Polyelectrolyte Complexes with pH-Tunable Solubility

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Received May 16, 2006; Revised Manuscript Received August 29, 2006

ABSTRACT: Polyelectrolyte complexes (PECs) with pH-tunable solubility were formed from a random copolymer of diallyldimethylammonium (DADMA) and acrylic acid (AA) having a net positive charge as polycation and poly(styrenesulfonate) as polyanion. Quasi-soluble PEC nanoparticles could be produced from solutions of exceptionally high polymer concentration by manipulating pH and ionic strength. The aggregation of the PEC nanoparticles, reversible using either changes in salt concentration or pH, exhibited well-defined hysteresis. Dynamic and static light scattering as well as transmission electron microscopy showed well-defined particles produced in the quasi-soluble limit. Viscosity and titration experiments supported a core−shell model for the dispersed nanoparticles and revealed preferential ion pairing of DADMA with sulfonic acid groups over AA units.

Introduction

Polyelectrolyte complexes, PECs, are made by mixing solutions of oppositely charged polyelectrolytes. These amorphous complexes, in which the constituents are blended at the molecular level, have been the subject of growing interest since Michaels et al. carried out the first systematic studies with synthetic polyelectrolytes.[1,2] PECs have been used for large-scale industrial applications as flocculants, coatings, and binders.[3] PECs have also been applied as vehicles for gene delivery,[4−6] microencapsulation,[7,8] and proton exchange membranes for fuel cells.[9,10] Many studies have focused on the fundamentals of PEC formation.[11−17]

Though conveniently prepared, PECs are generally infusible and insoluble in common solvents.[17,18] Processing PECs is usually done using the polyelectrolyte multilayer method[19,20] or by preparing "quasi-soluble" complexes (qPECs), which are stable colloidal suspensions of PEC nanoparticles, under specific conditions.[22−27] According to Kabanov,[28] a successful approach to preparing qPECs involves mixing polyelectrolytes with weakly acidic ionic groups (such as polycarboxylates) and significantly different molecular weights (the ratio of the degree of polymerization of "host polyelectrolyte", HPE, to "guest polyelectrolyte", GPE, is often greater than 20:1)[29] at nonstoichiometric mixing ratios (the molar ratio of HPE to GPE is typically in the range 1.4−5).[29,30]

Nonstoichiometric PECs may be considered as block copolymers containing free hydrophilic segments of host polyelectrolyte chains and relatively hydrophobic guest−host domains.[26,31,32] Zintchenko et al.[33] reinforce earlier notions that qPECS are preferentially formed under conditions of weak acids, differences in molecular weight, dilute solution, excess of a longer chain component, and the presence of salt in solution. A relatively narrow range of mixing ratios was also specified.[33] The type of polyelectrolytes used for complex formation was shown to be critical. Interestingly (in contradiction to the results described below), PECs with PSS were shown to be unable to form soluble complexes.[33,34]

Recently, copolymers containing charged and neutral blocks were synthesized and combined[35,36] to yield "block ionomer complexes".[37−40] Because of the stabilizing role of the neutral, hydrophilic block, stoichiometric complexes, such as those made from poly(ethylene oxide)-*block*-poly(sodium methacrylate) with poly(*N*-ethyl-4-vinylpyridinium bromide), were reported to be water-soluble.

Although the general conditions for formation of soluble PECs have been established for some time, there are a couple of disadvantages with the classical qPEC systems. The first limitation is the polymer concentration. Classical soluble PECs are only formed in highly dilute solutions (normally in the range of 0.2−1 mM).[34,39,41,42] Restricting conditions to only dilute solution prevents the preparation of complex liquids with interesting non-Newtonian behavior. Another limitation of current qPECs is the lack of a practical method to control the solubility. While PECs may be dissolved in "ternary" solvents containing organic components, a high salt concentration is required to effect solubility. Removal of salt to reprecipitate the PEC is rather cumbersome. Other approaches such as pH stimulus[29,39] are rarely seen in the literature.

In this paper, we present a "stimulus responsive" polyelectrolyte complex, made from widely available components, whose solubility can be reversibly controlled by tuning the pH of the solution.[43] The concentration of the soluble complex reached 0.1 M. The solubility of this PEC could also be reversibly controlled by the presence of added counterions (salt). Such responsive nanoparticulate materials may have applications in pharmaceutics, where self-assembled nanoparticles such as PECs are under intensive scrutiny as drug and gene delivery systems.[4−6]

Experimental Section

Materials. Poly(styrenesulfonic acid), PSS (Sigma Polymer Products, Inc., $M_w \sim 64\,000$ g mol^{-1}, $M_w/M_n = 1.4$, dn/d$c = 0.193$ mL g^{-1}), was used as polyanion. The polycation was a random copolymer: poly(diallyldimethylammonium chloride)-*co*-poly(acrylic acid) (PDADMA-*co*-PAA) (Nalco Inc., Merquat 281, $M_w \sim 230\,000$ g mol^{-1}, $M_w/M_n = 2.6$, dn/d$c = 0.169$ mL g^{-1}) with a mole fraction of acrylic acid (AA) repeat units of 0.36. Concentrations of polymer solutions were based on the repeat unit. The average molecular weight of the copolymer repeat unit, comprising 64 mol % DADMAC and 36 mol % AA, was $(0.64 \times 161.5) + (0.36 \times 72) = 129.3$ Da.

