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THE INFLUENCE OF "HYDROPHOBICITY" ON THE COMPOSITION AND DYNAMICS OF POLYELECTROLYTE COMPLEX COACERVATES

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Abstract

Various types of specific interactions are believed to supplement the major entropic driving forces responsible for spontaneous liquid-liquid phase separations in mixtures of oppositely-charged polyelectrolytes. Among these interactions, hydrophobicity has recently been probed experimentally *via* the synthesis and complex formation of polyelectrolytes bearing hydrophobic pendant groups or backbones. In this work, poly(4-vinylpyridines), P4VP, were N-alkylated with chains from one to six carbons in length. The fully-alkylated polycations were complexed with sodium poly(methacrylate) to yield polyelectrolyte complexes or coacervates, PECs. Counterintuitively, PECs made with N-methyl to N-butyl P4VP were *less* stable to the addition of salt the longer the alkane chain, being easier to dope and having a lower critical salt concentration for dissolution. In contrast, the linear viscoelastic response of these PECs varied little. A transition in doping and properties was observed with N-pentyl and -hexyl chains, the latter having much higher modulus and much less sensitivity to salt concentration. Small angle X-ray scattering suggested a new morphology for the N-pentyl and -hexyl P4VP PECs, with interacting/phase separating alkane chains providing a transition into hydrophobicity-dominated PECs.

Introduction

Well-mixed hydrated blends of negative and positive polyelectrolytes spontaneously form when individual solutions of these charged polymers are combined.[1, 2, 3, 4] The blends,[5] termed polyelectrolyte complexes or polyelectrolyte coacervates (PECs for both), a fascinating form of soft matter, have recently reignited interest from many fields. Both natural and synthetic charged macromolecules may produce PECs, although the details sought by researchers depends on their

discipline. The study of biomacromolecular coacervates gained much impetus from the work of Bungenberg de Jong and colleagues at the beginning of the last century.[6, 7] The finding that coacervation of biopolyelectrolytes, such as RNA, may explain the formation of membraneless organelles has invigorated research from the biological perspective.[8, 9] The first PECs made from synthetic polyelectrolytes tended to be solid-like and suggested applications in the materials and biomedical fields.[1, 10]

The high density of charges within PECs has stimulated much recent theory[11, 12, 13, 14, 15] and experiment[16, 17, 18] aimed at understanding both the driving forces for the formation of PECs from solution and their physical properties. While these driving forces are often assumed to be electrostatic in nature, entropy actually dominates PEC association:[1, 19] the release of polyelectrolyte counterions offers much more entropy change than does mixing two polymers.[20]

Entropic driving forces are supplemented with a variety of other potential interactions which are specific in nature and thus generate enthalpy changes.[21] These interactions include differences in hydration, hydrogen bonding and other dipolar interactions, and an overlapping class of interactions that fall under the umbrella of "hydrophobicity."[22, 23] The latter has been extensively examined and argued in the area of protein folding.[24, 25, 26] The interpretation of hydrophobic effects remains elusive, even for (much simpler) synthetic uncharged polymers.

It is generally thought that adding -CH_2- units to a polyelectrolyte makes it more hydrophobic. As with small molecules, increasing the length of alkane chains connected to charged repeat units in a polyelectrolyte eventually renders the polymer insoluble in water. From a synthetic perspective, it is relatively straightforward to program increasing hydrophobicity into a polyelectrolyte to investigate its effect on PEC formation and properties.[18] Recently, -CH_2- pendant groups on the polyelectrolyte repeat unit,[27] or interspersed with charged units,[18, 28] have been used to evaluate the influence of hydrophobicity on dynamics and composition in PECs. Alternative approaches involve modifying the hydrophobicity of the polymer backbone[29] or the pendant groups.[30] These studies have found that an increase in hydrophobic content results in

an increase in PEC stability, consistent with the notion that hydrophobic interactions reinforce electrostatic ones.[31]

In the present work, a series of N-alkylated poly(4-vinylpyridiniums) was prepared from the same poly(4-vinylpyridine) starting material and complexed with poly(sodium methacrylate) to yield soft complexes/coacervates. The PECs were doped with NaCl solutions to provide compositions over the complete range of [NaCl] to the point where the PECs completely dissociated (at the "critical salt concentration"). Counterintuitive results were obtained, which recommend caution in the use of "hydrophobicity" as a concept in PEC formation.

