

Microrheological Study of Polyelectrolyte Collapse and Reexpansion in the Presence of Multivalent Counterions

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ABSTRACT: We studied salt-induced changes in the viscosity of dilute solutions of a flexible polyelectrolyte, sodium poly(styrenesulfonate) (PSS), using particle tracking microrheology. It was found that PSS responds distinctively different to the presence of various trivalent cations. In addition to the well-known polyelectrolyte precipitation and redissolution, we also observed more subtle changes in solution viscosity, which can be attributed to chain collapse and reexpansion. In particular, the addition of indium chloride (InCl_3) to PSS solutions leads to polyelectrolyte chain collapse at low salt concentrations, followed by chain reexpansion upon further increasing the salt concentration, as evidenced by an increase in relative viscosity of the polyelectrolyte solution; at even higher salt concentrations, the relative viscosity decreases and chains exhibit a secondary collapse. The salt concentration at which the onset of reexpansion is observed is a function of the polyelectrolyte molecular weight and concentration; rescaling of the data suggests that the key physical quantity is the number of indium ions per polyelectrolyte repeat unit. Upon the addition of chloride salts with other trivalent cations (AlCl_3 and GaCl_3), PSS undergoes precipitation and redissolution, which makes it difficult to draw conclusions about chain expansion at high salt concentrations. A determining factor for the polyelectrolyte response seems to be the size of the trivalent ions. The study provides the first clear experimental evidence in support of recent claims based on numerical simulations [Hsiao and Luijten, *Phys. Rev. Lett.* **2006**, 97, 148301; Hsiao, *J. Chem. Phys.* **2006**, 124, 044904; Hsiao, *Macromolecules* **2006**, 39, 7125]. The hypothesis is that the association of multivalent ions with the monovalent charges on the polyelectrolyte chain leads to charge inversion and that intramolecular repulsion between associated trivalent ions results in chain reexpansion.

Introduction

Polyelectrolyte-counterion interactions have attracted considerable attention due to their relevance for biological and therapeutic applications.^{1,2} Polyelectrolytes are water-soluble macromolecules with ionizable groups along the backbone. The strong Coulombic repulsion between nearby charges tends to stretch the polymer chain; these interactions are moderated by the presence of counterions that screen the Coulombic repulsion. Computer simulations and theoretical studies,^{3–9} as well as experiments,^{10–16} have suggested that many biological and synthetic polyelectrolytes undergo macroscopic phase transitions upon addition of multivalent counterions or charged small molecules due to attractive interactions between like charges in solutions. These attractive Coulombic interactions often lead to formation of insoluble multimolecular aggregates of multivalent ions and polyelectrolytes, which can be redispersed at even higher salt concentrations. Recent numerical simulations by Hsiao and Luijten, however, predict that single macromolecules can show a more subtle response to the addition of multivalent counterions, i.e., chain collapse and reexpansion instead of precipitate formation and redissolution.^{4–6} In these single polyelectrolyte simulations, which were designed to represent the flexible poly(styrenesulfonate) (PSS), the bridging of chains via ions is prevented, and intramolecular ion-polymer interactions dominate. The work suggests that the strongly attractive interactions between multivalent counterions and monovalent charges on the polyelectrolyte backbone lead to effective charge inversion of the polymer chain. As a result, the repulsive Coulombic forces between the associated multivalent counterions reswell the polyelectrolyte chain, as indicated by an increase in the radius of gyration. The simulations showed that the effect strongly depends on counterion valency and size,

with larger, more heavily charged counterions inducing the strongest effects. These results should be predictive of experimental behavior in dilute polyelectrolyte solutions, but no clear experimental evidence has been reported for a model polyelectrolyte like PSS.

In close analogy with this predicted chain collapse and reexpansion, the reelongation of single molecules of collapsed DNA in the presence of the multivalent counterion was observed experimentally.¹⁶ In other work, the conformation of poly(styrenesulfonate) (PSS) was studied in the presence of counterions (La^{3+}) using small-angle neutron scattering (SANS) in the semidilute regime.^{18–22} One study suggests that the polyelectrolyte chains appear to be thicker and that the conformation of chains changes at higher concentrations of multivalent counterion, which may result from bridging phenomena between chains or from counterion condensation.¹⁹ However, because the studies were carried out in the semidilute regime, it is difficult to draw unambiguous conclusions about single-chain conformation.

