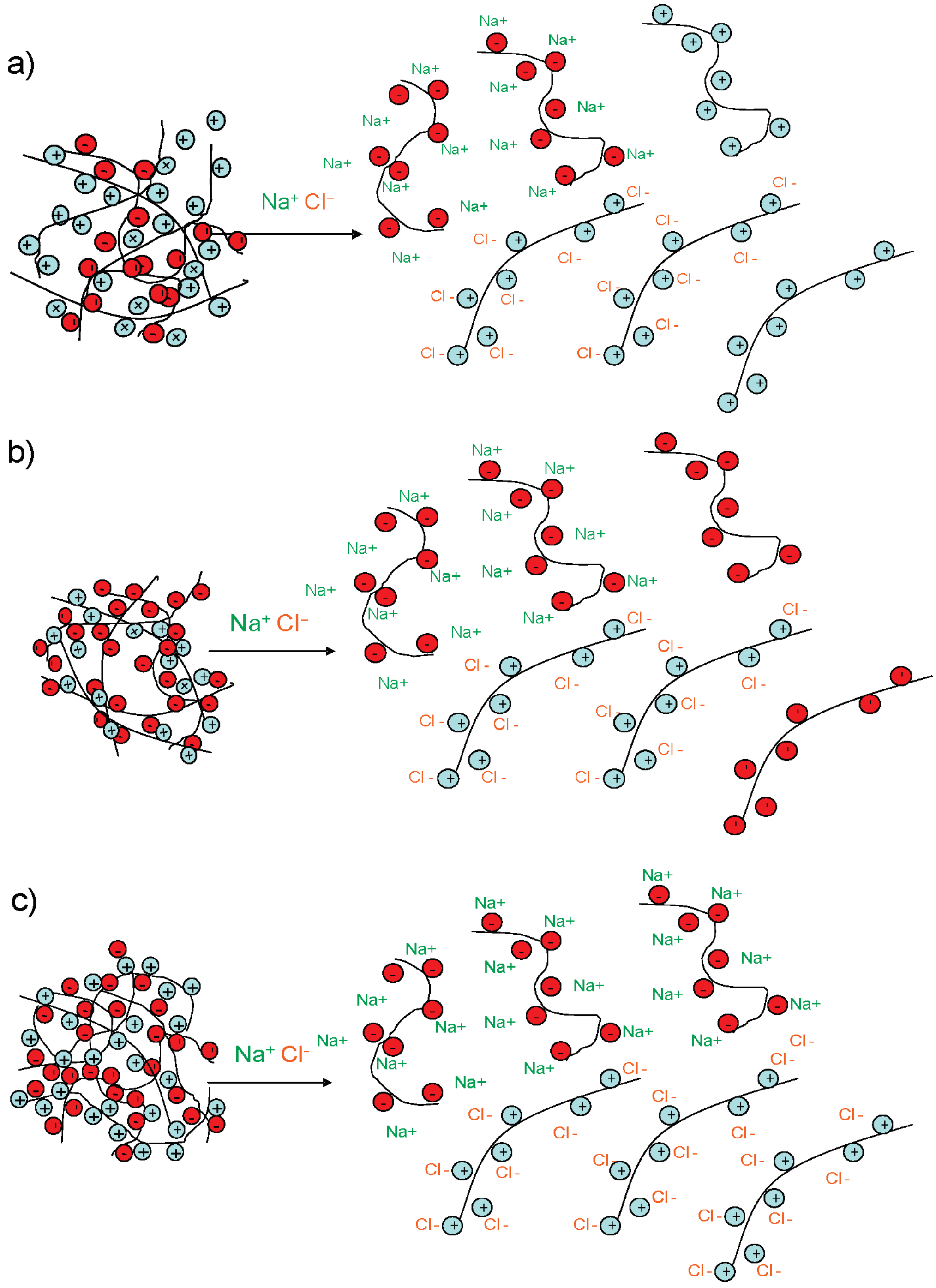

Figure 5. Schematic illustration of the screening of polyacid and polybase ionic groups by added salt and required amount of salt for screening of potentially interacting carboxylate ($-COO^-$) and ammonium ($-NH_3^+$) groups of differently composed systems: (a) and (b) polyelectrolyte acid/base unit ratio 1:2 and 2:1, respectively; (c) 1:1 acid/base unit stoichiometry.

as well as precipitate–coacervate and coacervate–solution transitions that vary with the salt concentration are observed. It is interesting to note that the change from precipitate to coacervate requires less salt when approaching 1:1 stoichiometry of acid/base units, but the opposite is observed for the coacervate–solution transition. Whereas the physical meaning of the first phenomenon (precipitate–coacervate phase transition between 40 and 60 wt % PAANa occurring at lower salt concentration as compared to the stoichiometrically more unbalanced 30 and 70 wt % compositions) is not clear yet, the significantly increasing amount of added salt required for the transition coacervate phase to solution, with maximum salt concentration for 1:1 stoichiometry of anionic/cationic units, can be understood by considering salt screening effects.