Experimental

Materials

Poly(4-vinylpyridine) (P4VP, molecular weight ~ 200,000) was obtained from Scientific Polymer Products. Iodomethane (99%), 1-bromoethane (98%), 1-bromopropane (99%), 1-bromopentane (98%), 1-bromohexane (98%), tetraethylammonium hydroxide (35 wt% in H_2O), NaCl, KBr, NaOH and $K_2S_2O_8$ were used as received from Sigma-Aldrich. 1-bromobutane (98% Merck), methacrylic acid (MAA, 99% Alfa-Aesar), DMF (99.8%, VWR), diethyl ether (98%, VWR), D_2O (99.9%, Cambridge Isotope), and ethanol (KOPTEC) were used as received. All aqueous solutions were prepared using deionized water (18 MΩ cm, Barnstead Nanopure Diamond). For radiolabeling experiments, $^{22}Na^+$ (half-life 950 days, positron, γ emitter, E_{max} = 511 keV, produced with a specific activity of 914 Ci g^{-1}) from Perkin-Elmer Life Sciences was used to prepare "hot" $^{22}Na^+$ stock solution of 100 μCi in 1.0 mL H_2O.

Synthesis of Poly(methacrylic acid sodium salt) (PMA-Na)

85 mL MAA were mixed with inhibitor removal beads and stirred for 4 h. This MAA and 1.32 g of $K_2S_2O_8$ were added to 1.9 L water in a three-neck flask. The solution was heated at 60 °C under

N_2 for 24 h with stirring. The poly(methacrylic acid) crude product was neutralized with NaOH, dialyzed against water for 48 h, then freeze-dried to obtain PMA-Na.

Quaternization of P4VP

To prepare methylated P4VP (PQVP-C1), 10 g P4VP (0.095 mol) was dissolved in 250 mL DMF dried with molecular sieve. Three equivalents (0.285 mol) of iodomethane were added and the mixture was stirred under Ar for 12 h at room temp. For C2 - C6 alkylated P4VP (PQVP-C2 to C6), reactions were carried out under Ar at 60 °C for 24 h with three equivalents of C2 to C6 bromoalkanes, respectively. Products PQVP-C1 thru PQVP-C6 were precipitated in diethyl ether, washed with another 500 mL diethyl ether, then dried under vac at 40 °C for 24 h. The degree of quaternization was verified to be > 95% using FTIR. The IR characteristic band of P4VP at 1414 cm^{-1} disappeared completely and the 1600 cm^{-1} band shifted to 1640 cm^{-1} on quaternization (see Supporting Information Figure S1 for the spectra).[32]

Preparation of PECs

To prepare a PMA/PQVP-C1 PEC, 50 mL of 0.125 M PMA-Na in 0.5 M KBr and 50 mL of 0.125 M PQVP-C1 in 0.5 M KBr were mixed. 10 mL of this solution was then transferred to a 50 mL centrifuge tube, and the pH was adjusted to 11 to fully ionize PMA-Na. 40 mL of DI water was then added to the centrifuge tube to precipitate the PMA/PQVP-C1 PEC. The “dilution” technique of going backwards from completely dissolved PEC to precipitated PEC by decreasing the salt concentration[33] (also termed “desalting”[34]) allows the individual component polyelectrolytes to thoroughly mix before they interact to form PECs. KBr was used in this step because most of the PQVPs had bromide counterions.

The PEC was centrifuged at 6000 rpm for 24 h, the supernatant was removed, and fresh water was added to the centrifuge tube to wash out KBr. The salt-free PEC was rinsed at room temp for 24 h, with water replacements every 8 h. Finally, the PEC was annealed in water at 50 °C for 24 h to ensure full mixing and pairing of polyelectrolytes. The PEC was dried under vac at 70 °C for

24 h and ground into a powder using a coffee grinder. PECs PMA/PQVP-C2, PMA/PQVP-C3 and PMA/PQVP-C4 PECs were prepared with the same procedure.