The aim of this study is to elucidate the complex phenomena of polyelectrolyte-counterion interactions in dilute solutions by measuring the viscosity response of polyelectrolyte solutions upon addition of multivalent counterions. In the dilute regime, the viscosity of polymer solutions is directly linked to the hydrodynamic volume occupied by each chain; intrinsic viscosity is an oft-used quantitative measure of the chain size and conformation. The hard-sphere theory, first developed by Einstein²³ and later augmented by Batchelor,²⁴ predicts that the relative viscosity of a hard sphere suspension will vary as a quadratic function of volume fraction. The theory can accurately relate particle size and concentration to suspension viscosity when interparticle interactions are negligible. Although polyelectrolytes are certainly not hard spheres, in dilute solutions and in the absence of significant shear flow, this is a viable approximation. Prior work in our group has shown the validity of this approach to measure the unfolding of proteins in the

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presence of a denaturing salt.²⁵ Similarly, changes in polyelectrolyte conformation due to the presence of counterions are expected to be readily detected through increased (swelling) or decreased (collapse) viscosity. A systematic study of polyelectrolyte solution viscosity in the presence of various counterions thus seems a viable strategy to investigate the predictions of Hsiao and Luijten.^{4–6}

There is a large body of experimental work dealing with the rheology of PSS in the absence of added salt, as was summarized comprehensively by Boris and Colby.²⁶ However, much less experimental data is available with regards to the rheological behavior of PSS solutions with added salt, and the available studies mostly focus on monovalent counterions.²⁶ In addition, experimental studies on the rheology of polyelectrolyte solutions mostly take place in the semidilute regime because traditional rheometers are relatively insensitive for low-viscosity aqueous solutions and interfacial effects can interfere with measurements.

In comparison with conventional rheology, particle tracking microrheology (PT μ R) is more sensitive at low sample viscosities and thus more suitable for accurately measuring viscosities of dilute polyelectrolyte solutions with and without added salts. The method is also relatively fast and requires only microliter-sized samples. With the image processing power of current desktop PCs, the acquisition and analysis of accurate particle trajectories take ca. 5 min per measurement and can be mostly computer-controlled without operator interference. The small sample size and automation of the data analysis procedure enable for \sim 250 measurements per day in contrast with 5–10 samples per day on a conventional rheometer; this is sufficient to generate rheological phase diagrams for macromolecules.²⁷ PT μ R also has the potential for performing high time resolution kinetic measurements,²⁸ using the ability of high-speed video analysis to detect viscosity responses associated with polyelectrolyte–counterion interactions under changing solvent conditions.

Passive microrheology uses thermal fluctuations to evaluate sample viscosity and the resulting Brownian motion of probe particles to evaluate sample viscosity. The mean-squared displacement (MSD) of embedded spherical probes in a Newtonian fluid is described by the Stokes–Einstein equation:

$$\text{MSD} = \langle \Delta \vec{r}(\tau)^2 \rangle \equiv \langle |\vec{r}(t + \tau) - \vec{r}(t)|^2 \rangle = \frac{dk_b T}{3\pi\eta a} \tau \quad (1)$$

where the thermal energy, $k_b T$, promotes the displacement of spheres of radius a as a linear function of the lag time, τ . The dimensionality d of the trajectories is generally equal to 2 in conventional microscopy.^{28–30}

In this paper, the goal is to study the interaction of PSS polyelectrolyte with several chloride salts (NaCl, CaCl₂, AlCl₃, GaCl₃, InCl₃, TlCl₃, LaCl₃, and CeCl₃) with cations of different sizes (ca. 0.05–0.12 nm) and valencies (1–3) using PT μ R. PSS polyelectrolyte solution rheology and phase behavior were studied over a wide range of salt concentrations as well as with different monomer concentrations and molecular weights of PSS polyelectrolyte. The results suggest that PSS polyelectrolyte solution rheology and phase behavior associated with polyelectrolyte–counterion interactions are dependent on the counterion valency, counterion size, monomer concentration, and molecular weight of PSS polyelectrolyte. In agreement with the simulations of Hsiao and Luijten, we found that PSS polyelectrolyte exhibits a reexpansion upon the addition of certain trivalent salts. The counterion size seems to be an important indicator of the occurrence of the reswelling transition. Most importantly, the transition also depends on the molecular weight and concentration of the PSS polyelectrolyte. In order to investigate the reversibility of the rheological response to salt addition, transient microrheological experiments were performed in a dialysis cell. Finally, viscosity measurements on several

PSS polyelectrolyte solutions with different InCl₃ salt concentrations were also performed on a controlled-stress rheometer for comparison.

Experimental Section

The sodium poly(styrenesulfonate) samples were purchased from Scientific Polymer Products. Three NaPSS samples with different molecular weights were used in this study and they are denoted as **PSS1M** ($M_w = 1\,188\,400$, $M_w/M_n = 1.18$, and Lot 02), **PSS500K** ($M_w = 505\,100$, $M_w/M_n = 1.24$, and Lot 490411002), and **PSS125K** ($M_w = 126\,700$, $M_w/M_n = 1.17$, and Lot 100605003). Sodium chloride (NaCl) was purchased from Fisher Scientific and calcium chloride dihydrate (CaCl₂·2H₂O) was purchased from Alfa Aesar. Aluminum chloride hexahydrate (AlCl₃·6H₂O), gallium chloride (GaCl₃), indium chloride (InCl₃), thallium chloride tetrahydrate (TlCl₃·4H₂O), lanthanum chloride (LaCl₃), cerium chloride (CeCl₃), and Pluronic F-127 were purchased from Aldrich. All purchased chemicals were used without further treatment. The solutions were prepared by using deionized water and passed through a syringe filter with nominal pore size 0.2 μ m.