Aqueous solutions of 1−100 mM PDADMA-*co*-PAA and PSS were prepared by diluting polymer stock solutions with the ultrapure

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10.1021/ma061098q CCC: $33.50
Published on Web 10/18/2006

(Barnstead E-pure) water. In all experiments, pH was controlled by dilute acid (HCl) or base (NaOH).

The complex was formed by mixing two polymer solutions at different molar ratios, defined as the molar ratio of copolymer to PSS. Thus, a 1.5:1 polyelectrolyte complex system indicates that this complex was formed by mixing copolymer with PSS at a molar ratio of 1.5:1.

Transmission Electron Microscopy. TEM images were acquired on a JEOL-2010 high-resolution transmission electron microscope at 200 kV with a point resolution of 0.23 nm. Polyelectrolyte complex solutions (10 μL) were cast onto Cu grids (300 mesh) covered with thin amorphous carbon film, and the solvent was left to evaporate in air. The distributions of diameters of the PECs were evaluated using image analysis and processing software (MetaMorph).

Turbidimetric and Viscometric Analysis. Turbidity was measured at 500 nm with a UV−vis spectrophotometer (Lambda 3A, Perkin-Elmer)[14,44,45] to monitor the state of dispersion/solubility of the complex. A high absorbance indicated a high degree of polyelectrolyte aggregation. A solution was considered "soluble" when the absorbance was lower than 0.05. When the absorbance was between 0.05 and 0.4, the polyelectrolyte complex existed as a milky white solution, but the suspension was stable. When the absorbance was higher than about 0.4, the complex began to precipitate out from the solution.

A Cannon-Fenske type viscometer (no. 418, capillary tube size #50) was used to measure the dynamic viscosity at 30 ± 0.1 °C. The viscometer constant was 0.003 966 cSt s^{-1} at 30 °C.

Determination of d*n*/d*c* Values for PSS, PDADMA-*co*-PAA, and qPEC. A Wyatt Optilab-DSP (λ_0 = 690 nm) interferometric refractometer was used to determine the refractive index increment, d*n*/d*c*, values (offline at 25 °C) of PSS and PDADMA-*co*-PAA. Six samples of each polymer in the concentration range of 0.1−1.0 mg mL^{-1} (prepared by serial dilution in 50 mM phosphate buffer pH = 7.14, 0.15 M NaCl) were injected using a 1.0 mL sample loop in line with a six-port injector valve and a syringe pump (Sage Instruments, model 352). The d*n*/d*c* of the complex was taken as the average of the PDADMA-*co*-PAA and PSS refractive index increments. Solvents and polymer solutions were filtered using 0.02 and 0.2 μm filters, respectively. The calibration constant of the instrument (2.289×10^{-4}) was determined using standard NaCl solutions in the concentration range 0.1−1.0 mg mL^{-1}. Data analysis was done using DNDC software (version 5.2) provided by Wyatt Technologies.

Static Light Scattering Analysis. The weight-average molecular weights and polydispersity indices of PSS and PDADMA-*co*-PAA were determined using an Agilent 1100 pump in series with a size exclusion chromatograph (SEC), column (TSK-Gel, 17 μm polymer column, 300 × 7.5 mm, Tosoh Biosciences, covering the molar mass range 4×10^3−1×10^6 g mol^{-1}), and a TSK-GEL guard column, coupled to a DAWN-EOS light scattering detector (K5 type flow cell, Wyatt Technologies) equipped with a GaAs laser (λ_0 = 690 nm) and a Wyatt Optilab-DSP interferometric refractometer. The experiments were performed at 25 °C (mobile phase: 50 mM phosphate buffer, pH = 7.1, 200 ppm of NaN_3, 50 mM or 0.5 M NaCl for PSS and PDADMA-*co*-PAA respectively). Samples (50 μL of 5 mg mL^{-1} polymer solution) were injected into the column using a syringe pump (Sage Instruments, model 352) with a six-port injector. The HPLC setup used was connected to a degasser and a temperature-controlled column. Toluene was used to calibrate the DAWN-EOS instrument (calibration constant 7.99×10^{-4}) and to establish the correct reading for the 90° detector. The other fixed angle detectors (22.5°−147.0°) on the DAWN-EOS were normalized to the calibration detector using 5 mg mL^{-1} (in 50 mM phosphate buffer, pH = 7.1, 200 ppm of NaN_3, 0.5 M NaCl) of dextran (48 600 g mol^{-1}, Sigma). Solvents and polymer solutions were filtered using 0.02 and 0.2 μm filters, respectively. Data analysis was done using ASTRA for windows software (version 4.81.07, Wyatt Technologies).