The critical salt concentration, CSC, of each PEC was determined using 50 mg of dry PEC in 1.5 mL centrifuge tubes. Solutions with increasing [NaCl] were added to each of the tubes and they were centrifuged at 15,000 rpm for 15 min. At the CSC, the hydrated PEC phase disappeared. This transition to single phase was well defined and occurred for a [NaCl] change of only 0.01 M.

The dilution method was used to prepare PEC samples at specific NaCl concentrations for phase compositions: PECs were first dissolved in sufficiently concentrated NaCl, water was then added to decrease the [NaCl] to the desired level. For example, to make PMA/PQVP-C1 in 0.2 M NaCl, 50 mg of PMA/PQVP-C1 powder was dissolved in 0.3 mL 1.0 M NaCl in a 1.5 mL centrifuge tube. Then, 1.2 mL water was added to the PEC solution to dilute the NaCl to 0.2 M. The sample was centrifuged at 9000 rpm for 24 h to obtain a clear PEC phase topped by a dilute phase.

Insoluble in water at room temperature, PQVP-C5 and PQVP-C6 had to be dissolved in solvent containing organic solvent. On the other hand, the sodium salt of PMA was soluble only in water. Thus, the acid form of PMA was neutralized with tetraethylammonium (TEA) hydroxide to yield PMA-TEA, which was soluble in solvents containing organic solvent. Figure S2 in Supporting Information shows the ^{1}H NMR spectrum of PMA-TEA and indicates a 95% degree of neutralization of the H-form of PMA. PECs were prepared by mixing stoichiometric amounts of PMA-TEA and PQVP-C5 or C-6 in a 3:7 volume ratio of water:ethanol. The PECs were centrifuged at 6000 rpm for 24 h, the PEC phases were collected and dried at 60 ^{o}C under vac then ground into powders.

For salt concentrations near the CSC, the dilute phase above PECs PMA/PQVP-C1 to -C4 had a yellow tinge, indicating the presence of polymers. UV–visible spectroscopy (UV–Vis) experiments were conducted on a Cary 300 Bio UV–Vis spectrometer to determine the

concentration of polyelectrolyte in the dilute (supernatant) phase of these PECs. 10.0 mg of PEC were added to vials containing 10 mL of aqueous NaCl, which was allowed to equilibrate for 48 h at room temp. UV-Vis spectra were collected for the resulting supernatants. A standard in which the concentration of NaCl was greater than the critical salt concentration was used to determine the polyelectrolyte extinction coefficient using the absorbance of the PQVP peak at 258 nm. These extinction coefficients were used to calculate the supernatant polyelectrolyte concentration of the other samples.

Attenuated Total Internal Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy

ATR-FTIR spectra of hydrated PECs were collected using a ThermoScientific Nicolet iS20 with a Pike MIRacle universal ATR attachment fitted with a single-reflection diamond/ZnSe crystal and a high-pressure clamp. A stainless steel well was machined to fit onto the crystal plate to allow solid samples immersed in solution to be pressed onto the crystal while preventing the evaporation of water. PECs soaked in water for 24 h were placed on top of the ATR crystal inside the well and immersed in water. Pressure was applied to samples with a high-pressure clamp. The resolution was 4 cm^{-1}.

^{1}H NMR

The stoichiometries of PECs were determined using ^{1}H solution NMR (Bruker AVANCE 600 MHz). Dry PEC powders were dissolved in 1.0 M NaCl in D_2O at a sample concentration of 10 mg mL^{-1} and 256 scans were averaged. Peak areas corresponding to protons in the positive and negative polyelectrolyte were integrated (Supporting Information Figure S3).