Amine-functionalized and carboxylated fluorescent-latex particles (nominal size 1 μ m in diameter in suspensions at 2 wt %) were purchased from Invitrogen. Because these charge-stabilized particles tend to aggregate in the presence of polyelectrolytes and multivalent counterions, they were PEGylated to create sterically stabilized particles by slightly adjusting procedures which were first reported by Kim and co-workers.³¹ A Pluronic F-127 solution was prepared by dissolving 47 mg of Pluronic F-127 in 2.25 mL of deionized water. Then 0.25 mL of fluorescent particle suspension (\sim 2 wt %) was added to the Pluronic solution and stirred (300 rpm) for 3–5 h. Then 0.045 mL of toluene was added, and the suspension stirred for an additional 2 h. After that, the vial with the suspension was immersed in a 90 °C water bath, while blowing air across the surface to strip off toluene for about 20–30 min. Centrifugation (4100 rpm and 12 min, Eppendorf MiniSpin) was used to collect the particles, followed by washing the particles five times (redispersion in 1.8 mL of deionized water, centrifugation to collect the particles, and removal of 1.8 mL of supernatant). Finally, the particles were redispersed in 0.2–0.25 mL of deionized water to create suspensions of PEGylated amine-functionalized or carboxylated fluorescent-latex particles for use in PT μ R. The particle size after functionalization was characterized by measuring the diffusion coefficient in DI water at a known temperature.

For steady state microrheological measurements, 30 μ L of the polymer solution was mixed well with 30 μ L of the salt solution and 2 μ L of PEGylated particle suspension was added. The resulting solution was vortex-mixed for about 10 s, followed by brief sonication for about 5 s to ensure the dispersion of tracer particles. The viscosities of the polyelectrolyte solutions were determined as a function of salt concentration by evaluating the trajectories of PEGylated amine-functionalized or carboxylated fluorescent-latex particles and applying the Stokes–Einstein equation for the diffusion of the probe particles (eq 1). An inverted Leica DM-IRB microscope equipped with an analogue 640 \times 480 pixel CCD camera (COHU 4920, Poway, CA) was used to monitor the Brownian motion of the fluorescent-latex particles at 30 frames/s and recorded digitally using specialized acquisition software (OpenBox). Image analysis software (IDL, Research Systems Inc.) identifies the particle trajectories, tabulating the time evolution of particle positions.²⁸ Individual movies were 83 s long (2500 frames) and included an average of 500 trajectories. The spatial resolution of the entire setup is 0.157 μ m per pixel for a 63 \times objective. For all polymer solutions, the linear scaling between MSD and τ was found to be valid through all measurable times (data not shown). Subsequently, the slope ($\Delta\tau/\Delta\text{MSD}$) is used to calculate the viscosity η (eq 1). The experimental errors of the viscosity measurements due to sample preparation and statistical data analysis were estimated implicitly by repeating experiments multiple times. It was found that viscosities could be determined with less than 5% error at low salt concentrations (for viscous solutions) and less

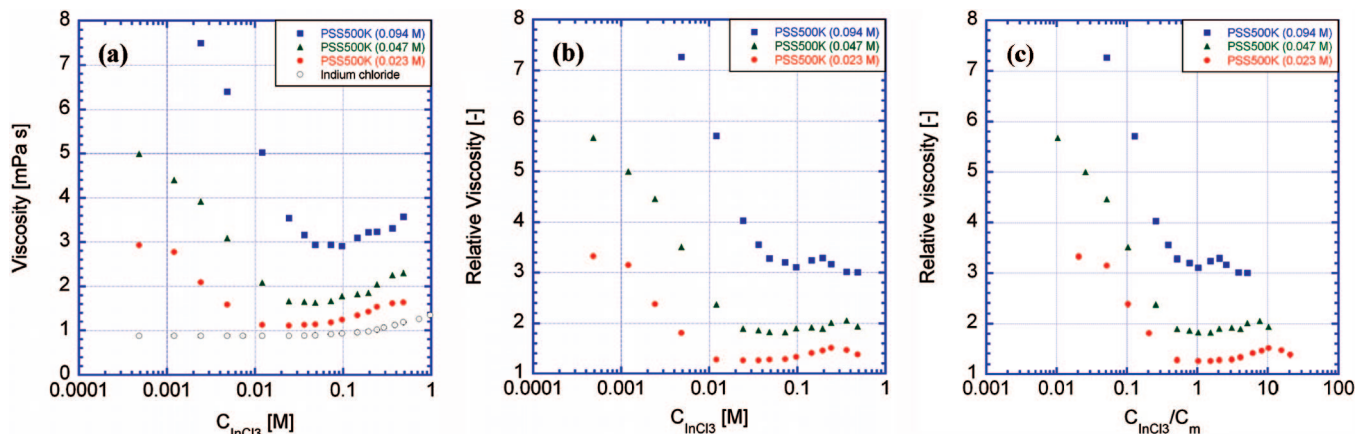


Figure 1. (a) Viscosity and (b) relative viscosity of **PSS500K** with different monomer concentrations C_m (0.094, 0.047, and 0.023 M) as a function of InCl_3 salt concentration. (c) Relative viscosity of **PSS500K** as a function of InCl_3 salt/monomer molar ratio.

than 3% error at high salt concentrations, where the phenomena of interest occurred: chain reexpansion and secondary collapse. As a result, error bars are smaller than the data symbols in most plots presented below.

