The Thiouronium Group for Ultrastrong Pairing Interactions between Polyelectrolytes

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Abstract

Various charged groups may be used as a repeat unit in polyelectrolytes to provide physical interactions between oppositely-charged polymers leading to liquid-liquid phase separation. The materials formed thus are termed polyelectrolyte complexes or coacervates, PECs. The strength of pairing between positive, Pol^+, and negative, Pol^-, repeat units, depends on the specific identity of the monomer repeat unit. In this work, the pairing strength of the thiouronium group, a cation closely related to guanidinium, is evaluated using a polythiouronium polyelectrolyte. Polymers containing guanidinium, notably polyarginine, a peptide, are known for their unusual behavior, such as the formation of like-charge ion pairs and hydrogen bonding. It is shown here that some of this behavior is carried over to polythiouroniums, which results in exceptionally strong interactions with polyanions such as polysulfonates and polycarboxylates. The resilience of the polythiouronium/Pol^- interaction was evaluated using the buildup of polyelectrolyte multilayers at various salt concentrations, and by breaking up preformed PECs with high concentrations of

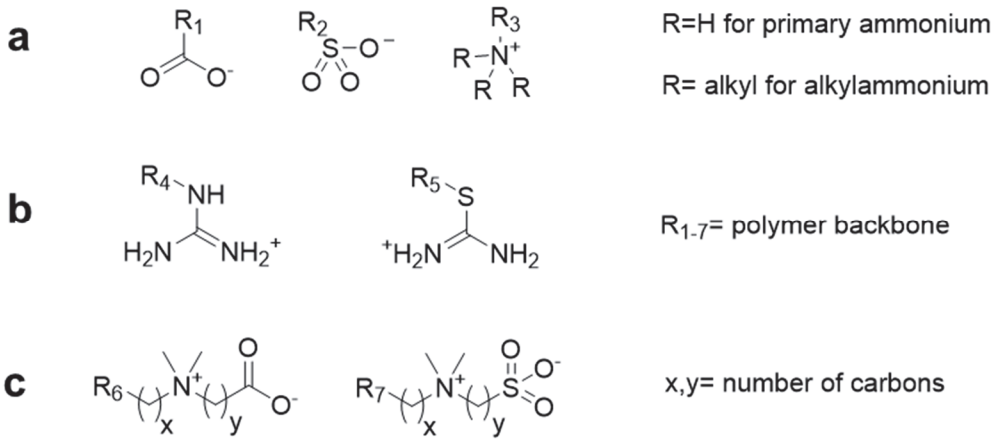
added salt. The thiouronium group even interacts strongly enough with polymeric zwitterions to enable complexation with this nominally weakly-interacting, net-neutral polymer.

Introduction

Oppositely-charged polyelectrolytes can associate in solution via ion pairing or "electrostatic" interactions.[1] Their association results in a liquid-liquid phase separation into solid-like hydrated polyelectrolyte complexes, PECs, or liquid-like coacervates. These morphologies reflect the water content and degree of association of the constituent polyelectrolytes. Most of the reported PEC polyanions bear a carboxylate or sulfonate group while polycations usually comprise an amine or an ammonium functionality (Scheme 1a). For example, polycarboxylates, known to form weak complexes, are mostly used to prepare coacervates while polyaromatic sulfonates form less hydrated, tough complexes.[1, 2] Interactions between polyelectrolytes are also widely used in preparing ultrathin films of polyelectrolyte complex, via layer-by-layer assembly of oppositely charged polyelectrolytes.[3, 4] The properties of the polyelectrolyte multilayer, PEMU, are controlled by the functional groups incorporated.[3, 5] This degree of compositional control has led to a number of applications ranging from electronics, to separations[6-8] to antifouling and antibacterial surfaces.[8-11] One aim of PEMUs in biomaterials is to provide a surface that prevents cell attachment.[9, 11] Various factors affecting the properties of PEMUs, such as the nature of the polyelectrolyte[11-13] and ionic strength, have been extensively studied. For example it is generally known that deposition at high salt concentrations will yield thicker films[13] and the type of polymer will affect its biological properties[11].

Polyelectrolytes tend to interact strongly when they form a less hydrated polymer ion pair.[13-15] The addition of a salt to the solution to which a PEMU is exposed is used to challenge pairing between positive, Pol^+, and negative, Pol^-, polyelectrolyte repeat units. Weakly-bound ion pairs break easily in the presence of salt.[16] The strength of the polymer interactions dictates

whether the film will grow linearly or exponentially.[9, 13, 17] PEMUs having polystyrene sulfonate, PSS, tend to grow linearly[11, 13] unlike a multilayer containing carboxylate[11, 13] (Scheme 1) .



Scheme 1. a) Functional groups (from left to right) carboxylate, sulfonate, and amine or alkylammonium b) Structural similarities between guanidinium (left) and thiouronium (right) c) two common zwitterion functional groups carboxybetaine (left) sulfobetaine (right).