The critical salt concentration established for reaching the coacervate to solution transition of a given polyacid/polybase ratio can be related to the amount of theoretically possible ion pairs between PAA carboxylate and PAH ammonium groups. It is inferred that the experimentally determined amount of salt required for obtaining minimum turbidity or clearing is in correspondence to the number of potentially interacting oppositely charged groups of the employed anionic and cationic polyelectrolytes as schematically illustrated in Figure 5.

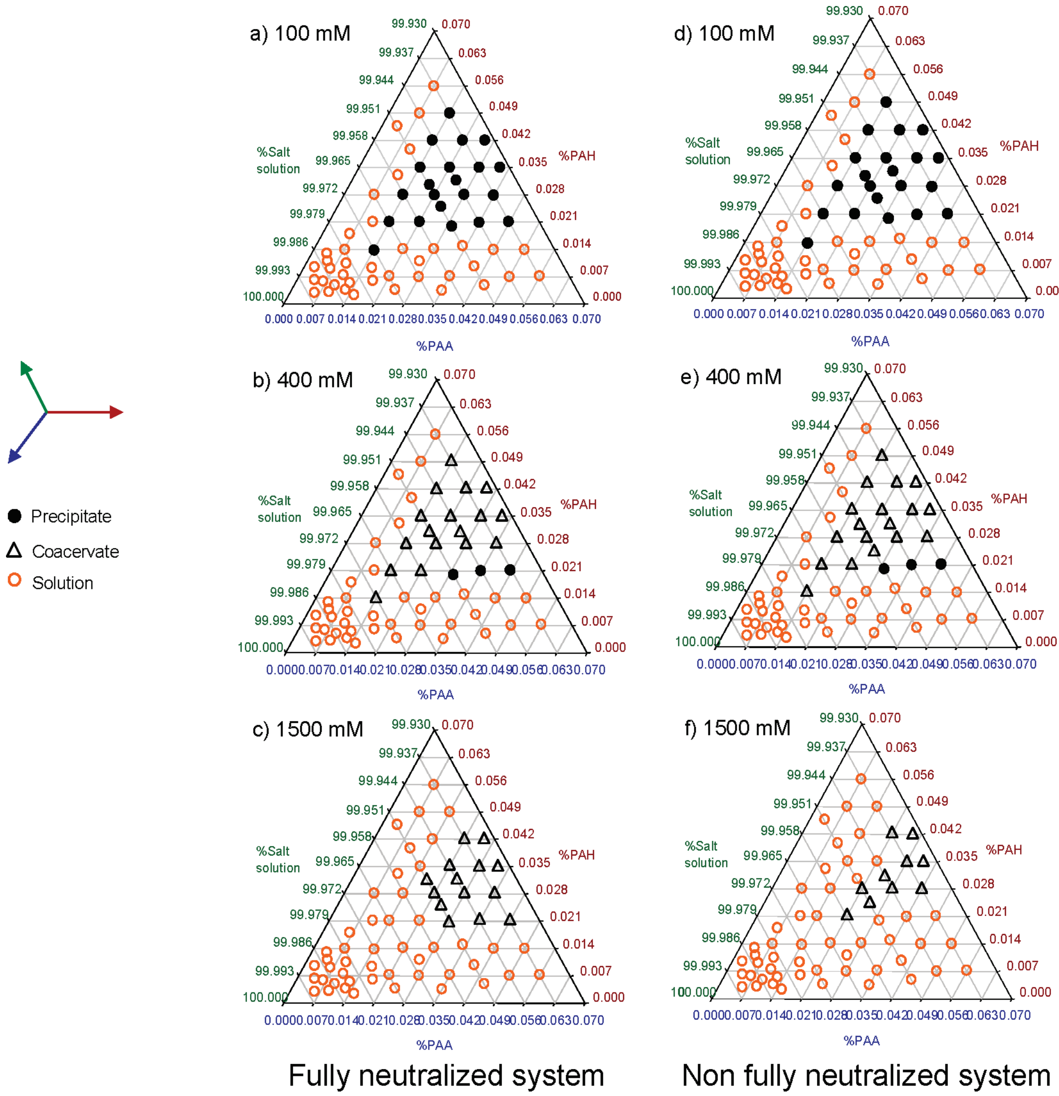

Figure 6. Ternary phase diagrams of PAA/PAH mixtures (0.05 wt % total polymer concentration) in aqueous NaCl salt solution (100, 400, and 1500 mM); fully neutralized system: PAANa solution of pH = 8.6 employed (a−c); partially (not fully) neutralized system: PAA solution of pH = 7 employed (d−f).

For example, in a PAANa/PAH mixture with a mole ratio of acid to base repeat units of 1:2 (Figure 5a), the amount of added salt required to dissolve the polymer ion complex is equivalent to the mole concentration of the carboxylic groups (two chains carrying red anionic charges). The same holds true for the reverse mole ratio of 2:1 (Figure 5b: two chains carrying blue cationic charges). However, in case of acid/base unit stoichiometry, the ionic charges of all polymer chains have to be screened (Figure 5c). Correspondingly, the experimentally determined critical salt concentration is about 1/2 to 2/3 of the value determined for the stoichiometric 1:1 ratio (see Figure 4) which requires the maximum amount of salt to dissolve the coacervate (Figure 5c).

The above picture is in accordance with ideas developed for the formation and dissolution of weak polyelectrolyte multilayers, where both the requirement of adding a sufficiently large amount of salt, and that less added salt is needed for both high excess of polyanion or polycation, were similarly discussed.[34]

Ternary phase diagrams of PAANa/PAH mixture established for different salt concentrations illustrate how the precipitate, the coacervate, and the solution region depend on the composition of the system (Figure 6). These phase diagrams describe the composition of the salt containing aqueous polyelectrolyte system as consisting of the components PAANa (PAA fully neutralized with NaOH), PAH (hydrochloride of poly(allylamine), and aqueous NaCl solution of a given NaCl concentration (100, 400, and 1500 mM).