The molecular weight of qPEC was determined from Zimm's formalism at low concentrations (Debye plots) using the DAWN-EOS detector in batch mode. Solutions of complex (mole ratio of PDADMA-*co*-PAA:PSS was 1.5:1) were prepared in 50 mM phosphate buffer (pH = 7.1, 200 ppm of NaN_3) to a final concentration of 0.5 mg mL^{-1}. Samples were injected into the flow cell using a 1.0 mL sample loop in line with a six-port injector valve and a syringe pump (Sage instruments, model 352). The solvent (phosphate buffer) was introduced first, followed by the complex and solvent again. To determine the molecular weight of qPEC, $R(\theta)/K^*C$ was plotted vs $\sin^2(\theta/2)$ (Debye plot) and extrapolated to zero angle (see Supporting Information). Since we are using a single concentration, a zero concentration fit degree was used instead of the regular first-order polynomial.

Dynamic Light Scattering Analysis. Quasi-elastic (dynamic) light scattering (DLS, Wyatt Technology Corp.) was used to measure the apparent hydrodynamic radius, R_h (nm), of the PDADMA-*co*-PAA/PSS complex at various pH values and salt concentrations. An optical fiber receiver was mounted in the head of detector number 13 (108°) and coupled to an avalanche photodiode in the autocorrelator. The experiment was done in a scintillation vial with collection duration of 2 min and collection interval of 0.5 s. The molar ratio of copolymer to PSS in this experiment was constant at 1.5:1 (by mixing 12 mL of 1 mM copolymer and 8 mL of 1 mM PSS). For comparison, DLS of PSS (1 mM in 50 mM phosphate buffer with no NaCl at pH 7.1) and of PDADMA-*co*-PAA (1 mM in 50 mM phosphate buffer with no NaCl at pH 7.1) was also recorded. Cumulant analysis of the autocorrelation function of the qPEC was done using Wyatt QELS ver 5.27w.06 software to provide a DLS polydispersity index.

Potentiometric Titration. Potentiometric titration was performed with a calibrated pH meter (Accumet Basic AB15, Fisher Scientific) fitted with a glass/reference combination electrode. Both PDADMA-*co*-PAA copolymer solutions and PDADMA-*co*-PAA/PSS complex solutions were titrated with 9.61 mM NaOH.

Results and Discussion

The random copolymer, PDADMA-*co*-PAA, used in this work contained both pH-dependent units (acrylic acid, AA, uncharged at low pH, negatively charged at high pH) and pH-independent units (diallyldimethylammonium, DADMA, positively charged at any pH). When the copolymer was at sufficiently low pH, the AA units were completely protonated, the copolymer chain had only positive charges from the DADMA units, and the net charge density per repeat unit was +0.64. At sufficiently high pH, AA units were fully ionized so that the copolymer chain had both positive charges (0.64 mole fraction) and negative charges (0.36 mole fraction). Therefore, the net charge density per repeat unit of the PDADMA-*co*-PAA at high pH was +0.28.

The complex was formed by mixing the PDADMA-*co*-PAA solution with the PSS solution (1 mM each) at a volume ratio of 1.5:1 to yield a molar ratio of copolymer to PSS of 1.5:1. Since the initial pH of the mixture was 4.1 due to protons generated from the AA units, the formed complex was insoluble. Under these conditions, the ratio of the positive charge (quaternized ammonium on DADMA units, pH-independent) to the negative charge (from PSS) is approximately 1:1 ($1.5 \times 0.64 = 0.96$). The complex was resolubilized by adding dilute NaOH solution to a final pH of 8.5. As imaged by TEM, the unaggregated qPECs were essentially spherical (as seen in Figure 1A).

The average dimension of the nanoparticles was determined to be 37.5 ± 3 nm (from a sample of 150 particles imaged with 200K magnification). A histogram showing an unexpectedly narrow size distribution of nanoparticles is presented in Figure 1B. The fact that the distribution is narrower than that obtained by QELS, and the average size is also larger, may be an indication that the smaller particles are not all being sampled, or the particles have flattened on drying.

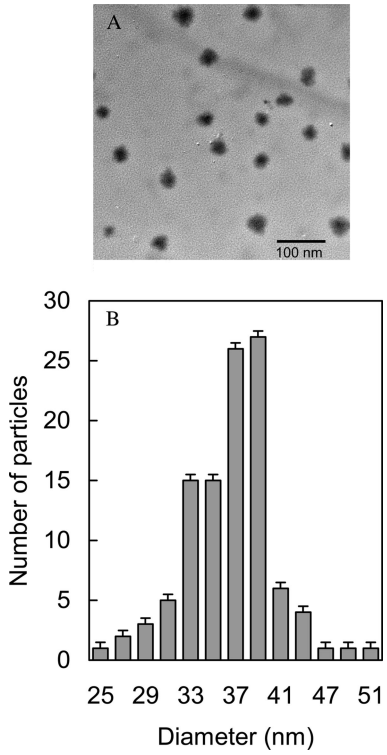

Figure 1. (A) TEM image of polyelectrolyte complexes made by mixing PDADMA-*co*-PAA with PSS at a volume ratio of 1.5:1. No salt was added, other than that generated by the neutralization and complexation processes. (B) A histogram showing the size distribution determined from a sample of 150 nanoparticles of complex. The sizes of particles were measured manually from the image.