Size Exclusion Chromatography (SEC)

SEC was used to determine the number-average molecular weight, M_n, weight-average molecular weight, M_w, and polydispersity, $Đ = M_w/M_n$, of PMA-Na. 50 µL 2 mg mL^{-1} PMA-Na in 0.3 M $NaNO_3$, filtered through a 0.2 µm filter, was injected through a TSK guard column in series with a 17 µm 300 × 7.5 mm^2 Tosoh Biosciences TSK-GEL G5000PW column and a 13 µm 300 × 7.8 mm^2

Tosoh TSK-GEL GMPWxl column at a flow rate of 1.0 mL min^{-1} (see Supporting Information Figure S4) using 0.3 M $NaNO_3$ as mobile phase. Absolute molecular weights were determined with a 13-angle light scattering detector (DAWN-EOS, Wyatt Technology) in series with a differential refractive index detector (rEX, Wyatt). The *dn/dc* of PMA-Na in the mobile phase was 0.2397 mL g^{-1} as determined on the refractometer. PMA-Na had M_w, M_n, and Đ of 3.52 x 10^5, 2.68 x 10^5 and 1.32, respectively. The molecular weights of the PQVP series were assumed to be those of the starting PVP (ca. 2 x 10^5 g mol^{-1} according to the manufacturer) with the alkane chain and iodide or bromide ion counterions.

Isothermal Titration Calorimetry (ITC)

Polyelectrolyte solutions were dialyzed (3,500 molecular weight cutoff tubing, SnakeSkin™, ThermoFisher) against deionized water for 2 days, with water replacement every 12 h. The solutions were then freeze-dried (Labcono, FreeZone 105). QVP-C1 was dialyzed against 2 M NaBr for 2 days with salt solution replacement every 12 h prior to dialysis against water to exchange the counterion from iodide to bromide. The polyelectrolyte powders were dried at 110 °C for 4 h, then quickly transferred to an Ar-filled glovebox to be weighed.

ITC was performed using a VP-ITC (MicroCal Inc.) calorimeter. The ITC was calibrated with an internal y-axis calibration followed by a standard titration between hydrochloric acid and Tris base. All samples were degassed for 10 min at room temperature. Approximately 300 µL of a 10 mM polycation solution with 0.05 M NaCl were loaded into the syringe. 10 µL of the syringe solution were manually discharged from the syringe to relieve any back pressure from the loading process. Prior to filling, the sample cell (1.4138 mL) was washed with 0.5 mM polyanion solution with 0.05 M NaCl. The syringe was rotated at 260 rpm in the sample cell with an injection size of 4 µL per aliquot at a rate of 0.50 µL s^{-1}, with 240 s between injections. The heat flow was recorded as a function of time at 25.0 °C for all samples (Supporting Information Figure S5). Enthalpies were calculated by summing the total heat generated to the end point with a correction for the background dilution enthalpy.

Small Angle X-Ray Scattering (SAXS)

SAXS measurements were performed on a Bruker NanoSTAR system with an Incoatec IμS microfocus X-ray source. The primary beam was collimated with cross-coupled Göbel mirrors and a 3-pinhole system providing a Cu K_α beam (λ = 0.154 nm) with a size of about 0.15 mm at the sample. The 2D scattering pattern was obtained using Vântec-500 detector located at a distance of 25.87 cm from the sample. Samples were prepared by adding 10 mg of dry PEC powder and 20 μL 0.10 M NaCl to a 6 x 6 mm aluminum foil pocket with a foil thickness of 20 μm. The foil pocket was crimped shut and allowed to sit for 24 h. The pockets were sealed against a stainless-steel sample holder with a 4 mm diameter hole for the beam.

For the PMA/QPVP-C5 sample, the Q-range was extended with the use of the synchrotron 5-ID-D beam source at Argonne National Laboratory. PEC samples were placed in 1.5 mm diameter thin wall capillaries. The direct beam position and detector distances were calibrated using lanthanum hexaboride, silver behenate and a silicon diffraction grating. Scattering intensities were calibrated using a glassy carbon standard. The data were collected along the center axis of each sample at x-ray energies of 17 keV and a wavelength of 0.7293 Å.