Pendant zwitterionic functionalities, having balanced negative and positive charges, exhibit particularly weak interactions with other charges.[18, 19] This weak interaction, coupled with a high degree of hydration, is commonly exploited to endow surfaces with antifouling properties.[20-22] Polyzwitterions have been incorporated in PEMUs by either being sandwiched between the polycation and polyanion[23-25] or by copolymerizing the zwitterionic monomer with an anionic monomer to provide persistent net-negative charge.[26-28] Alternatively, polycarboxybetaine polyzwitterion may be partially charged by lowering the pH sufficiently to provide a partial net positive charge which allows for PEMU formation.[29-32] In contrast, when pH-insensitive sulfobetaine was used, there is no loss of zwitterionic character at various pH but weak interaction reported with poly(diallydimethylammonium chloride), PDADMAC, was enough to provide for weak multilayering, resulting in a thickness limit and multilayer desorption at 0.5 M NaCl.[23-25, 33, 34]

Because so many properties of complexed polyelectrolytes depend on the specific pair of functional groups used to prepare them, there has been increasing interest in quantifying or ranking the strength of Pol^+:Pol^- interactions.[2] Polyarginine, a polycation, contains the guanidinium group. The guanidinium functionality keeps the polymer portonated over a wide pH range ($pK_a \sim 13$),[5, 35] and tightly binds with anions due to strong electrostatic and hydrogen-bond interactions.[5, 36-38] It also has the ability to form “columbic defying” like-charge ion pairs.[39, 40] Guanidinium can either destroy electrostatic pairing or form one of the strongest ion pairs.[38, 41, 42] Polyarginine has been highlighted for its antibacterial properties[5, 43, 44] and importance in cell-penetrating peptides[39, 45-51]due to its ability to diffuse across membranes and through films.[11, 52, 53] In its salt form, guanidinium is used for protein denaturation, while as part of a polymer, polyarginine/guanidinium forms an insoluble complex.[38, 41, 42]

The use of synthetic polyelectrolytes containing guanidine, mostly as a comonomer, has been reported by Müller et al. for making multilayers with controlled protein adsorption properties.[54] Multilayers from poly(styrene sulfonate), PSS, and polyallylamine, PAH, grafted with up to 29% guanidine were shown by Cao et al. to be stable and to interact more strongly with anions.[5] Renken et al.[55] and Hartig et al.[56] prepared biodegradable PECs from polymethylene-*co*-guanidine and biopolysaccharides such as alginate. Sadman et al.[38] showed that complexes from

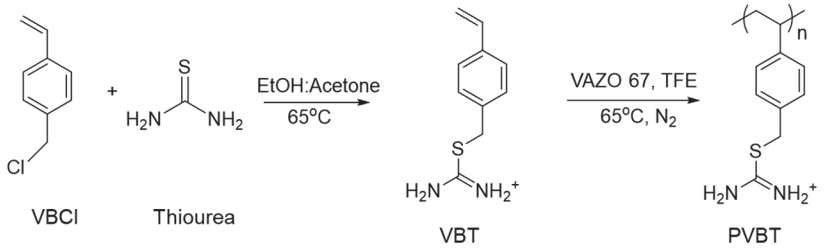
PSS and guanidine-derivatized PAH were more resistant to salt swelling, a measure of interaction strength, than PAH homopolymer.

Seeking another example of a strongly-interacting polycation, we explore here the layer-by-layer assembly of poly(vinyl benzyl thiouronium), PVBT, that bears a positively charged moiety similar to that of guanidinium (Scheme 1b). Multilayering characteristics consistent with strong thiouronium-anion interactions were observed: thin, tightly bound films may be assembled in solutions with high salt concentration. The strong association is evident when PVBT is compared to a polyelectrolyte system made of poly(3-methacryloylaminopropyl trimethylammonium chloride), PMAPTAC and PSS alongside a copolymer with a low percentage (10%) of PVBT. The interaction is strong enough to complex and layer a polymeric sulfobetaine zwitterion without altering the pH, copolymerizing or embedding the polyzwitterion in a polycation/polyanion multilayer.

Methods

Reagents. 4-Vinylbenzylchloride (VBCl) was from Scientific Polymer Products. Thiourea, acryloyl chloride, [3-(methacryloylamino)propyl]trimethylammonium chloride solution (MAPTAC) in 50 wt% water, acetone, 2,2,2-trifluoroethanol (TFE), ammonium persulfate and tetraethylammonium bromide (TEABr) were obtained from Sigma Aldrich. N,N'-dimethylethylenediamine and 1,3-propane sultone were from Alfa Aesar. Diethyl ether, ethanol (EtOH) methanol (MeOH) and dimethylsulfoxide (DMSO) were from VWR chemicals. Initiators 2,2′-azobis(2-methylbutyronitrile) (VAZO-67) and 2,2'-azobis[N-(2-carboxyethyl)-2-methylpropionamidine] hydrate (VA-057) were from Miller-Stephenson Chemical Co and Wako respectively. Sodium acetate (NaAc) was from Fisher Scientific and deuterium oxide (D_2O) from Cambridge Isotope Laboratories. Deionized water (18.2 MΩ cm, Milli-Q) was used to prepare all aqueous solutions.