The M_w of the complex ($dn/dc = 0.181$ mL g^{-1}) was evaluated from Debye plots (in microbatch mode) by measuring the light scattering of a dilute sample of complex (0.5 mg mL^{-1}) at different angles (θ). Extrapolation to $\theta = 0$ gave the apparent M_w of the complex (540 800 g mol^{-1}), which, at low concentrations (<1 mg mL^{-1}), approaches the real M_w. It was not possible to perform chromatography of the qPEC on our column. Therefore, we were unable to determine M_w/M_n for the qPEC. However, analysis of the QELS autocorrelation function of the qPEC in terms of the normalized second cumulants indicated a polydispersity index of 0.35. For comparison, the QELS polydispersity index of PSS and PDADMA-*co*-PAA was 0.33 and 0.42, respectively.

Effect of pH on the PDADMA-*co*-PAA/PSS Complex. The turbidity of the complex solution, monitored as a function of pH at different temperatures (25, 45, and 65 °C), is presented in Figure 2. The complex was formed by mixing the PDADMA-*co*-PAA solution with the PSS solution (1 mM each) at a volume ratio of 1.5:1. The pH was adjusted with dilute HCl or NaOH solution. No salt was added, other than that generated by neutralization of the carboxylates on the AA units.

Upon mixing the two polymer solutions, the complex formed was insoluble and the absorbance increased to 0.8 (Figure 2). As the solution pH was gradually increased by adding NaOH, the absorbance reading decreased gradually, indicating the change of the solubility of complex from an insoluble state to soluble state. At pH > 5.7, the complex completely dissolved and formed a homogeneous solution. The soluble complex was observed to be stable for many months. When pH was then gradually decreased, a hazy solution was formed between pH 4.5 and 5.5, and the complex slowly precipitated out as the pH was further decreased. Repeating the pH cycle resulted in the same observations, indicating a reversible and reproducible process.

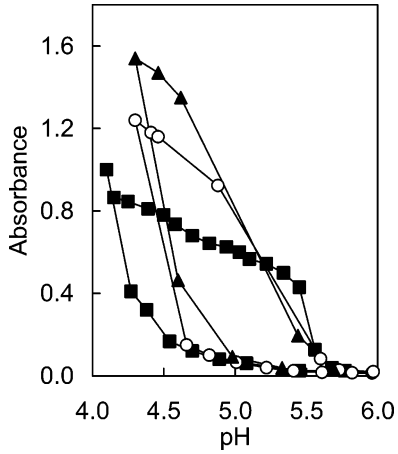

Figure 2. Turbidity (indicated by absorbance at 500 nm) vs pH of a PDADMA-*co*-PAA/PSS polyelectrolyte complex solution. The mixing ratio of PDADMA-*co*-PAA to PSS was 1.5:1. No salt was added. Experiments were conducted at 25 °C (solid squares), 45 °C (open circles), and 65 °C (solid triangles).

An interesting hysteresis is apparent in Figure 2. Over the range at which the complex dissolves/reprecipitates the increasing and decreasing pH tracks do not overlay. Such hysteresis, where the state of a system is at nonequilibrium and depends on the history of the stimulus, is often observed in gels made from cross-linked homopolymers or copolymers.[47−49] Recent work on the pH cycling of polyelectrolyte complexes prepared by the multilayering method also reveals a memory effect, where the complex, existing as a thin film, can exist as one of two states, depending on the pH history.[50] The stability of weak PECs assembled as polyelectrolyte multilayers as a function of pH has also been investigated.[51,52]

Similar turbidity experiments were conducted at 45 and 65 °C (Figure 2). The higher the temperature, the narrower the hysteresis between increasing and decreasing pH, as one would expect for a kinetically limited process.

In order for the molecular rearrangements that would be required to effect transitions between precipitated and soluble PECs, the ion-pairing interactions between the positive (PDADMA) and negative (mostly PSS) repeat units have to be labile. According to studies on the exchange of complexed polyelectrolytes in solution,[31] it was shown that the quaternary ammonium/styrenesulfonate interaction is nonlabile. Salt may be added to reduce interactions and facilitate polyelectrolyte rearrangements within a complex.[53,54]

Dynamic light scattering (DLS) experiments (Figure 3) were performed to follow the apparent size of the PEC particles as a function of pH at two salt concentrations (0.01 and 0.08 M). In this case, the complex was formed by mixing the PDADMA-*co*-PAA solution with the PSS solution (1 mM each) at a volume ratio of 1.5:1, after adjusting their solution pH to 9.5 with dilute NaOH. The soluble complex was passed through 0.2 μm filters. The hydrodynamic radius of the soluble nanoparticles was in the range 18−22 nm when the pH was above 8.5. Below pH 5.5, the PEC aggregated rapidly and its R_h could not be evaluated. Significant hysteresis was observed in the DLS experiments, more so than in turbidity. For DLS, following precipitation, the pH had to be increased to 9.5 to return the qPEC particles to their minimum size. The difference between the two techniques is due to the difference in sensitivity—the turbidity registers the complex as soluble even when it is partially aggregated.