The homogeneity of all solutions was investigated by quantifying the deviation of the particle displacement probability distribution from a Gaussian, which is indicative of a homogeneous sample; in particular, the ratios of the second and fourth moment of the distribution were compared.^{32,33} From this analysis, it could be concluded unambiguously that all samples showed Gaussian displacement distributions, and no statistically significant heterogeneity could be observed.

For transient microrheological measurements, the sample preparation methods were the same as for the steady state microrheological measurements, except that 3 μL of PEGylated amine-functionalized or carboxylated fluorescent-latex particles was added to slightly increase the particle concentration. We used the dialysis cell setup developed by Sato and Breedveld.³⁴ Rapid and reversible changes of sample solvent composition can be achieved by switching the external flow through a supply system of syringe pumps, tubing, and valves. The solvent is pumped into the reservoir with 10 mL/h flow rate, and the viscosity response in the sample chamber was measured by switching between two InCl_3 salt concentrations, 0.5 and 0.05 M.

Results and Discussion

The evidence of polyelectrolytes chain collapse and reexpansion upon addition of a trivalent salt was reported, and the effect of monomer concentration on chain collapse and reexpansion was investigated. Figure 1a shows the absolute viscosity of pure salt solutions and **PSS500K** solutions at different polyelectrolyte concentrations as a function of InCl_3 concentration. To emphasize the viscous contributions of the polyelectrolyte, the relative viscosity $\eta_r = \eta_{\text{solution}}/\eta_{\text{salt}}$ is presented in Figure 1b. The polyelectrolyte concentrations is expressed as the concentration of monomer repeat units in the solution, C_m , and experiments were performed at 0.094, 0.047, and 0.023 M. Precipitation of **PSS500K** was not seen for InCl_3 salt. Instead, as can be seen in Figure 1b, the relative viscosity decreases with the addition of InCl_3 salt, followed by a small, but significant, increase of relative viscosity once more salt was added. The data support the work of Hsiao and Luijten,^{4–6} who observed an initial decrease of relative viscosity due to the collapse of the polyelectrolyte chains as a result of Coulombic screening by the counterions and subsequent increase of solution viscosity. Comparison of parts a and b of Figure 1 shows that at high salt concentration there are two contributions to an increase in viscosity: the solvent viscosity and a secondary effect related to the polyelectrolyte, presumably chain expansion. The

relative viscosity data highlights the polyelectrolyte contribution and proves that the chains indeed undergo a change in conformation when the salt concentration is increased. As described above, an increase of relative viscosity is indicative of an increase of effective polyelectrolyte volume fraction and thus chain size. The collapse concentration C_z is defined as the salt concentration at which the chains reach their most compact state and the lowest relative viscosity occurs. The minimum volume fraction, ϕ_{min} , at the collapse concentration C_z was calculated using the hard-sphere Einstein–Batchelor theory with coefficients constrained to 2.5 and 6.2 and the molecular weight of PSS equal to 505 100. From this analysis, the minimum volume fractions, ϕ_{min} , at 0.023, 0.047, and 0.094 M are 8.6%, 21%, and 42%, respectively. And the corresponding effective minimum radii of the PSS coils at these different monomer concentrations show thus the following order: $(r_{\text{min}})_{0.094 \text{ M}} \sim (r_{\text{min}})_{0.047 \text{ M}} > (r_{\text{min}})_{0.023 \text{ M}}$. It is worthy to point out that $(r_{\text{min}})_{0.094 \text{ M}}$ might be underestimated by the hard-sphere theory because the volume fraction is greater than 20%, so that multiparticle interactions cannot be ignored.²⁹ The results show that the more dilute the monomer concentration, the smaller value the minimum of volume fraction is. The effect has also been predicted by computer simulations.⁵

The computer simulations also predict that this collapse concentration C_z is proportional to monomer concentration.^{4,6} As shown in Figure 1b, the data show that the minimum in relative viscosity indeed shifts to lower salt concentration at lower monomer concentration. By replotting the data in Figure 1c as a function of ratio of counterion and polyelectrolyte concentration, this effect is highlighted. Although it is hard to unambiguously assign the exact collapse concentration C_z for each curve, it seems that after rescaling the minima for all solutions occur at values of C_{InCl_3}/C_m between 0.7 and 1. According to computer simulations, if the counterions and monomers have the same size, the smallest value of squared radius of gyration, R_g^2 , occurs for C_s near the equivalence concentration C_z^* , the salt concentration at which the total charge of the z -valent cations neutralizes the bare polyelectrolyte charge, $C_z^* = 3/C_m \sim 0.333$ for trivalent counterions and $C_z^* = 4/C_m \sim 0.25$ for tetravalent counterions.⁴ If the counterions are half the size of the monomers, the minimum radius of gyration (R_g^2) was found at salt concentrations much higher than C_z^* , with $C_{z=3}/C_m$ between 0.5 and 0.8 for trivalent counterions and $C_{z=4}/C_m$ between 0.25 and 0.35 for tetravalent counterions.^{4,5} The supposed reason for this shift is that the effective valency of the salt reduces due to a strong increase in counterion–coion attraction. Consequently, the chains are not neutralized at C_s , but more salt must be added to reach the minimum of R_g^2 .^{4,5} In