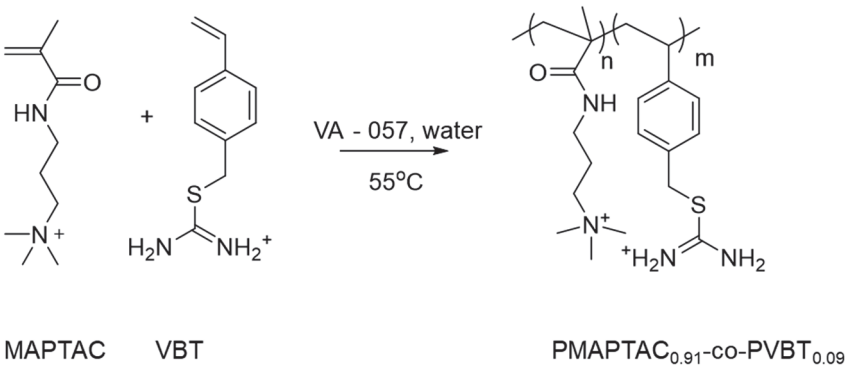
Synthesis of VBT. The positively charged monomer VBT was synthesized from VBCl and thiourea modifying a literature procedure:[57, 58] to a solution of VBCl (3.05 g, 20 mmol) in 22.9 mL ethanol and 22.9 mL acetone, thiourea (1.38 g, 18 mmol) was added and the mixture stirred for 18 h under reflux at 65 °C. After precipitation of the concentrated reaction mixture into diethyl ether, the crude product was recrystallized using a 1:4 ratio of methanol to diethyl ether to yield VBT (60 % yield). ^{1}H NMR (600 MHz, D_2O, Supporting Information Figure S1) δ 7.43 (d, J = 7.9 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 6.70 (dd, J = 17.7, 10.9 Hz, 1H), 5.81 – 5.75 (m, 1H), 5.26 (d, J = 10.9 Hz, 1H), 4.31 (s, 2H).



Scheme 2 Synthesis of the cation monomer and polycation PVBT

Synthesis of PVBT and PMAPTAC-*co*-PVBT. VAZO-67 8.4 mg (1%) was added to 4.4 mmol PVBT in 20 ml TFE. The mixture was left to stir at 65 °C for 24 h under N_2. The crude polymer was precipitated using diethyl ether and purified by dialysis against water (SnakeSkin, molecular

weight cutoff, MWCO = 3500) at 4 °C for 48 h. The PVBT homopolymer was isolated after freeze-drying (yield 81%). ^{1}H NMR (600 MHz, D_2O, Figure S1) δ 7.09 (s, 4H), 4.29 (s, 2H), 2.50 – 0.28 (s, 3H).



Scheme 3 Synthetic scheme of $PMAPTAC_{0.91}$-co-$PVBT_{0.09}$

Copolymer. MAPTAC (g, 4.5 mmol) and VBT monomer (0.115 g, 0.5 mmol) were mixed with 21 mg (1 %) VA-057 in 10 mL water and stirred at 55 °C under N_2. The copolymer was purified by dialyzing against water for 48 h and recovered by evaporating the water at 70 °C under reduced pressure (60% yield). The composition was found to be $PMAPTAC_{0.91}$-*co*-$PVBT_{0.09}$ by ^{1}H NMR (600 MHz, D_2O, Figure S2) δ 7.09 (d, J = 145.6 Hz, 4H), 4.32 (s, 2H), 3.32 (t, J = 8.0 Hz, 15H), 3.10 (s, 97H), 2.30 (s, 4H), 1.97 (s, 19H), 1.68 (s, 13H), 0.97 (d, J = 84.2 Hz, 23H).

Synthesis of PMAPTAC and PMA PMAPTAC and PMA were synthesized following an aqueous radical polymerization procedure.[59] PMA was neutralized using sodium hydroxide to pH 9 before being precipitated and dried under vac at 65 °C.

Synthesis of AEDA, AEDAPS and PAEDAPS. AEDA and AEDAPS were synthesized following literature procedures,[18, 60] where a coupling reaction between N, N-dimethylethylenediamine and acryloyl chloride and AEDA (Figure S3) and 1,3- propane sultone was used to yield both products, respectively. A free radical copolymerization in an aqueous solution was performed to obtain PAEDAPS[18] (Figure S4). Additional details on synthesis and characterization are provided in Supporting Information.

Dynamic Light Scattering (DLS). The PVBT aggregate size was determined by dynamic light scattering using a goniometer system (ALV CGS-3-A0-111, Langen, Germany) equipped with a He-Ne laser (λ = 632.8 nm, 22 mW) and a vertically polarized light. At an angle of 30° and temperatures ranging from 25 to 90 °C, measurements were taken in 10 mm capped cylindrical borosilicate glass tubes through a reservoir filled with a refractive index matching liquid (toluene). The polymer samples of 1 mg/mL concentration were prepared in aqueous 0.01 and 0.1M TEABr and then filtered through a 0.1 μm Milipore filter. By pseudo-cross-correlation of the signals from two photomultipliers, the intensity autocorrelation function $g^{(2)}(q,\tau)$ where $q = 4\pi n_D \sin(\theta/2)/\lambda$ was obtained with suppressed noise using ALV correlator software V.3.0. The hydrodynamic radius R_h was calculated using CONTIN analysis, the distribution of R_h represents the average hydrodynamic radius of all polymer molecules in solution.

^{1}H-NMR Studies. The VBT monomer (5 mg mL^{-1}) stability in D_2O (with 0.5 μL DMSO as standard) and 0.5 M sodium acetate was analyzed using ^{1}H-NMR (Avance-600 MHz, Bruker). The VBT peak position and integration were monitored vs. time.

Multilayer Buildup. Polyelectrolyte multilayers were built manually on double-side-polished silicon (Si 100) wafers of thickness 775 μm. The substrates were cleaned in "piranha" (70% H_2SO_4/30% H_2O), rinsed with water and dried with N_2. The Si wafers were dipped for 10 min in 10 mM (based on the repeat unit) polymer solutions followed by three 1 min rinses. All polymer solutions were prepared in water for TEABr concentration ranging from 0 to 2M, expect PVBT in 2M TEABr was dissolved in 1:1 water:acetone. The PAEDAPS system included polymer solutions dissolved in 0.4M NaAc.