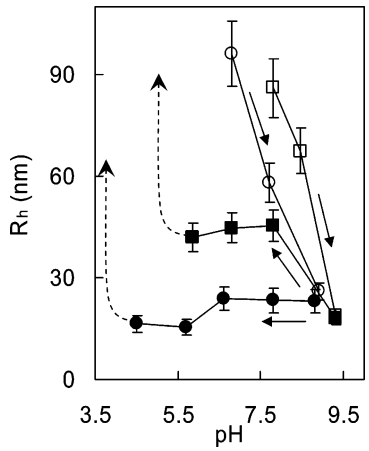

Figure 3. Hydrodynamic radius, R_h (nm), of copolymer/PSS complex vs pH of the solution. The mixing ratio was 1.5:1 (12 mL of 1 mM copolymer and 8 mL of 1 mM PSS). Circles and squares indicate NaCl concentrations of 0.01 and 0.08 M NaCl, respectively. Filled symbols refer to decreasing pH (starting from 9.5).

Higher salt concentrations led to larger R_h, indicating anti-polyelectrolyte behavior, which is expected for complexes with well-paired positive and negative charges. The hysteresis between precipitation and redissolution decreased with higher salt concentration (Figure 3) due to enhanced mobility of polyelectrolyte chains. The particles in higher salt concentration were easier to destabilize by lowering the pH (Figure 3) due to enhanced screening of surface charge.

The apparent hydrodynamic radius of the individual component polyelectrolytes was measured for comparison. At pH 8, with 0.01 M NaCl present in the polymer solutions, the R_h of copolymer was 21.3 ± 2.3 nm, and that of PSS was 7.1 ± 1.5 nm. Only one mode was observed on the autocorrelation function, indicating the polymers were sufficiently screened by salt to suppress an additional "slow mode" that is often observed in polyelectrolyte solutions.[55] On the other hand, the rms radius, R_g, measured by static light scattering for qPEC, PSS, and PDADMA-*co*-PAA were 22.3 ± 0.6, 12.0 ± 0.3, and 33.6 ± 1.2, respectively. The ratio R_g/R_h serves as an indication of the compactness of a molecule and increases as it becomes more compact. The fact that the ratio is 1.09 for the (heavier) PEC but 1.58 and 1.69 for PDADMA-*co*-PAA and PSS, respectively, suggests that the PEC is less hydrated and more compact.

To rationalize these observations, we refer to a structural model of PECs (Figure 4) proposed by Dautzenberg.[3,56,57] According to the model, PEC particles consist of a polymer charge neutral core surrounded by a charged shell of the component which happens to be in excess at nonstoichiometric mixing ratios. This shell stabilizes the particle, via electrostatic repulsion, with respect to further coagulation.

For the present system, when the mixing ratio is 1.5:1, under low pH with fully protonated PAA, the ratio of the positive charge (quaternized ammonium on DADMA units, pH-independent) to the negative charge (from PSS) is approximately 1:1. Since all the charges are balancing each other to form the neutralized core, no net charges remain to form the shell that stabilizes the "quasi-soluble" particles and they aggregate. In addition, the protonated carboxylic groups may interact through hydrogen bonding,[58−61] which would further drive aggregation. When the pH increases, additional negative charge appears via ionization of carboxylates, and the charge balance between positive and negative polymer repeat units is no longer stoichiometric. The excess negative charge is extruded to the surface.[62,63] The additional negative charges exist as a stabilizing charged shell and the bulk precipitate phase separates into nanosized quasi-soluble particles. The process appears to be completely reversible, as numerous precipitation/resolubilization cycles may be performed with the same batch of PEC. The TEM images are of insufficient contrast to provide definitive proof of this core−shell structure, but the DLS measurements in Figure 3 (decreasing pH) show an interesting and reproducible dip in R_h just before aggregation, which we ascribe to dehydration of the shell due to neutralization just before aggregation (Figure 4).

Viscometry and Titration Studies. To elucidate the proposed structural model of the complex, further studies employing viscometry and potentiometric titration techniques were carried out to confirm the state of association of the qPEC. In particular, we wished to address the possibility that the polyelectrolytes may be completely dissociating at high pH, rather than remaining complexed as suggested by the model above.

The viscosity of the complex solution was monitored as a function of mixing molar ratio (Figure 5). Experiments were done at pH 8.5−9.5 to keep the complex soluble. Copolymer solution was added to the PSS. The viscosity of the solution started to decrease as the molar ratio of copolymer to PSS gradually increased, until a minimum was obtained at a molar ratio between 1.5:1 and 2:1.

Then the viscosity increased again with further increase of the copolymer:PSS molar ratio. In a complementary experiment, PSS solution was added to the copolymer solution, and a similar trend was observed. The results were similar when no salt or 0.05 M NaCl was present in the complex solutions.

To interpret the viscosity data of Figure 5, it is understood that the PEC particles are more compact, and therefore contribute less to viscosity, than individual polyelectrolyte molecules. Polymer charge in the core of a quasi-soluble PEC particle is well balanced, and the charge is essentially screened by the high charge density of other polyelectrolyte segments. A polyelectrolyte/polyelectrolyte ion pair is also less hydrated than a polyelectrolyte compensated by a small counterion (i.e., Na^+ or Cl^-).