At low salt concentration (100 mM, Figure 6a), a solution region is found only for very low concentration of either PAA (below 0.028 wt %) or PAH (below 0.021 wt %). At and above these polyelectrolyte concentrations, a precipitate but no coacervate is observed.

When increasing the salt concentration to 400 mM (Figure 6b), a limited precipitate region is found only for (around) 0.021 wt % PAH and certain concentrations of PAANa at and above about 0.028 wt %. Higher concentrations of either PAANa or PAH result in a coacervate region.

For significantly higher salt concentrations, e.g., 1500 mM NaCl, a precipitate is not observed anymore. The coacervate region is shifted toward higher polyelectrolyte concentration, meaning a broadening of the solution region and a corresponding narrowing of the coacervate region (Figure 6c).

3.2.2. Effect of pH on the Polyelectrolyte Complex Formation. The strongest interaction between oppositely charged polyelectrolytes is electrostatic in nature. Since PAA is a weak polyacid, it is expected that the degree of dissociation of carboxyl groups, i.e., the fraction of carboxylate groups affects the interaction with the cationically charged PAH. In this context it has been reported for polyion complex between synthetic polyelectrolytes[26] as well as biopolymer polyelectrolytes[23] that the electrostatic polyion complex is mainly influenced by the degree of neutralization (i.e., the degree of dissociation) of the polyanion, the pH of the system, and the ionic strength.

From the pK_a values of poly(acrylic acid) PAA (pK_a = 4.5) and of poly(allylamine hydrochloride) PAH (pK_a of amine group = 8.5[7]) it is clear that for pH > pK_a of PAA most of the carboxyl groups of PAA are in the $-COO^-$ form. On the other hand, PAH will be increasingly deprotonated with increasing pH; i.e., the number of $-NH_2$ amino increases accordingly. This means that, in a certain pH range, the PEC is being formed between not fully charged polyelectrolyte macromolecules.