PEMU Characterization. The thickness at every bilayer and that of the film were measured with an ellipsometer (L116S, Gartner Scientific) using a 632.8 nm laser at a 70° incidence angle. The measurement was made 4 times per sample using a PEMU refractive index of 1.55 and a 1 nm oxide layer was subtracted from the total measured value to yield the final thickness.

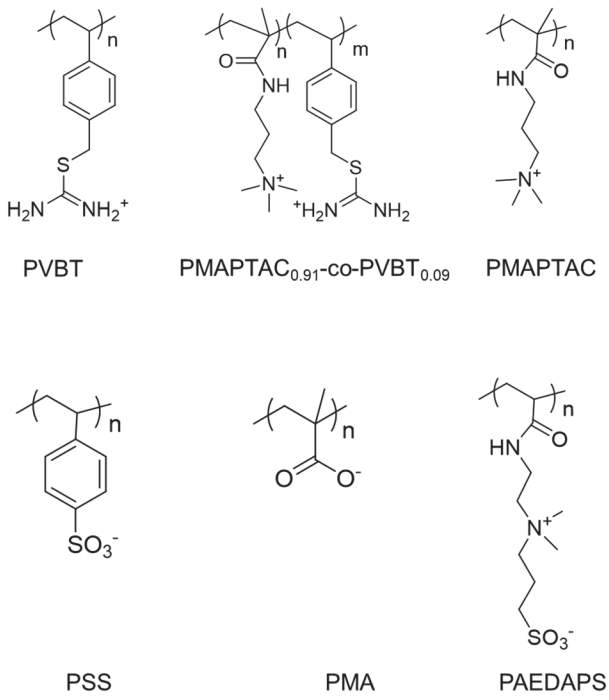
The PEMU composition was confirmed with infrared (IR) spectroscopy using a nitrogen purged FTIR (Nicolet Avatar 360 with a DTGS detector) spectrometer. Spectra were taken averaging 100 scans at a resolution of 4 cm^{-1}.

Imaging. The topography of the PEMUs was obtained using a MFP-3D AFM (Asylum Research Inc., Santa Barbara, CA) with an ARC2 controller and silicon TESPA-V2 probes (Bruker, radius = 10 nm, spring constant = 42 N m^{-1}). The cantilever was adjusted to 5% below its resonance frequency and AC mode (intermittent contact) was employed. To obtain the film roughness a scan size of 1 x 1 μm of 1 Hz was used.

UV-Vis Turbidimetry. The solution turbidity of different complexes (1 mM) in pure water was measured by recording the absorbance at 400 nm using a UV-vis spectrophotometer (Cary 100 Bio; Varian Instruments). The normalized absorbance was plotted as a function of salt (TEABr) concentration.

Results and Discussion

The polymers selected for multilayering (Scheme 4) are a mix of commonly used positive and negative charged polymers with aromatic and aliphatic functionalities. In particular, a comparison of aliphatic tetraalkylammonium (PMAPTAC) and the aromatic thiouronium (PVBT) was made. Aliphatic tertiary ammoniums are known to form weak complexes,[2, 42] while guanidinium can form strong interactions of various kinds.[38, 42] However, the most striking property of guanidinium is the ability to form "like-charge" ion pairs in water which distinguishes it from other polyelectrolytes.[39] The monomeric salt form is used to break strong interactions and denature proteins.[38, 41] These strong interactions allow polyarginine (polyguanidiniums) to pass through a cellular membrane by interacting with the phosphate group in the lipid bilayer.[39] It is anticipated that the thiouronium will share these unusual properties of guanidinium. Concerning polyanions, aromatic sulfonates form stronger complexes than carboxylates with their positive counterparts.[2] The polyzwitterion PAEDAPS, known to form weak (or no) complexes with polyanions and polycations, was used to evaluate the relative strength of PVBT at the highest salt concentrations.



Scheme 4. Structures of polyelectrolytes and polyzwitterion used

Polythiouronium Solution Behavior

The VBT repeat unit has been used previously as a precursor for the benzylthiol functional group. We have used polyelectrolytes grafted with benzylthiols to make multilayers and to derivatize multilayer surfaces with thiols.[57, 58, 61] The strength of interaction of this positive charge with negative polyelectrolytes has not been investigated but can be compared to the guanidinium moiety due to the structural similarities. The monomer was recrystallized before use to remove any deprotected thiols. The PVBT polymer was prepared using radical polymerization in TFE and was characterized using NMR spectroscopy. Because the thiouronium group is usually employed as a precursor to thiols, via base-induced hydrolysis, the stability of the VBT monomer under neutral and mildly basic conditions was assessed by recording ^{1}H-NMR spectra of VBT in D_2O and in 0.5 M NaAc (pH ≈ 9.2) for 3 weeks. The monomer showed no signs of hydrolysis in both solutions. In fact, hydrolysis is typically carried out at much higher pH.

It was quickly found that solutions of PVBT were difficult to prepare and work with. This is to be expected if the range of unusual interactions reported for guanidinium are carried over to its thiouronium "cousin." For example, positive-positive attractive interactions would tend to drive aggregation and poor solubility. Thus, while PVBT could be dispersed in water, a number of salts were found to cause precipitation, including NaCl. TEABr and sodium acetate, occupying different ends of the Hofmeister series,[37] were found to be suitable as added salts without inducing precipitation. A summary of the solubility of PVBT in various added salts is given in Table S1, Supporting Information. Interestingly, PVBT was soluble in guanidinium chloride but not guanidinium thiocyanate. In addition, size exclusion chromatography was not possible due to the affinity of PVBT for surfaces, even positively-charged ones. The size of PVBT in solution was therefore estimated by DLS. Figure 1 shows an autocorrelation function of solution PVBT with a single decay and an R_h distribution with an average R_h of 25.4 nm at 296.8K that remains stable for at least 24 h. This R_h is consistent with polymer chains in the 100s of kDa molecular weight

range, or aggregates of a few smaller chains. Aggregation, indicated by large average R_h and long tails to even larger R_h, was observed for many conditions.