Before the point of charge equivalence, the concentration of the free polymer molecules being titrated decreases as complex is formed. The viscosity decreases accordingly. Around the mixing ratio of 1.5−2:1 essentially no free polyelectrolyte exists

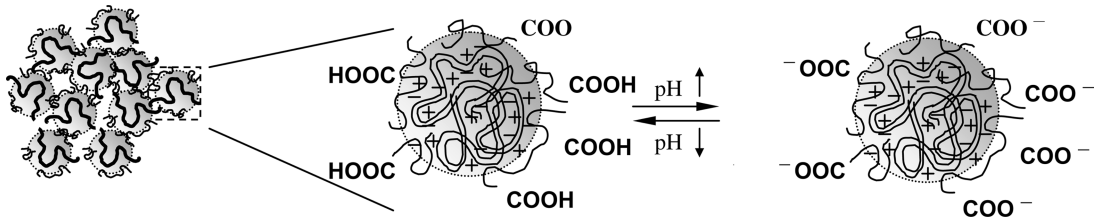

Figure 4. Structural model of the polyelectrolyte complexes. At low pH, the aggregation of the complex particles yields precipitates (left and middle). At high pH, carboxylic groups are deprotonated. The additional negative charges reside on the surface and repel each other, inhibiting aggregation so that the particle of complex is soluble (right).

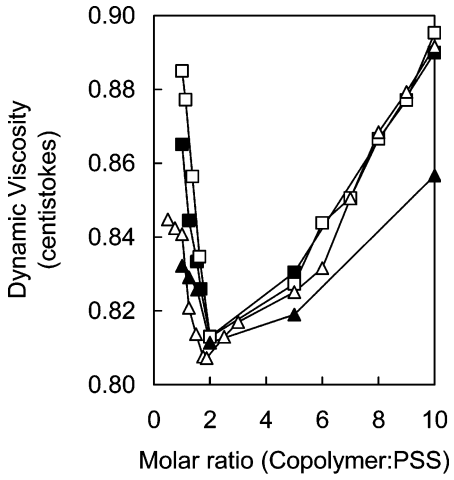

Figure 5. Dynamic viscosity vs mixing ratio of PDADMA-*co*-PAA to PSS. The pH of the complex solution was maintained in the range 8.5−9.5. Polymer concentrations were 10 mM each. Solid squares, adding PSS to the copolymer, no salt present; open squares, adding the copolymer to PSS, no salt present; solid triangles, adding PSS to the copolymer, 0.05 M NaCl; open triangles, adding the copolymer to PSS, 0.05 M NaCl.

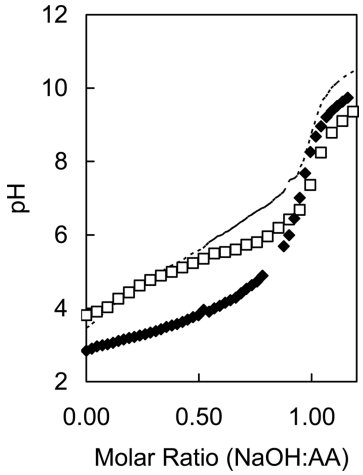

Figure 6. Titration curves of PDADMA-*co*-PAA/PSS complex (molar ratio 1.5:1, 10 mM, open squares); PDADMA-*co*-PAA (10 mM, solid diamonds); and homopolymer PAA (10 mM, dotted line) titrated by NaOH (10 mM). 0.1 M NaCl was present in each solution.

in the solution so that a minimum viscosity is observed. Further addition of polyelectrolyte leads to an excess of this polymer and an increase in viscosity. The result supports the thesis that when the complex is dissolved at high pH the two polymer molecules are still associated. If this were not the case, adding one polymer to the solution would have simply increased the overall polymer concentration and thus increased the solution viscosity. The viscosity as a function of pH was also studied, and the results are presented in the Supporting Information.

The pK_a values of the free copolymer and the complex, as well as homopolymer PAA, in 0.1 M NaCl were measured by means of potentiometric titration, as shown in Figure 6. Titration curves and pK_a values of these systems were also determined at different ionic strengths as summarized in the Supporting Information.

The only titratable groups in these systems are the acrylic acid units on the copolymer chain. The pK_a of copolymer PDADMA-*co*-PAA in 0.1 M NaCl solution is 3.81, which is in good agreement with pK_a's reported in the literature.[62,63] Compared to the pK_a of acetic acid (4.7), the carboxylic group