3.2.2.1. Mixtures of PAH and Less than Fully Neutralized PAA. First, the 1:1 stoichiometric PAA/PAH mixture prepared with partially neutralized PAA that showed an almost quantitative PEC formation (Figure 2) was investigated, including studying effects of different NaCl salt concentrations. In these studies, a PAA solution adjusted to pH 7 by partial neutralization of the carboxyl groups with NaOH was employed. At pH 7, about 99.7% of the carboxyl groups are neutralized, corresponding to a $[-COO^-]/[COOH]$ group ratio of about 316 in a salt-free aqueous solution (calculation based on the classical relation $\mathrm{pH} - \mathrm{p}K_a = \log(\alpha/(1-\alpha))$, with α = degree of dissociation; and $\mathrm{p}K_{a(\mathrm{PAA})} = 4.5$).

As in case of the PAANa/PAH system (fully neutralized PAA employed), a precipitate is formed for the partially neutralized system (aqueous PAA of pH 7 employed) as well. The turbidity increases for a small amount of added salt (100 mM concentration), and the precipitate changes to the coacervate phase for practically the same (200 mM) salt concentration (Figure 3). However, the phase transition from coacervate to solution occurs already at about 2 M salt concentration, i.e., at a significantly lower salt concentration as compared to the fully neutralized PAANa/PAH system where the coacervate−solution transition is observed only at 3 M salt concentration (Figure 3).

This is a consequence of the much lower degree of dissociation of PAA at pH = 7, considering that only the ionized $-COO^-$ carboxylate but not the free $-COOH$ carboxylic groups are capable to interact with the $-NH_3^+$ ammonium groups of PAH to form a coacervate. For pH = 8.6 of the aqueous solution of fully neutralized PAA (PAANa), a group concentration ratio $[-COO^-]/[COOH]$ of about 12 590 can be calculated, whereas for pH = 7 the ratio $[-COO^-]/[COOH]$ is only 316. This translates to differences in the number of electrostatic interactions between carboxylate groups of PAA and the PAH polycation; i.e., a lower critical salt concentration to dissolve the coacervate is required for the system prepared with the partially (not fully neutralized) PAA, as was experimentally observed.

Schematically, the change from a fully to a partially neutralized PAA means a decrease of ionized groups along the polyacid which is reminiscent of an increase of the separation of ionized groups along the polymer chain. (Note: for an acrylic polyelectrolyte, ideally, the ionizable segment length a = 0.25 nm.[16,45]) Thus, the observed effects of the interplay between pH and salt concentration on the stability of the coacervate region are in qualitative agreement with theoretical predictions that the critical salt concentration significantly decreases when increasing the input parameter a in the calculation of the stability boundaries.[16]

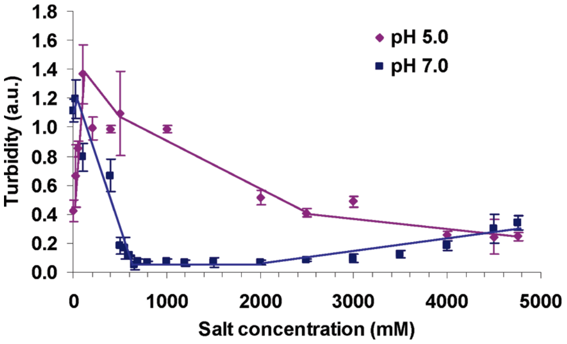

Figure 7. Turbidity of 1:1 acid/base units stoichiometry PAA/PAH mixtures (0.02 wt % total polyelectrolyte concentration) as a function of pH and salt concentration; the pH of both the aqueous polyanion and polycation solution was adjusted prior to mixing; (◆): pH = 5; (■): pH = 7.

In another set of comparative experiments, 1:1 stoichiometric mixtures of PAA and PAH (0.02 wt % total polyelectrolyte concentration) were prepared by employing polyelectrolyte solutions where the pH of both aqueous PAA and aqueous PAH was adjusted to pH 5 and pH 7 prior to mixing. As shown in Figure 7, the turbidity values obtained at pH = 5.0 are higher than those at pH = 7.0. In both cases the initial turbidity is lower than observed for the higher concentrated (0.05 wt % total polyelectrolyte) mixture of PAH (pH ∼ 3) with fully (pH ∼ 8.5) or partially (pH = 7.0) neutralized PAA (see Figure 3); this may be ascribed to the different total polyelectrolyte concentrations. However, the turbidity of the system with adjusted pH = 5 was always higher and never came down to the level of the other systems. Single phase solutions were not obtained for the low-pH system even at about 5 M salt concentration.