Linear Viscoelastic Response (LVR)

A DHR-3 stress-controlled rheometer (TA Instruments) in oscillation mode equipped with 20 mm parallel plate geometry was used to study the LVR of hydrated PECs. A custom-designed stainless-steel reservoir with a cap to prevent water evaporation was used as the bottom geometry. Samples were soaked in 0.01 M NaCl for 24 h before the rheology experiments. Samples were loaded on the bottom plate (reservoir), squeezed to a thickness of 100 μm and trimmed to remove excess material. The reservoir was then filled with 0.01 M NaCl. Frequency sweep experiments were carried out from 0.01 to 100 Hz at temperatures ranging from -5 °C to 65 °C. Temperature ramp experiments were carried out from 0 °C to 60 °C with a ramp rate of 1 °C min^{-1} at 0.1 Hz and 1% strain. Amplitude experiments were performed before each trial to ensure all data were within the linear viscoelastic regime.

Radiolabeling Experiments

The amount of salt in PECs as a function of the solution salt concentration was determined precisely using NaCl labeled with ^{22}Na isotope. Labeled NaCl solutions with various concentrations were prepared at a specific activity of 5×10^{-4} Ci mol^{-1}. For example, 5.6 μCi $^{22}Na^{+}$ was spiked into 45 mL 0.25 M NaCl to label the NaCl. For scintillation counting, a vertical RCA 8850 photomultiplier tube (PMT) powered at -2300 V and a Philips PM6654C frequency counter were used. The gate time of the frequency counter was 10 s, and the pulse threshold was -20 mV.

Depending on the viscoelastic properties of the sample, two radiolabeling methods were used. For solid-like PECs ([NaCl] < 0.2 M), starting from the lowest salt concentration, PEC samples were soaked in 50 mL 0.04 M NaCl in a 50 mL centrifuge tube for 24 h before use. The 0.04 M NaCl was replaced with 10 mL of 0.04 M NaCl “hot” solution and PEC samples were allowed to equilibrate for 24 h to allow the $^{22}Na^{+}$ tracer to exchange with unlabeled Na^{+}. PEC samples were dab-dried and weighed. These radiolabeled PECs samples were directly placed on top of a 3 mm thick disc of plastic scintillator sitting horizontally on the end of the PMT and counted. PEC samples were then transferred into a “hot” NaCl solution with a higher salt concentration, and the same procedure repeated. To convert counts to moles of NaCl, a calibration curve was created for each “hot” solution by adding 10-50 μL aliquots on top of the plastic scintillator. The total counts of each sample ranged from 400,000 to 2,700,000, with respective counting errors of 0.2% and 0.1%.

For liquid-like PECs ([NaCl] > 0.2 M), where PEC samples could not be easily transferred to the top of the PMT or re-collected, an extraction method was used: after doping in labeled 0.25 M NaCl, the solution was removed carefully with a pipette and the wall of the centrifuge tube was dried completely with a wipe. The entire tube was weighed, and 10 mL of unlabeled 0.25 M NaCl was added to the centrifuge tube to exchange the radioisotope out of the PEC over a period of 24 h. 500 μL of the exchanged solution was added to a plastic vial containing 5 mL liquid

scintillation cocktail (LSC), and the activity of the sample was determined by counting the vial on top of the PMT. The remaining solution was removed, and the next labeled NaCl was added. A calibration curve was made by adding 10 - 50 μL aliquots of labeled NaCl into 5 mL LSC.

After all salt concentrations had been measured, PECs were rinsed in water for 3 days, with water replacement every 24 h, dried under vac at 120 °C for 24 h and weighed to record the dry weight of PEC.

Results and Discussions

Hydrophobicity in Polyelectrolytes

A common strategy for increasing hydrophobicity in small molecules and polymers is to attach increasingly longer alkane chains -C_nH_{2n+1}, or increasing numbers of the same chain, to them. In polyelectrolytes, these chains sprout from the charged repeat unit, or they are dispersed as neutral comonomers in the chain, for example in "polysoaps."[35] Quaternized polyvinylpyridines have been used for antimicrobial coatings[36] and to probe the influence of hydrophobicity in "polyplexes" for gene delivery.[37]