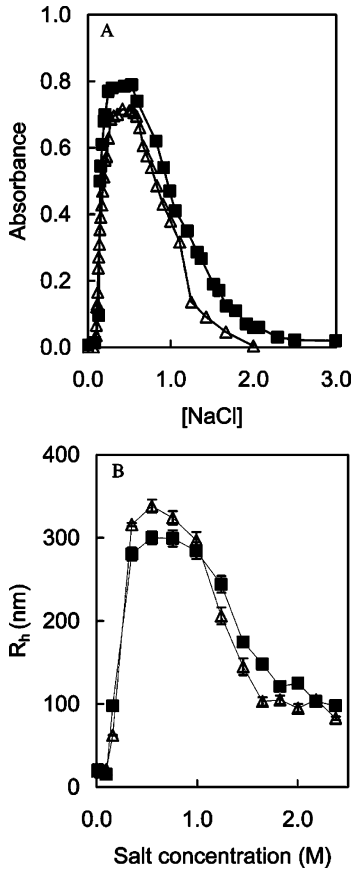

Figure 7. (A) Turbidity (absorbance at 500 nm) vs concentration of NaCl for the PEC. The molar ratio of copolymer to PSS was 1.5:1. The pH of the solution was maintained in the range 8−8.5. (B) Hydrodynamic radius, R_h (nm), of the same PEC. Squares: increasing salt concentration; triangles, decreasing salt concentration.

in the copolymer is more acidic, which is due to the local ion pairing between AA and the permanent positive charges of PDADMA on the copolymer chain.[31,64]

A significant change in titration behavior of PDADMA-*co*-PAA was observed when it formed a complex with PSS. The pK_a of the complex in 0.1 M NaCl solution was 5.31, much higher than that of free copolymer. This is because when forming complex with PSS, SS units pair with DADMA units, making them unavailable for ion pairing with carboxylic groups. This observation is further evidence that free carboxylates exist at the surface of quasi-soluble PECs.

Effect of Ionic Strength on the PDADMA-*co*-PAA/PSS Complex. The solubility of the complex at high pH as a function of the concentration of NaCl is presented in Figure 7. A salt-free complex solution was prepared by mixing 1 mM copolymer solution with 1 mM PSS solution at a ratio of 1.5:1. The solution pH was adjusted to be in the range 8−8.5.

Salt concentration was first increased by adding solid NaCl to the solution of complex. The volume change due to the increment of salt concentration was negligible. The complex remained soluble as long as the salt concentration was below 0.1 M. At higher concentrations, the complex began to precipitate out until the strongest turbidity was reached at around 0.5 M NaCl. After that point the complex began to dissolve again. The complex became completely soluble when the salt concentration was higher than 2 M.

In the second experiment, soluble complex solution was prepared in the presence of 2 M NaCl. Salt concentration was decreased by adding salt-free complex solution to the 2 M salt solution. Decreasing the salt concentration by dilution in this

way essentially backtracked the prior data, reaching a maximum in turbidity at about 0.5 M NaCl. The complex was completely dissolved when the salt concentration was less than 0.1 M with no apparent turbidity. Precipitation/redissolution under the influence of added salt appears to be reversible.

To explain the observations summarized by Figure 7, it is useful to consider the following simplified representation for the association of two oppositely charged segments from different, or the same, polyelectrolyte:

$$Pol^{+}Cl^{-}_{(aq)} + Pol^{-}Na^{+}_{(aq)} \rightleftharpoons Pol^{+}Pol^{-}_{(p)} + Na^{+}_{(aq)} + Cl^{-}_{(aq)} \quad (1)$$

where Pol^{+} represents a polycation repeat unit with chloride counterion and Pol^{-} represents a polyanion repeat unit with a sodium counterion. The subscript "p" refers to the PEC phase, and "aq" refers aqueous phase. The driving force is the (entropic) release of counterions[2,65,66] along with their waters of hydration. In the presence of excess salt, the reverse of eq 1 occurs, swelling the complex as polymer−polymer ion pairs are gradually replaced by more hydrated polyelectrolyte−counterion pairs.[67]

According to Kabanov,[68−70] there are three different regimes of response of PECs to the addition of NaCl: at very low ionic strength (<0.01 M), PECs shrink because of the shielding of the charges by salt; within a certain range of ionic strength, the screening of the electrostatically stabilizing shell around the PEC particles leads to insoluble PECs; further addition of salt leads to complete dissociation of the PEC. In our case, when the salt concentration exceeds about 0.1 M, there is sufficient screening of negative charges to lead to aggregation. The transition to this regime is rather sharp, suggestive of a true phase transition. Further addition of salt swells the complexes. The addition of sufficient amounts of salt (>2 M for the present system) completely dissociates the complex.

A similar salt effect was observed for polyelectrolyte complexes in multilayers (polyelectrolyte multilayers).[71−75] A recent example from our group[71] studied the decomposition of multilayers comprising the same polyelectrolytes used here. Two different stimuli were applied individually, change in salt concentration and change in pH, to effect decomposition of the thin film.[71] However, simultaneous application of these stimuli was not effective in decomposing the multilayers. Clearly, the decomposition strategies may not be cooperative. For example, if one tries to decompose a precipitated PEC by increasing the pH (Figures 2 and 3), additional salt (Figure 7) screens the charges that have been created by the pH change, encouraging aggregation.