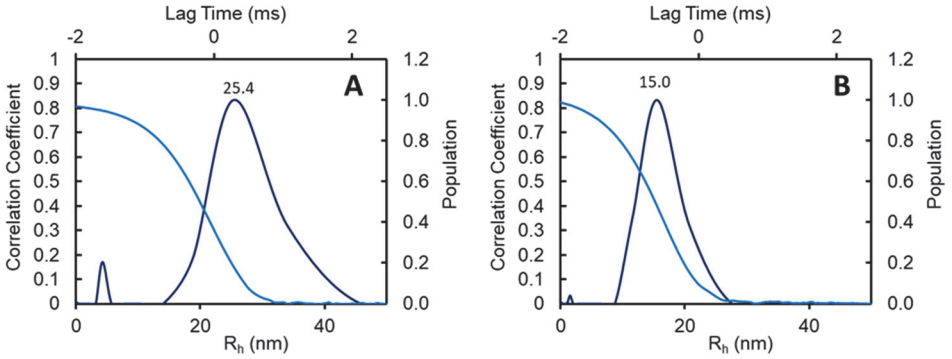


Figure 1. Autocorrelation function and hydrodynamic radius, R_h, distribution of PVBT in 0.1M TEABr at an angle of 30°, 1 mg mL^{-1} concentration at a) 296.8K; b) 339.8K

The ability of the thiouronium moiety to form "like-charge" ion pair and hydrogen bonding causes the polymer to aggregate in solution.[39] However, increasing the ionic strength has little effect on polymers complexed by hydrogen bonding.[62] Figure 2 compares the apparent R_h of PVBT in 0.01 M and 0.1 M TEABr. The size of PVBT decreases in both [TEABr] on heating. In 0.1 M TEABr, Figure 2 shows first a decrease, then an increase in PVBT size with temperature, interpreted to show the breaking first of *intermolecular* (Figure 3a) then *intramolecular* (Figure 3b) hydrogen bonds to reach a minimum which possibly represents the size of a single polymer chain (Figure 1b and 3c). The R_h increase at 349 K and 358 K (Figure 2) could also be explained by the expansion of the polyelectrolyte in solution in response to increasing temperature, which has been observed for other polyelectrolytes.[63]In 0.01 M TEABr the decrease in size was more marked (Figure 2), and though the data at the highest temperatures suggests the size might start to increase, temperatures were limited by the apparatus to 360 K. Any decreased condensation of counterions caused by a temperature increase[64] would increase the effective charge on the polymer and would assist with the expansion of chain. The continued decrease of size in 0.01M TEABr beyond 340 K is consistent with the claim that more salt decreases "like-charge" ion pairing:[65] both pairing and H-bonding would tend to collapse and/or aggregate PVBT which means interactions can be eliminated if both [TEABr] and temperature are high enough.

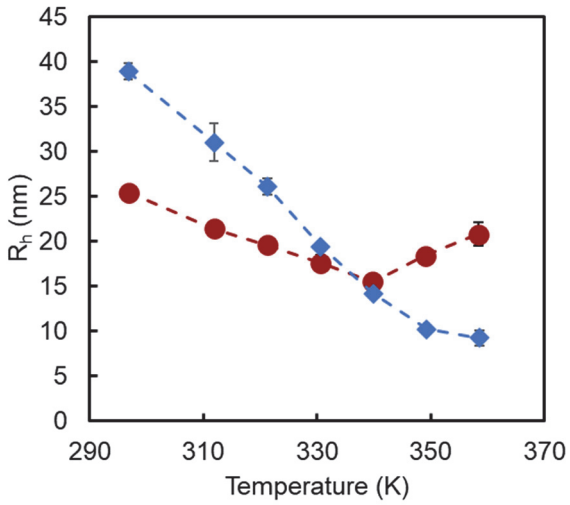


Figure 2 The variation of the apparent hydrodynamic radius (R_h) of 1 mg mL^{-1} PVBT in 0.1M TEABr (●) and 0.01M TEABr (♦) as a function of temperature.

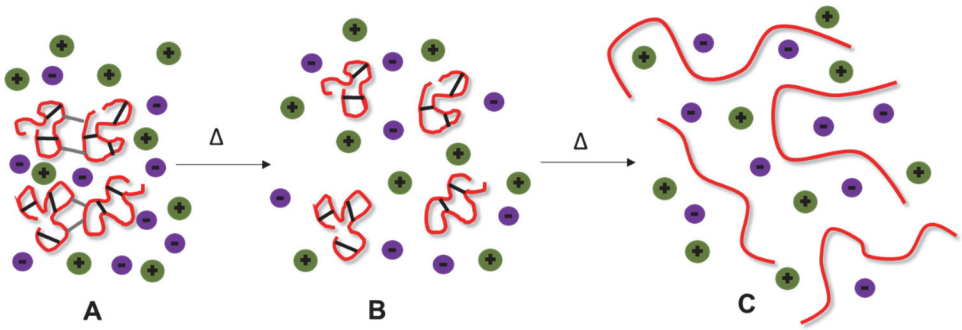


Figure 3 Representation of the dissociation of PVBT (red) aggregate (A) with intermolecular (grey) and intramolecular (black) H-bonds in tetraethylammonium (green) bromide (purple) solution first into individual chains with intramolecular H-bonds (B), which break on further heating (C).