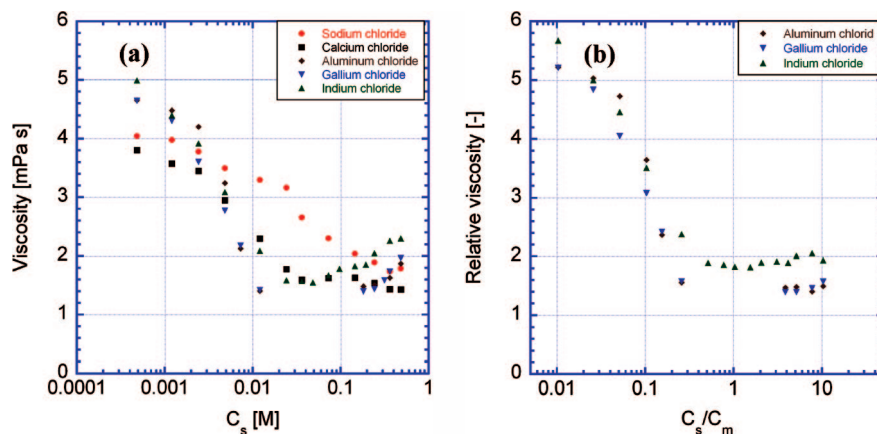


Figure 2. (a) Viscosity of PSS500K ($C_m = 0.047$ M) as a function of salt concentration and (b) relative viscosity of PSS500K ($C_m = 0.047$ M) as a function of salt/monomer molar ratio. The errors of the data are within 5%.

comparison with the predicted C_z/C_m from simulations, Figure 1c show excellent agreement, since the ion size of In^{3+} (0.076 nm) is slightly less than half the size of the monomer (styrenesulfonate) (see Supporting Information).

It is important to point out that ion bridging between chains via multivalent counterions and intermolecular polyelectrolyte–polyelectrolyte interactions could affect the solution rheology. However, for the two most dilute samples, the relative viscosity remains below 2 at all salt concentrations, which is clearly in the dilute regime, so that one can expect the interactions between polyelectrolyte chains are negligible and ion bridging between chains is unlikely to happen. Therefore, the increase of relative viscosity at higher salt concentration must predominately be attributed to intrachain interactions, in particular to chain swelling due to the counterion condensation on the chains leading to charge overcompensation. Recent studies have shown that multivalent counterions can overcompensate the macromolecular charge on DNA when present at high salt concentration.^{3,7,35,36} Such charge inversion of negative-charged PSS polyelectrolyte by In^{3+} counterions is likely to happen here as well. This is consistent with the decrease of relative viscosity at very high salt concentrations (>0.25 M, see Figure 1), since the chloride anions will eventually electrostatically screen the charge-inverted PSS polyelectrolyte chains at sufficiently high concentrations. The swelling effect is most obvious and convincing at the lower polyelectrolyte concentration. One might expect that higher polyelectrolyte concentrations show a stronger increase in relative viscosity, but it is important to realize that these samples also require a higher salt concentration, thus enhancing the electrostatic screening effects and obscuring the charge inversion and swelling. There are no literature data to compare these findings of secondary collapse with, since no simulation work has ever done at relatively high salt concentrations (>0.1 M).

Next, the effects of counterion valency and size on the stability and viscosity of polyelectrolyte in solution were investigated. Figure 2 shows the viscosity of PSS500K as a function of salt concentration and relative viscosity of PSS500K as a function of trivalent salt/monomer molar ratio. The monomer concentration of PSS500K studied here was 0.047 M. Mono- and divalent chloride salts (NaCl and CaCl_2) are included for comparison. Precipitation and redissolution of PSS500K were observed for GaCl_3 and AlCl_3 salts, but not for NaCl, CaCl_2 , or InCl_3 salts. The polyelectrolyte precipitation and redissolution have been well studied and reported in the literature,^{3,8–15} and the observations in this study are in accordance with previous work. Also, the observed chain collapse and reexpansion in this study have been predicted by numerical simulations.^{4–6} The initial decrease in relative

viscosity due to electrostatic screening is stronger for cations with higher valency, as expected. Upon the addition of monovalent salt (NaCl), the repulsions between polyelectrolyte charges are screened more gradually, and the solution viscosity decreases continuously with increasing salt concentration. For divalent salt (CaCl_2), the solution viscosity decreases much stronger at lower salt concentration, and the polyelectrolyte chain collapses to a more compact state. The apparent minimum in absolute viscosity for CaCl_2 can fully be attributed to the increasing salt solution viscosity; when the relative viscosity was calculated, no reswelling could be observed.