It is interesting that much less hysteresis was observed with cycling salt (Figure 7) than with cycling pH (Figures 2 and 3). According to the model outlined above, negative charges created as a result of carboxylate ionization create bulk instability in aggregated PECs. Rearrangement of this charge is required to produce the core−shell arrangement depicted in Figure 4. Specifically, nonlabile DADMA−PSS ion pairs must be broken and rearranged. However, these ion pairs are not perturbed by the pH change, and excess driving force, caused by ionization beyond the equilibrium point, is required for the bulk phase change. In comparison, when salt is used to control the dispersion of the PEC, the concentration of NaCl is usually in the >0.5 M range. This concentration of salt moderates the DADMA−PSS ion pairing, allowing faster polyelectrolyte interdiffusion[54,71] and a response that is closer to equilibrium (i.e., with less hysteresis).

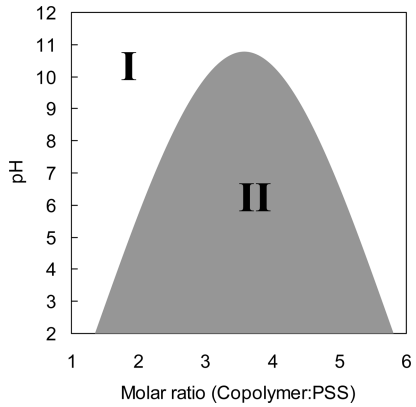

Figure 8. Phase diagram of solubility of the complex at different pH as a function of polyelectrolyte mixing molar ratio (concentration of polymers is less than 0.1 M and the concentration of NaCl is less than 0.1 M). Area I represents the soluble region of the PDADMA-*co*-PAA/PSS complex in the aqueous phase, while area II represents the insoluble region.

Several other factors relating to the robustness of the system have been investigated using the 1.5:1 PEC. For example, the precipitate, isolated by centrifugation and dried, could be dissolved at high pH. Further, the order of mixing had no effect on the experiment results. Another significant finding of this work is that the concentration of the soluble complex could be as high as 0.1 M. Prior work on PECs[31,76] emphasizes the use of very dilute polyelectrolyte solutions to avoid aggregation.

The solubility behavior of the PDADMA-*co*-PAA/PSS system with other molar ratios (copolymer to PSS 1:1, 1.2:1, 1.3:1, 1.4:1, 2:1, and 3.6:1) was investigated in this work, and the results are provided as Supporting Information. To summarize the solubility behavior of the PDADMA-*co*-PAA/PSS complex, a descriptive phase diagram is presented in Figure 8.

Briefly, between the molar ratios of 1:1 and 3.6:1 (on the left side of region II), negative charges are in excess, stabilizing the complex nanoparticles from aggregation. The higher the molar ratio, the more negative charge is needed to compensate the permanent positive charges and the higher the pH required to dissolve the PECs (e.g., pH 4.4 for 1.4:1 system, 4.8 for 1.5:1 system, and 5.5 for 2:1 system). At even higher molar ratio (>4:1, the right side of region II), the PEC will behave more like a classical nonstoichiometric qPEC, with particles stabilized by excess positive charge.

PECs with pH-dependent properties similar to those illustrated above could be employed as enteric coating materials for gastrointestinal drug delivery: the coating would be insoluble in a gastric acid environment and soluble in intestinal fluid. Enteric coatings have traditionally been reserved for drug substances that cause gastric irritation, produce nausea if released in the stomach, or are destroyed by acid or gastric enzymes.[77,78] The water-based coating materials currently used to achieve enteric properties include anionic polymethacrylates (copolymers of methacrylic acid and either methyl methacrylate or ethyl acrylate),[79] cellulose-based polymers such as cellulose acetate phthalate, hydroxypropyl methylcellulose,[80,81] and hydroxypropyl methylcellulose acetate succinate,[82] polyvinyl derivatives such as polyvinyl acetate ethylene phthalate,[83] soft gelatin capsules,[84,85] sodium alginate matrices,[86] and silicone microspheres.[87]

Conclusions

The stability, to changes in salt and proton concentration, of the PEC prepared with the pH sensitive (weak)/pH insensitive

(strong) combination of repeat units on a polyelectrolyte molecule is comparable whether the complex is prepared as a solution precipitate or as a multilayer. Excess polymer charge, produced by ionization of weak acid, is thought to be extruded to the surface[62] and, compensated with counterions, is much more hydrated than core polyelectrolytes.

The titration results reveal selective complexation of one charged repeat unit (SS) over another (AA). When there is competition for DADMA units between SS and AA, pairing with SS is favored, leaving the hydrophilic AA units to stabilize the quasi-soluble PEC nanoparticle. Such preference is reasonable, given that the interaction energy between DADMA and SS is about 15 kJ mol^{-1} stronger than that between DADMA and AA.[71] The effectiveness of the hydrophilic carboxylate shell at stabilizing the qPEC is probably the main reason atypically high qPEC concentrations may be achieved. The resulting nanoparticle suspensions are likely to have interesting and complex viscosity behavior. They may also be interesting vectors for biomolecules, such as proteins and polynucleic acids (DNA and RNA).

Acknowledgment. This work was supported by a grant from the National Science Foundation (DMR-0309441).

Supporting Information Available: Additional turbidity vs salt or pH measurements at various mixing ratios and additional polymer and complex titration curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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MA061098Q