Multilayer Comparisons

The differences in properties between PVBT and PMAPTAC were probed using differences in layer-by-layer assembly with three polyanions. The thickness versus number of layers during PEMU growth can be either linear or nonlinear. The latter, sometimes termed "exponential" growth, is caused by mobility of one or both of the polyelectrolytes or their "extrinsic" counterion-compensated sites.[66, 67] True exponential growth only occurs under demanding quasi-equilibrium conditions.[67] However, nonlinear growth is taken as evidence of mobility within the PEMU and is generally interpreted to reflect weak interactions between positive and negative polyelectrolytes.[66,68, 69]

Figure 4 shows the buildup of PVBT and PMAPTAC with PSS and PMA in TEABr "salt" concentrations ranging from 0.1 M to 2 M. PMAPTAC/PMA did not form a multilayer even at low

salt concentrations (Figure 4A). For PMAPTAC/PSS, at 0.1 M salt (Figure 4A) the multilayer follows a slight nonlinear trend, it increases in thickness greatly at 1 M salt (Figure 4B) and does not form a multilayer at all at 2 M of the organic salt (Figure 4C). In contrast, the PVBT system keeps growing linearly, even at the highest salt concentrations (Figure 4C) and the multilayer formed is thin and has a constant thickness. In other words, the PVBT system shows little sensitivity to salt concentration, an indication of strong interactions.

The similar behavior between the PVBT/PSS and PVBT/PMA system is somewhat surprising due to the different interaction strengths of the two polyanions in complexes.[2] The difference between PMAPTAC and PVBT becomes more prominent at higher salt concentrations (Figure 4B-C). This contrasting behavior is interpreted as follows: PMAPTAC and PVBT both interact by ion pairing, which is weakened by higher salt concentration, whereas PVBT has additional attractive interactions, also peculiar to guanidinium, including hydrogen bonding. [5, 42]

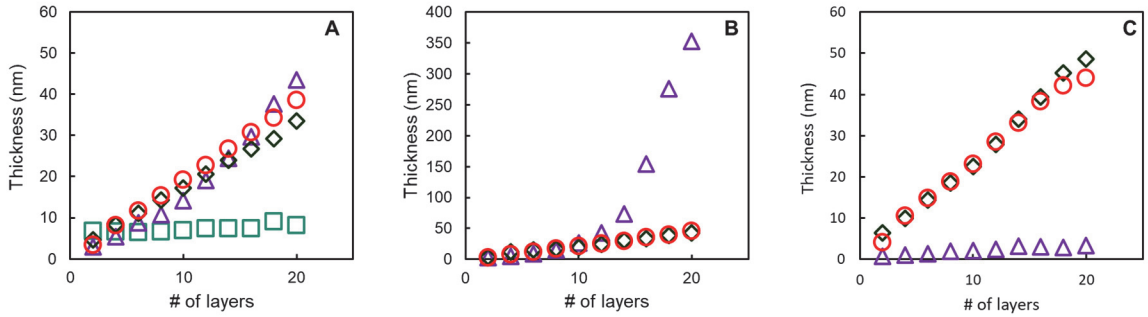


Figure 4. Thickness vs number of layers for PEMUs made from PMAPTAC/PMA (□), PMAPTAC/PSS (△), PVBT/PSS (○) and PVBT/PMA (◊) constructed in a) 0.1M, b) 1M and c) 2M TEABr. Room temperature.

Polylysine and polyarginine have been compared extensively in the literature when it comes to phospholipid interactions. Polyarginine has been known to interact more strongly and penetrate the lipid bilayer easily.[42, 52] The exterior surface of the lipid bilayer is largely made up of the zwitterion phosphorylcholine. The ability of the guanidinium moiety to interact with the phosphate groups prompted the use of PAEDAPS in multilayer formation. Figure 5 depicts the linear growth of PVBT/PAEDAPS multilayer in 0.4 M NaAc whereas no PMAPTAC/PAEDAPS multilayer could be constructed under these conditions.

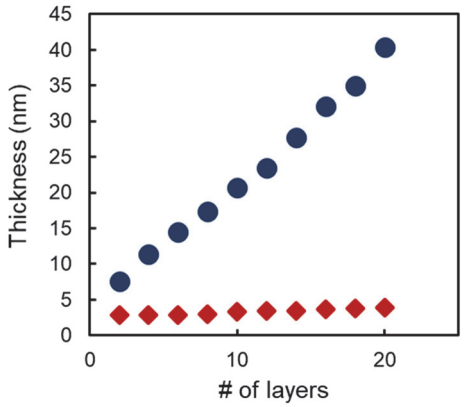


Figure 5. Thickness vs. number of layers for PMAPTAC/PAEDAPS (♦) and PVBT/PAEDAPS (●) PEMUs in 0.4 M NaAc. Room temperature.

IR spectroscopy offers a way to confirm the chemical composition of the PEMUs built. Characteristic peaks of the polycations; PMAPTAC (HNC=O ~1600 - 1700cm^{-1}), PVBT (benzene ring and C=N ~1500-1600 cm^{-1}), polyanions; PSS (S=O ~1300 cm^{-1}), PMA ($^{-}$OC=O ~1500 cm^{-1}) and polyzwitterion; PAEDAPS (HNC=O ~1600-1700cm^{-}1) used are present (Figure S10-S14).