Several studies suggest that the screening of electrostatic repulsions is sensitive to counterion valency and size and thus related to chain precipitation and redissolution (or chain collapse and reexpansion).^{4–6,9,11} For comparison, the ion size (ionic radii) of all the cations were retrieved from the literature and found to be in the following order:^{37,38} Ca^{2+} (~ 0.103 nm) $>$ Na^+ (0.0997 nm) $>$ In^{3+} (0.076 nm) $>$ Ga^{3+} (0.062 nm) $>$ Al^{3+} (0.050 nm). The calculation of the ionic radii in aqueous solutions is derived by subtracting the radius of a water molecule, r_{water} , from the mean internuclear distances, $d_{\text{ion-water}}$, between ions and the oxygen atoms of the surrounding water molecules, that is, $d_{\text{ion-water}} - r_{\text{water}}$.³⁷ These “bare” ionic radii of all the cations were calculated and are reported here. When the hydrated ionic radii are used instead for all the cations in this study, their ordering does not change. At very low salt concentration (<0.005 M), the screening of electrostatic repulsions by NaCl and CaCl_2 is stronger than trivalent salts (AlCl_3 , GaCl_3 , and InCl_3) as evidenced by the lower solution viscosity, suggesting that the charge screening is mainly contributed by the effect of counterion size. As the salt concentration increases (>0.005 M), for trivalent salts (AlCl_3 , GaCl_3 , and InCl_3), the effect of counterion valency takes over and results in stronger decreases of solution viscosity, which indicates the polyelectrolyte chains collapse to a more compact state. The results of these experimental investigations of the effect of counterion size on the polyelectrolyte chain size qualitatively support the simulation works.^{4–6} Further addition of trivalent salt lead to chain precipitation and redissolution for AlCl_3 and GaCl_3 salt, while chain collapse and reexpansion occurred in the presence of InCl_3 salt. The results are interesting and suggest that counterion valency is not the only factor influence polyelectrolyte phase behavior.

Experiments^{10–15} have shown that multivalent salts or molecules can induce phase separation in polyelectrolyte solutions if the salt or molecule concentration is higher than a critical value C^* . Above C^* , precipitation of polyelectrolyte occurs and the solution has two phases. The precipitate subsequently redissolves and the solution returns back to a single

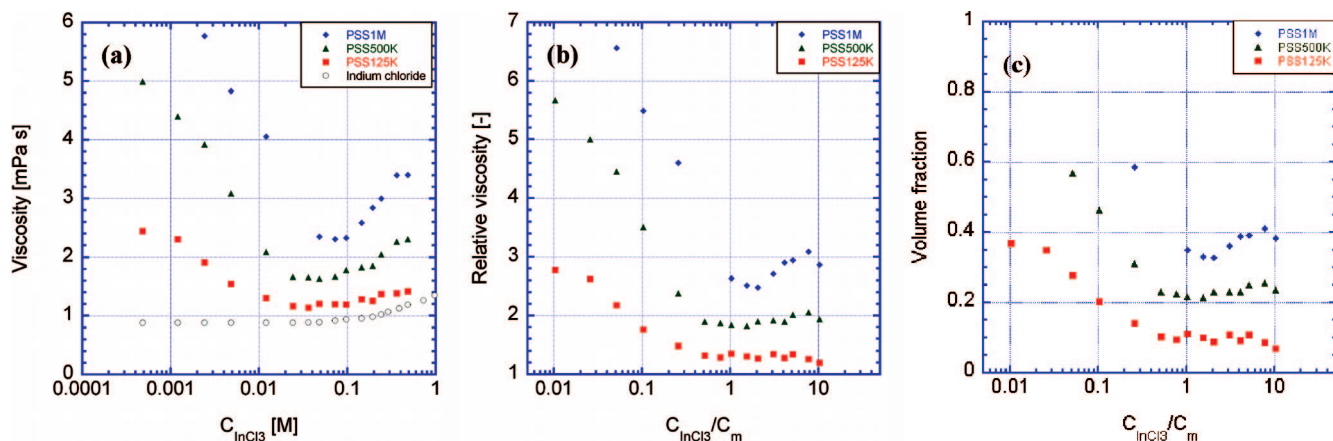


Figure 3. (a) Viscosity of PSS1M, PSS500K, and PSS125K ($C_m = 0.047$ M) as a function of InCl_3 salt concentration, (b) relative viscosity of PSS1M, PSS500K, and PSS125K ($C_m = 0.047$ M) as a function of InCl_3 salt/monomer molar ratio, and (c) volume fraction of PSS1M, PSS500K, and PSS125K ($C_m = 0.047$ M) as a function of InCl_3 salt/monomer molar ratio. The errors of the data are within 5%.