The situation at pH = 7 is not much different from mixing aqueous PAH with fully or partially neutralized PAA (Figure 3) as already discussed above: here both PAA and PAH are practically fully charged. The significantly higher turbidity in the coacervate regime under the low pH = 5.0 condition can be attributed to the fact that PAA is about 25% protonated and therefore in a less extended coil conformation and somewhat hydrophobized state as compared to higher pH.[26] It may be speculated whether the mixture really forms a true coacervate under these conditions or contains precipitate-like sites. In this context it is interesting to note that unusual thickness behavior has been observed in sequential adsorbed layer formation in the same pH range.[33]

Regarding the nonstoichiometric PAA/PAH mixing ratios, as was already discussed above (see section 3.2.1.1), the salt-free system prepared with partially neutralized PAA gave again only PEC precipitate, the amount of which varied with the PAA/PAH mixing ratio the same as observed for the mixture of fully neutralized polyelectrolytes (Figure 2). However, for all mixing ratios, the amount of precipitate formed with the partially neutralized PAA is always a little smaller than in case of the fully neutralized PAANa. This implies that the extent of PEC formation is related to the stoichiometry of potentially interacting anionic ($-COO^-$ carboxylate) and cationic (ammonium) units of the employed polyelectrolytes. It is surprising that only a relatively small fraction of nonionic $-COOH$ units causes such a small, but distinct, effect on the amount of formed PEC.

Nevertheless, it is obvious that partially neutralized PAA macromolecules are only capable to form PECs with

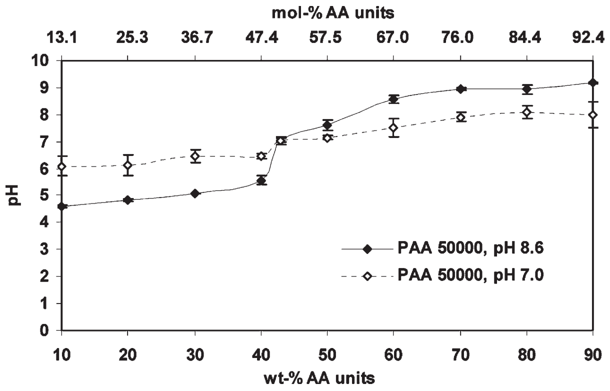

Figure 8. Final pH of PAA/PAH mixtures (0.05 wt % total polymer concentration) as a function of PAA content (in wt %); solid line (◆): fully neutralized PAA employed (PAANa solution of pH = 8.6); dashed line (◇): partially neutralized system (PAA solution of pH = 7 employed).

ammonium units of PAH via their anionic carboxylate groups; i.e., the amount of ionically bound PAH per PAA macromolecule is less than in the case of fully neutralized PAANa carrying exclusively carboxylate groups. This finding is phenomenologically in agreement with data reported in the literature for polyion complex formation between poly(methacrylic acid) and a polycation carrying charges in the backbone.[26] However, the compositional dependency on the degree of neutralization reportedly was only observed for degrees of neutralization below about 0.6, whereas in our studies the degree of neutralization of PAA at pH = 7 is much higher. This points to a significant influence of the polyelectrolyte architecture on PEC formation.

In this context, it is interesting to note that the pH values of the PAA/PAH mixtures (10−90 wt % PAA; 0.05 wt % total polyelectrolyte concentration) prepared with the partially neutralized PAA (pH = 7) vary only slightly between pH ~ 6−7.5 with the polyelectrolyte mixing ratio, whereas a comparatively sharp pH change from pH ~ 4.5−5.0 to pH ~ 7−7.5 and finally up to pH ~ 9.0 for large surplus of PAA was observed when the fully neutralized PAANa was employed (Figure 8). This is indicative of a buffer effect of the $-COO^-Na^+/-COOH$ units of PAA on the mixture which may also affect PEC formation.

As was already to be expected from the lower critical salt concentration required for the coacervate−solution transition of the 1:1 stoichiometric polyelectrolyte mixture (Figure 3), the same holds true for different mixing ratios of PAH with partially neutralized PAA (Figure 4). The shapes of the phase diagrams of both systems (prepared with fully neutralized PAANa or partially neutralized PAA) look the same, but for the partially neutralized system, the critical salt concentration required for the coacervate phase to single phase polyelectrolyte solution is lower than that for the fully neutralized system.