N-alkylated P4VPs have been used by Sadman et al. to prepare PECs with poly(styrene sulfonate), PSS.[27] Reporting results using C1-C3 materials, they found PECs were stiffer than those used in the present work. PECs made with polycarboxylates are generally weaker and less resistant to salt than those made with PSS,[38] possibly because PSS itself is more hydrophobic and may exhibit π-π or π-cation interactions with polycations. In addition, endothermic complexation (as is the case here) assists the unpairing of polyelectrolyte repeat units whereas PECs which complex exothermically may never dissolve in added salt.[39]

A distinct difference in solutions and PECs made with the two longest chains, -C5 and -C6, compared with C1-C4 PECs was noted. PQVP-C1 to -C4 were soluble in water at room temperature, whereas PQVP-C5 & -C6 were not. Transforming the PMA to the

tetraethylammonium salt allowed solutions of PMA to be prepared in water/ethanol mixtures, which could be combined with PQVP-C5 & -C6 to prepare PECs (see Table S1, Supporting Information). An appropriate solvent to dissolve these PECs for ^{1}H NMR stoichiometry measurements could not be found, and ITC could not be performed due to the lack of water solubility. Thus, the main focus was initially on PMA/QPVP-C1 to -C4 PECs.

The ratio of positive to negative repeat units, the stoichiometry, is an important variable in PEC viscoelastic properties. Off-stoichiometric PECs have lower storage and loss moduli (G' and G", respectively) and the glass transition temperature, T_g, is lowered compared to stoichiometric PEC.[40] The stoichiometries for each PEC, dissolved in 1.0 M NaCl, were measured with ^{1}H NMR spectroscopy (see Supporting Information Figure S3 for spectra) and are summarized in Table 1.

Table 1. Stoichiometry of PECs, enthalpy of complexation ΔH_{PEC}, critical NaCl concentration CSC, at room temperature

Cation in PEC	$Pol^+ : Pol^-$ ± 0.03	ΔH_{PEC} (J mol^{-1}) ± 100	CSC (M, NaCl)	^{1}CSC, Φ_{salt}
PQVP-C1	1.00 : 1.00	3220	0.58	0.016
PQVP-C2	1.01 : 1.00	3550	0.45	0.012
PQVP-C3	1.03 : 1.00	3870	0.44	0.012
PQVP-C4	1.03 : 1.00	4110	0.31	0.0084

[1] CSC in terms of volume fraction of salt

Calorimetry and Hydrophobicity

As in prior work,[27, 28, 41] it was assumed that adding successively longer carbon chains to the nitrogen would systematically make the PECs more hydrophobic. In fact, PVP with 5- and 6-carbon alkane chains (PQVP-C5 and PQVP-C6) were not soluble in water at room temperature,

such was their “hydrophobicity.” Two quantitative measures were employed to assess the degree of hydrophobicity. First, solution calorimetry was used to measure the enthalpy of complexation, ΔH_{PEC}. In a second measure of hydrophobicity, samples of hydrated PEC were pressed against a diamond FTIR-ATR crystal to record the water O-H stretching region, known to be sensitive to the environment in solution[42, 43] and in polymers.[44]

Sensitive calorimetry measurements (see Supporting Information Figure S5 for the individual results) returned the surprising result that ΔH_{PEC} increased slightly (became more *endothermic*) with the addition of more carbons (see Table 1), implying a *less favorable* driving force for PEC formation. This finding is not expected if increasing hydrophobicity drives complexation, as it could be reasoned that increasing hydrophobicity would result in increasingly exothermic complexation as the alkylated regions of PVP could “escape” from water on PEC complexation. However, as with many assumptions regarding hydrophobicity,[45] this assumption is not correct, since the enthalpy of hydration of ethane to heptane is actually *exothermic*, adding about -3.2 kJ mol^{-1} per carbon on the alkane chain at room temp.[46]