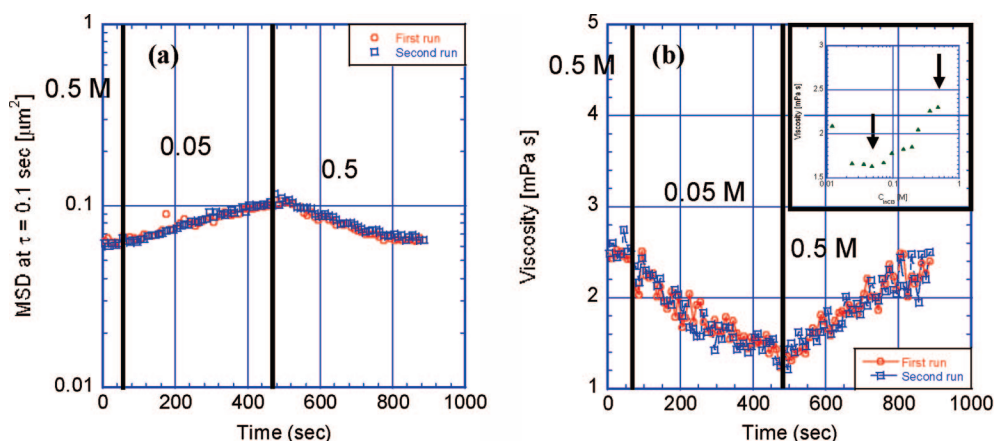


Figure 4. (a) Temporal evolution of tracer mean-squared displacement and (b) viscosity of PSS500K. The inset shows viscosity of PSS500K as a function of InCl_3 salt concentration. The monomer concentration of PSS500K is 0.046 M.

phase if the salt or molecule concentration is increased beyond a second critical value C^{**} . To get more insight in the influence of counterion size and specificity of the trivalent counterions on polyelectrolyte phase behavior, the precipitation regime of PSS500K ($C_m = 0.047$ M) was also determined in the presence of trivalent salts (CeCl_3 , LaCl_3 , TlCl_3 , InCl_3 , GaCl_3 , and AlCl_3). The ion size (ionic radii) of all the trivalent cations are found in the following order:^{37,38} Ce^{3+} (0.116 nm) \sim La^{3+} (0.114 nm) $>$ Tl^{3+} (0.084 nm) $>$ In^{3+} (0.076 nm) $>$ Ga^{3+} (0.062 nm) $>$ Al^{3+} (0.050 nm). Precipitation and redissolution of PSS500K were observed for CeCl_3 , LaCl_3 , GaCl_3 , and AlCl_3 , but not for InCl_3 and TlCl_3 . The range of precipitation regime, as quantified by $C^{**} - C^*$, was found to show the following order: $\text{Ce}^{3+} \sim \text{La}^{3+} >$ $\text{Ga}^{3+} >$ Al^{3+} , corresponding to the order of counterion size. The onset of precipitation was found to occur at $(C^*/C_m)_{\text{Al}} = 0.33 \pm 0.01$ for AlCl_3 salt, $(C^*/C_m)_{\text{Ga}} = 0.30 \pm 0.01$ for GaCl_3 salt, and $(C^*/C_m)_{\text{Ce}} \sim (C^*/C_m)_{\text{La}} \sim 0.2$ for CeCl_3 and LaCl_3 salts. For comparison, other researchers reported C^* to be approximately equal to $0.2C_m$ for LaCl_3 salt.¹⁵ The second critical value, the redissolution concentration C^{**} , exhibited the following order: $(C^{**}/C_m)_{\text{Ce}} \sim (C^{**}/C_m)_{\text{La}} >$ $(C^{**}/C_m)_{\text{Ga}} >$ $(C^{**}/C_m)_{\text{Al}}$, also corresponding to counterion size. The onset of redissolution was found to occur at $(C^*/C_m)_{\text{Al}} = 2.61 \pm 0.02$ for AlCl_3 salt, $(C^*/C_m)_{\text{Ga}} = 3.06 \pm 0.02$ for GaCl_3 salt, and $(C^*/C_m)_{\text{Ce}} \sim (C^*/C_m)_{\text{La}} \sim 10.5 \pm 0.4$ for CeCl_3 and LaCl_3 salts.

Chain collapse and reexpansion of PSS polyelectrolyte were also observed in the presence of TlCl_3 salt when the measurements were done right after the solutions were prepared. However, for TlCl_3 salt solutions, brown particulates precipitated

out of the pure salt solutions on the time scale of minutes at low salt concentration (< 0.01 M). For the PSS polyelectrolyte solution with added TlCl_3 salt, PSS polymer dissolved at first and then precipitated of PSS polymer along with brown particulate after several minutes. Because of these issues with instability of TlCl_3 salt solutions, no data are reported here with regards to precipitation and redissolution concentrations.