This finding is in agreement with the picture developed for the dependency of the coacervate−solution transition of the fully neutralized PAANa/PAH system on salt concentration (Figure 5): Since the critical salt concentration required for the coacervate−solution transition is only related to the number of contacts between anionic and cationic units, less salt is needed to dissolve the coacervate prepared from partially neutralized PAA and fully neutralized PAH because the charge density of this PEC system is less than in the case of a pair of fully neutralized polyelectrolytes.

The ternary phase diagrams of the PAA/PAH mixtures made with partially neutralized PAA (Figure 6d,e) look similar to the diagrams obtained with the fully neutralized PAANa (Figure 6a−c), also regarding the occurring of precipitate and coacervate phases for intermediate salt concentrations. The sole, but distinctive, difference is that the single phase solution regime is slightly broader for the system prepared with the not fully neutralized PAA, most clearly seen in the comparison of the phase diagrams for 1500 mM salt concentration. This again elucidates the strong effect of the ionic strength on PEC phase formation and its coupling with the fraction of ionic units in the polyectrolytes.

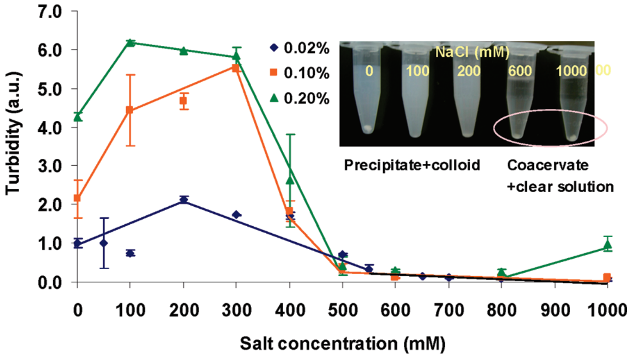

Figure 9. Turbidity of 1:1 acid/base units stoichiometry PAA/PAH mixtures of different total polymer concentration as a function salt concentration; the pH of both the aqueous polyanion and polycation solution was adjusted to pH = 7 prior to mixing; (◆) 0.02 wt % total polymer concentration; (■) 0.10 wt %; (▲) 0.20 wt %. The inserted photographs exemplify the appearance of the 0.10% or 0.20% systems in the high (three vials on the left) and low (two vials on the right) turbidity regime.

3.3. Effect of Total Polyelectrolyte Concentration on the Polyelectrolyte Complex Formation. Three different total polymer concentrations (0.02, 0.10, and 0.20 wt %) of 1:1 stoichiometrical PAA/PAH mixtures (all with pH = 7 adjusted prior to mixing) were employed, and the effect of the amount of added salt on the PEC formation as reflected from the turbidity was investigated. The turbidity data are given in Figure 9.

In this context it has to be emphasized that the concentration of either polyelectrolyte (as well as the total polymer concentration) is below the critical overlap concentration c^*, which was calculated from $c^* = 1/[\eta]$ by using the corresponding intrinsic viscosity−molecular weight relationships[46] (1.0 M aqueous NaCl; 25 °C) for PAANa ($[\eta] = 41.5M_w^{0.63}$ (in mL/g)) and PAH ($[\eta] = 13.9M_w^{0.714}$ (in mL/g)). For both polyelectrolytes, c^* is about 2.5 wt %.

At low total polyelectrolyte concentration (0.02 wt %), a change from cloudy coacervate to a clear solution is observed when the critical salt concentration is exceeded (at about 500 mM), meaning a dissolution of the polyelectrolyte complex. This behavior is similar to that found for the 0.05 wt % polyelectrolyte system (Figure 3), but the clearing temperature is shifted to significantly lower salt concentration.

Considering the known effect that the fraction of the counterions decreases with increasing polyelectrolyte concentration,[47] corresponding to a decrease in the Debye length,[45] this experimental observation is meaningful: the higher the polymer concentration, the higher the number of already condensed counterions, i.e., the lower is the salt concentration (added NaCl) needed for reaching the critical number of counterion condensation required to destroy the polyelectrolyte complex.