AFM images (Figure 6) revealed the presence of a few pores in the PMAPTAC/PSS system in 0.1 M and 1 M salt (Figures 6a and 6b) with a roughness of 3.17 and 4.7 nm, respectively. All polyelectrolyte surfaces show some degree of roughness, which tends to be greater for thicker layers made from stiffer combinations of polyelectrolyte.[69] The PMAPTAC/PSS film built in 1 M salt has the highest thickness, but the roughness does not increase drastically from that of the 0.1 M film. This can be explained by the ability of these polyelectrolytes to flow during assembly producing smooth films.[70] Figure 6b reveals larger pores at higher salt concentration. These porous films might have applications in filtration.[8] The film with the smoothest surface was the PVBT/PAEDAPS (Figure 6e) with a roughness of 0.4 nm followed by PVBT/PMA (2.55 nm roughness, Figure 6d) and PVBT/PSS (3.18 nm roughness, Figure 6c). The smoothness of the PVBT films could be due to the strongly bonded ion pairs (scheme 5) forming ultrathin films.[70, 71]

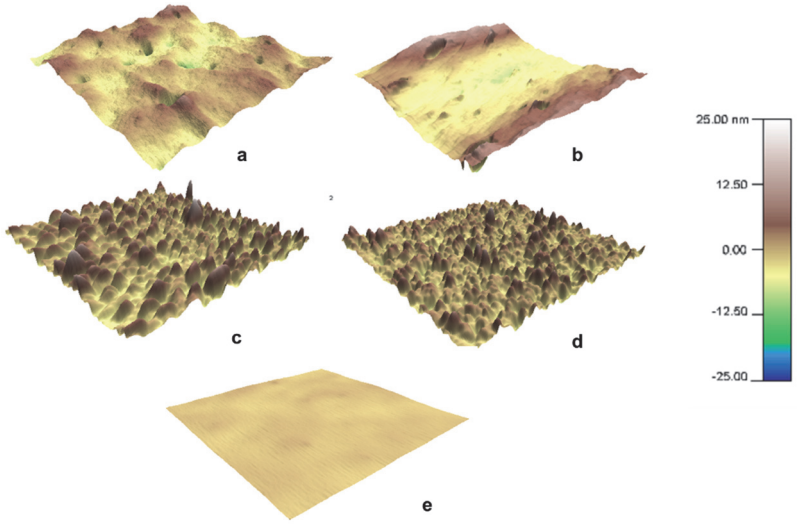


Figure 6. AFM images, 3D presentation, of a) PMAPTAC/PSS built in 0.1 M TEABr; b) PMAPTAC/PSS built in 1 M TEABr; c) PVBT/PSS built in 0.1 M TEABr; d) PVBT/PMA built in 0.1 M TEABr; e) PVBT/PAEDAPS built in 0.4 M NaAc. *X*-*Y* images are 1 x 1 μm.

The analogous denaturing ability of guanidinium (Gdm) chloride and tetrapropylammonium (TPA) chloride has been discussed in the literature.[37, 38, 65] Gdm and TPA are next to each other in the Hofmeister series. However TPA does not have the ability to hydrogen bond or form homo-ion stacking.[65] The efficacy of an ion to disrupt H-bonding, nonpolar and electrostatic interactions contributes to its denaturant properties. Thus, TPACl is a strong destabilizer by diminishing the stated interactions wherein both ions play a role at denaturing concentrations (~ 6 M). [37, 65] Halides like chloride, bromide and iodide have been shown to diminish protein aggregation.[37]

Salt Resistance

The strength of a PEC is usually evaluated by the ability of a salt to break ion pairs. Almost all combinations of polyelectrolytes, except polyguanidinium, swell when sufficient concentration of particular salts are used.[3] Effective salts for swelling/dissolution are commonly found at the hydrophobic end of the Hofmeister series (e.g. tetraalkylammonium). Hydrophilic ions, such as SO_4^{2-} and acetate, have low swelling strengths.[72] Turbidimetry is used to determine the salt concentration needed to form a homogeneous polycation and polyanion solution[73]. Figure 7 represents the salt resistance of various polyelectrolyte complexes. The stronger the complex, the higher the salt resistance. Thus, the polyelectrolyte complexes can be arranged from weak (PMAPTAC/PSS) to strong (PVBT/PSS) (Table1).

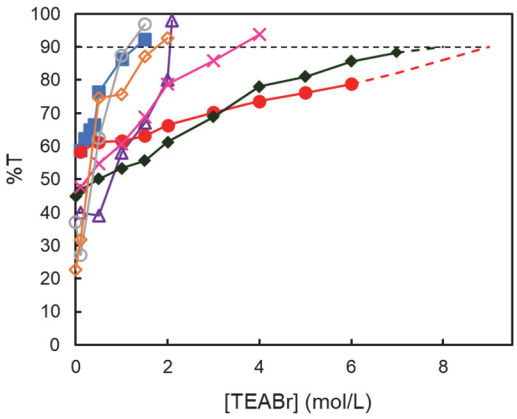
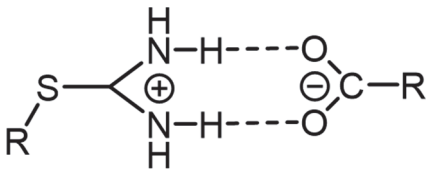


Figure 7. Percent transmittance of 1 mM $PMAPTAC_{0.9}$-co-$PVBT_{0.1}$/PMA (-○-), $PMAPTAC_{0.9}$-co-$PVBT_{0.1}$/PSS (-◊-),PMAPTAC/PMA (-■-), PMAPTAC/PSS (-Δ-),PVBT/PAEDAPS(-X-), PVBT/PMA (-♦-) (last data point extrapolated) and PVBT/PSS (-●-) (extrapolation to 90 %T shown) solutions vs the concentration of tetraethylammonium bromide. At 400 nm and room temperature. Dotted line shows 90% T.