Finally, the effect of chain length (or molecular weight) on the collapse and reexpansion of polyelectrolytes was investigated. Figure 3 shows the viscosity of polyelectrolytes with different chain lengths (or molecular weight) as a function of InCl_3 salt concentration and the calculated relative viscosity polyelectrolytes with different chain length (or molecular weight) as a function of InCl_3 salt/monomer molar ratio. The calculated volume fraction of PSS1M, PSS500K, and PSS125K ($C_m = 0.047$ M) as a function of InCl_3 salt/monomer molar ratio using hard-sphere theory is also included. The monomer concentration for PSS1M, PSS500K, and PSS125K was kept constant at 0.047 M. Only PSS1M was found to precipitate between 0.015 and 0.04 M salt concentration, while PSS500K and PSS125K remained in solution at all salt concentrations. As shown in Figure 3, the behavior is similar to that presented in Figure 1: the solution viscosity decreases upon addition of salt at low salt concentrations and then increases once more salt is added due to both the increase of solvent viscosity and polyelectrolyte chain expansion. When normalized to eliminate solvent effects, the relative viscosity emphasizes the polyelectrolyte effect, and the extent of expansion clearly depends on the molecular weight of PSS polymer, as shown in Figure 3b.

In Figure 3c, the changes of volume fraction in solutions with added salts give a clear trend and indicate the extent of expansion clearly depends on the molecular weight of PSS polymer. The higher molecular weight materials show a larger effect, as might be expected because of the scaling relations for the dimensions of polyelectrolyte coils as a function of molecular weight. The main precaution is that at very high molecular weights precipitation obscures the picture, so that the intermediate molecular weight **PSS500K** represents the most well-defined experimental scenario.

In order to cross-check that $PT\mu R$ is a valid method for measuring salt-induced viscosity changes of dilute polyelectrolyte solution in the presence of multivalent counterions, viscosity measurements on **PSS1M** and **PSS500K** were performed on a controlled stress rheometer (Anton Paar MCR300) at 0.05 and 0.5 M InCl_3 concentrations. The rheometer data showed Newtonian behavior for all samples in the low-shear region, and zero-shear viscosities of **PSS1M** in 0.05 and 0.5 M InCl_3 salt solutions were respectively 2.4 and 3.4 $\text{mPa}\cdot\text{s}$, in excellent agreement with the data shown in Figure 3. Similar agreement was found for the zero-shear viscosities of **PSS500K** in 0.05 and 0.5 M InCl_3 salt, respectively 1.7 and 2.3 $\text{mPa}\cdot\text{s}$.

An intriguing question that remains after the $PT\mu R$ experiments presented above concerns the reversibility of the observed viscosity response at different salt concentration. One would hope that the observed behavior represents equilibrium and that is possible to switch between collapsed and reexpanded swollen states by adjusting the salt concentration. Unfortunately, traditional methods do not enable in situ changes in salt concentration. A microdialysis cell for particle-tracking microrheology³⁹ enables such measurements by exposing a sample to different solvents via a dialysis membrane. For this study, a **PSS500K** solution at 0.046 M was initially prepared in InCl_3 salt solution at 0.5 M, exposed to 0.05 M for 420 s, and subsequently dialyzed against 0.5 M salt solution for 420 s; the sample was exposed to the same protocol two times. Figure 4 shows the temporal evolution of tracer mean-squared displacement (MSD) and viscosity of the **PSS500K** at lag time 0.1 s during these transient experiments. The MSD (Figure 4a) increases with the removal of salt and decreases again when the sample is dialyzed against high salt concentration (0.5 M). The corresponding viscosities (Figure 4b) were obtained from the Stokes–Einstein equation, eq 1. The experiments show that the viscosity response to solvent change was rapid and reversible. The microrheological viscosities in these transient experiments were found to be 1.6 $\text{mPa}\cdot\text{s}$ for 0.05 M InCl_3 salt solution and 2.4 $\text{mPa}\cdot\text{s}$ for 0.5 M InCl_3 salt solution, in good agreement with the steady-state microrheology shown in Figure 3. The minor deviations in viscosity originate from differences in sample concentration and treatment.

Conclusions

Here we demonstrated that particle tracking microrheology can accurately measure viscosities of dilute PSS polyelectrolyte solutions with added trivalent salts and generate rheological phase diagrams including information on both solution rheology and phase behavior. The interactions between PSS polyelectrolyte and trivalent counterions are complex and dependent on the counterion valency, counterion size, PSS polyelectrolyte molecular weight, and concentration. In addition of the well-known chain precipitation and redissolution, we also first found that PSS polyelectrolyte in a solution with added InCl_3 salt undergoes a chain collapse and followed by a reexpansion at

higher salt concentrations in support of the theoretical findings by Hsiao and Luijten. The results suggest the extent of reexpansion is not only a function of the PSS polyelectrolyte monomer concentration as predicted by Hsiao and Luijten but also a function of the PSS polyelectrolyte molecular weight.

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Supporting Information Available: Viscosity of aluminum chloride (AlCl_3), gallium chloride (GaCl_3), and indium chloride (InCl_3) salt solution as a function of salt concentration and details on estimation of effective monomer size. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

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