However, for higher polymer concentrations (0.10 and 0.20 wt %), only a change from cloudy solution containing flocculate (i.e., a precipitate phase) to a two-phase system consisting of a clear solution over a sticky gel phase (at the bottom, presumably a coacervate phase) was obtained as illustrated by the photographs of sample vials after centrifugation (inset in Figure 9). Further increase of the salt concentration did not dissolve the coacervate. To the contrary, in case of the 0.2 wt % system, an increase in turbidity occurred already at moderate salt concentration. This may be indicative to secondary aggregation phenomena[30] or could be simply a consequence of a decreasing solvent (aqueous NaCl solution) quality with increasing salt concentration.

Regarding the turbidity at a given salt concentration as related to the polymer concentration, the data (Figure 9) indicate a proportionality with the polymer concentration up to about 500 mM salt concentration where the transition from coacervate to solution (low polymer concentration of 0.02 wt %) or precipitate to coacervate (higher polymer concentrations) occur. The general trend of an increase in turbidity with increasing polymer concentration may be understood as resulting from a higher PEC volume fraction in the more concentrated systems.[22]

3.4. Effect of Temperature on the Polyelectrolyte Complex Formation.

The temperature may influence the PEC formation through a number of effects.[10] Whereas in the reported biopolymer-based PECs temperature-induced denaturation and related conformational changes play an important role, in the present case, the interplay between enhanced hydrophobic interactions, on the one hand, and increased polyelectrolyte dissociation, on the other, both coupled to a temperature increase, have to be considered primarily.

The temperature effect on the complex formation between the oppositely charged synthetic polyelectrolytes was studied for the temperature range 30−75 °C. The polyelectrolyte mixtures were prepared from aqueous PAA and PAH solutions that had been adjusted to pH 7 prior to mixing. Turbidity measurements were carried out after 1 day incubation time in each experiment.

In order to study the temperature effect without having to consider effects of ionic strength and pH, first salt-free system were investigated. The turbidity data obtained for various PAA/PAH compositions and temperatures are collected in Figure 10.

The shape of the curves in Figure 10a is similar as established for the mixtures of fully neutralized polyelectrolytes (Figure 2a); i.e., maximum turbidity is observed for 1:1 stoichiometry of acid/base units. Therefore, the same considerations as to the origin of the variation of turbidity with relative polyelectrolyte composition apply for the pH 7 system as well.

It is interesting that almost no temperature effect on turbidity is observed for large deviations from 1:1 stoichiometry; i.e., the very minor turbidity for 10, 20, and 90 wt % PAA at 30 °C did not change upon further temperature increase (Figure 10b). In contrast to this, for all other compositions a pronounced temperature effect that increases proportional to the approaching 1:1 stoichiometry is observed, with the strongest decrease of turbidity with increasing temperature for the 1:1 acid/base unit stoichiometry (Figure 10b). The 80 wt % PAA system almost completely dissolved at 75 °C, whereas all other compositions remained turbid. The temperature induced decrease in turbidity goes along with a decrease of the size of the flocculated precipitate.

The temperature and composition depending properties of the PEC systems can be attributed to the increasing degree of dissociation with temperature. Since the PEC formation is electrostatic in nature, the interaction between acid and base units decreases with increasing temperature, i.e., not only the number but also the bonding strength of intramolecular ion pairs decreases. Accordingly, in case of the extremely stoichiometrically misbalanced systems, PECs will be dissolved for the most part. As for the 30−70 wt % PAA containing systems, temperature increase may mean a partial transformation of precipitate to coacervate, or even dissolving (of coacervate), both processes resulting in a reduction of turbidity.