Table 1 summarizes the estimated TEABr concentration needed to solubilize different PECs. This concentration was recorded at 90% transmission. A copolymer with 10% VBT was synthesized to demonstrate the effect of a small fraction of thiouronium comonomer on complexation. PMAPTAC/PMA is the weakest complex, where 1.5 M TEABr was needed to break the ion pairs. When 10% thiouronium functionality was introduced to the PMAPTAC backbone, the salt resistance increased slightly attaining almost the same salt concentration as that of PMAPTAC/PSS. Using a strong polyanion, PSS, the salt resistance increased to 3 M with the positively charged copolymer. The strength of the PVBT interaction becomes clearer when 4 M of salt is needed to break the interactions with the weakly negative zwitterionic PAEDAPS. PSS is known to form stronger bonds than PMA thus a higher concentration of salt is needed to break the PVBT/PSS bond.

Table 1. Tetraethylammonium bromide (TEABr) concentration at ≥ 90% transmittance of different complexes

Complex Name	$[TEABr]_{\geq 90\%T}$ (M)
PMAPTAC/PMA	1.5
$PMAPTAC_{0.9}$-co-$PVBT_{0.1}$/PMA	2
PMAPTAC/PSS	2.1
$PMAPTAC_{0.9}$-co-$PVBT_{0.1}$/PSS	3
PVBT/PAEDAPS	4
PVBT/PMA	8-8.5
PVBT/PSS	9-9.5



Scheme 5. Representation of guanidinium “salt-bridge” like interaction with acetate.

This high salt concentration is explained by the “salt-bridge” interaction, the carboxylate and the sulfonate are strong hydrogen bond acceptors and thus forms two types of bonds with the thiouronium; electrostatic and hydrogen bonds[36, 39] (Scheme 5). The tetraethylammonium and the bromide weakly interact[37] and thus are able to disrupt the interchain polymer bonds and fully interact with the extended polymer chains (Figure 8). This shows that TEABr behaves similarly to TPACl and has a strong denaturing ability. Figure 8 summarizes the unusual requirements for pair breaking and solubility of PVBT/PMA: both hydrogen bonds and charge-charge interactions must be broken. NaCl does not offer H-bonding while guanidinium chloride does (Table S1, Supporting Information).

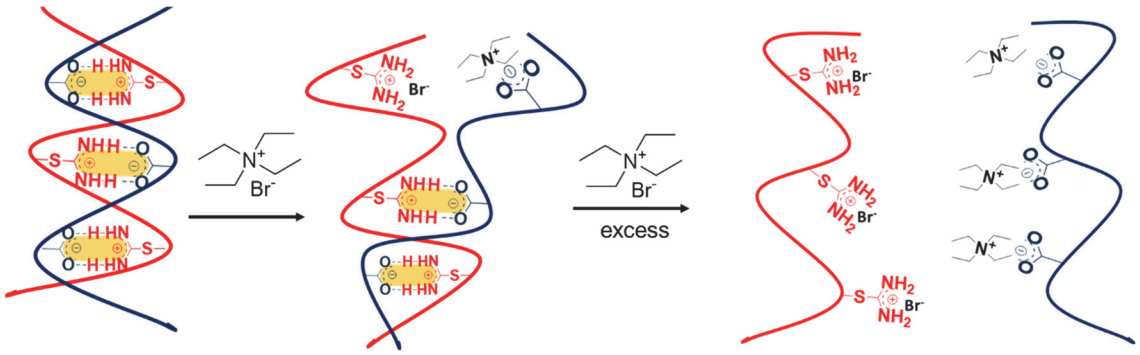


Figure 8. Representation of salt resistance between PVBT (in red) and PMA (in blue). Salt must be able to break both “electrostatic” as well as H-bonding interactions.

Conclusions

The thiouronium group is capable of strong interactions with negatively-charged moieties, which makes it a promising candidate for preparing rugged films and articles from polyelectrolyte complexes. It appears to share characteristics with guanidinium, such as the formation of like-ion pairs and hydrogen bonding, in addition to the usual charge pairing mechanism of the polycations to which it is compared here. These intra- and intermolecular forces also lead to aggregation, though weak, of polythiouronium in solution and unusual solubility characteristics in aqueous solutions of salts. These troublesome properties may be partially alleviated by incorporating the thiouronium group alongside more traditional cationic repeat units as copolymers.

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Notes

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Supporting Information

^{1}H NMR spectra of VBT monomer and PVBT; ^{1}H NMR spectra of PMAPTAC and PMAPTAC-co-PVBT; ^{1}H NMR spectra of AEDA and AEDAPS monomer and PAEDAPS polymer; ^{1}H NMR of VBT stability in D_20 and 0.5 M NaAc; multilayer buildup of PMAPTAC/PSS, PVBT/PSS and PVBT/PMA; FTIR spectra of multilayers on Si wafer. This information is available free of charge.

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