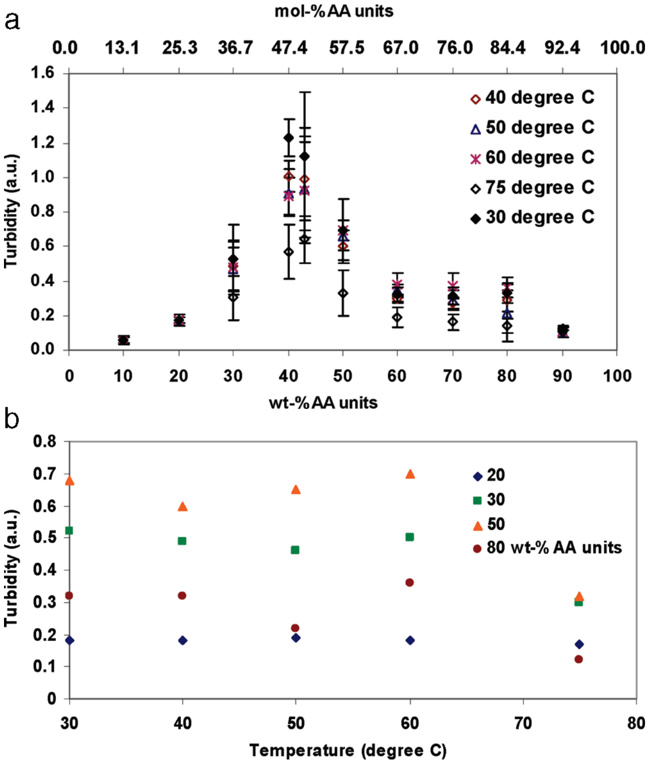

Figure 10. Change of turbidity of PAA/PAH mixtures (0.05 wt % total polymer concentration) as a function of PAA content (in wt %) and incubation temperature; polyanion and polycation solution was adjusted to pH = 7 prior to mixing: (a) turbidity vs polyelectrolyte mixing ratio for various temperatures; (b) turbidity vs temperature for selected mixing ratios.

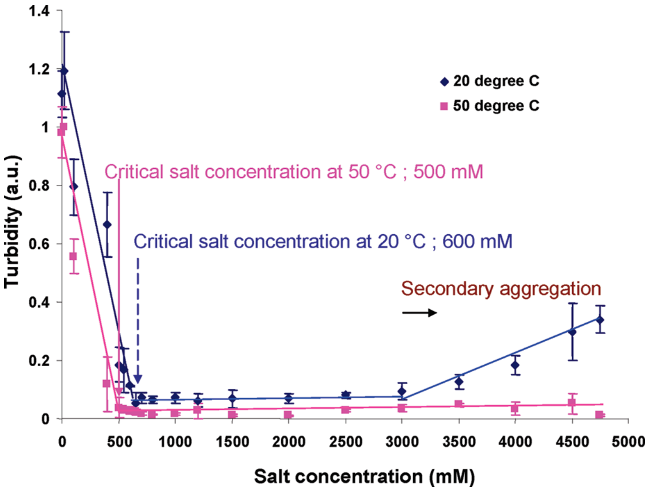

Figure 11. Turbidity of PAA/PAH mixture (1:1 acid/base units stoichiometry; 0.02 wt % total polyelectrolyte concentration) as a function of temperature and salt concentration; the pH of both the aqueous polyanion and polycation solution was adjusted to pH = 7 prior to mixing.

For a comparative study of the combined effect of temperature and salt concentration on PEC formation, a polyelectrolyte system of 1:1 acid/base unit stoichiometry was investigated at 20 and 50 °C (Figure 11). The critical salt concentration required for the precipitate−coacervate phase transition is shifted from 600 mM at 20 °C to 500 mM at 50 °C. This finding is in agreement with the earlier observations of the effect of added salt on PEC characteristics and also with the above-described temperature effect. It is interesting that for high salt concentrations a secondary aggregation effect[29,30] is observed at 20 °C when increasing the salt concentration beyond 3 M, but not at 50 °C. Since the secondary aggregation is believed to be related to the polyelectrolyte charge screening and the coupled coil shrinkage, a plausible explanation for the absence of this phenomenon at 50 °C is the higher degree of dissociation at that temperature.

4. Conclusion

The polyacid/polybase mixing ratio, total polymer concentration, salt concentration, pH, and the temperature mutually effect the formation of a polyelectrolyte complex (PEC). The development or disappearance of turbidity of the aqueous polyelectrolyte systems in combination with visual and microscopic observation allowed following the phase behavior as related to the various experimental parameters. Ternary phase diagrams characterizing the relation between the composition and ion strength of the systems and the two-phase precipitate or coacervate and single phase solution regimes could be established.

The critical salt concentration required for reaching the coacervate or solution of a given mixing ratio can be related to the amount of theoretically possible ion pairs between PAA carboxylate and PAH ammonium groups. Effects of the degree of neutralization of PAA and of the variation of pH on the complex formation can be associated with the polyelectrolyte charge density and screening of ionic groups along the polymer chain.

Molecular models explaining the effects of the polyelectrolyte mixing ratio and ionic characteristics of the system, as well as temperature effects on the formation of PECs and the redissolution of PECs, can be established based on systematic, quantitative data. Ongoing and further work will include the study of effects of varying the molecular weight of the polyelectrolytes on the phase behavior as well as to establish a general and theoretically founded understanding of coacervate formation.

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