ATR-FTIR spectra of the O-H stretching region in hydrated (i.e. soaked in water) PMA/PQVP-C1 to -C6 are shown in Figure 1. Figure 1A displays the expected increases in C-H methylene stretching bands with increasing alkane chain lengths. Small differences are observed in the shape of the O-H stretching envelope from 2750 to 3700 cm^{-1}. Changes to the low-wavenumber shoulder of this band are present if there is a difference in the water hydrogen-bonding network structure compared to bulk water.[42, 43] The zoom-in of Figure 1B normalizes the PEC water bands to that of bulk water at 3350 cm^{-1}, an area of the envelope impacted less by disruption to H-bonding. The changes in shape qualitatively illustrate less disruption to H-bonding by PMA/PQVP-C1 - C4 (“cosmotropic,” in the language of the Hofmeister series) and more disruption by PMA/PQVP-C5 and -C6 (chaotropic). Recent studies on the balance of ions within PECs have emphasized the influence of specific interactions between charged repeat units and ions.[47] These interactions and their enthalpies correlated to the degree of hydrogen bond

disruption in the water shell around the polymer/ion pair.[48] Although Figure 1 shows some evidence of H-bonding changes relative to bulk water, the perturbations in the water O-H band are much lower than those in, for example, poly(diallyldimethylammonium) paired with different counterions.[48]

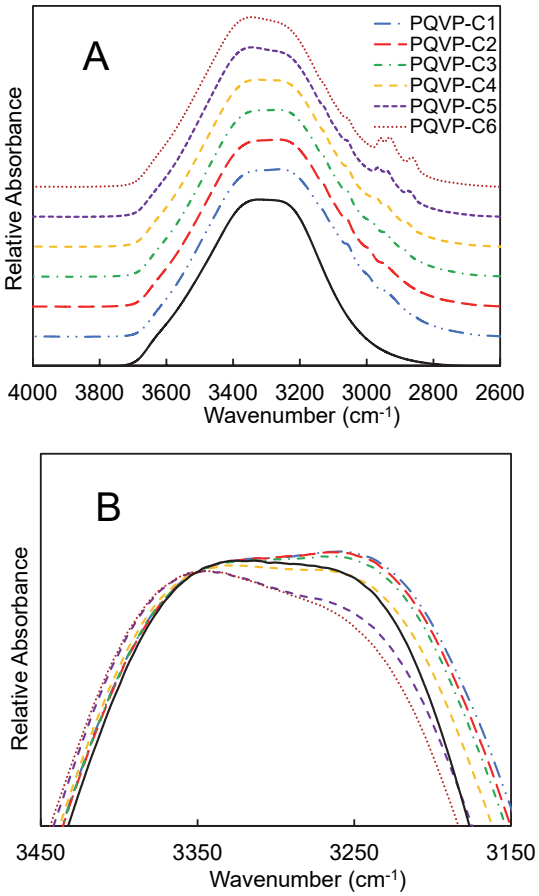

Figure 1. ATR-FTIR spectra of wet PMA/PQVP-C1 to -C6 coacervates showing **A**, the O-H stretching region from water in the PECs (spectra have been normalized and offset for clarity); **B**, zoom-in of the water bands normalized to the absorbance of bulk water at 3350 cm^{-1} to emphasize changes in shape. Room temperature. The solid black spectrum is that of bulk water.

Compositions of PECs in Response to Increasing Salt Concentration

The composition of PECs in response to salt concentration provides information on the phase space and stability of these materials. In a site-specific interaction model, added salt breaks charge pairs between positive, Pol^+ and negative, Pol^-, repeat units, as in Equation 1

$$Pol^+Pol^-_{PEC} + Na^+_s + Cl^-_s \rightarrow Pol^+Cl^-_{PEC} + Pol^-Na^+_{PEC} \quad [1]$$

where subscripts "PEC" and "s" refer to PEC and solution phase (or "dilute" phase), respectively. Pol^+Pol^- pairs are termed "intrinsic" charge compensation while Pol^+ or Pol^- balanced by a counterion are "extrinsic" sites.[33] At a sufficiently high $[NaCl]_s$ the polyelectrolytes may (or may not[39]) dissociate completely and the solution becomes single phase. This critical salt concentration, or "salt resistance"[7] is typically used as a measure of the strength of polyelectrolyte association.[17, 18, 41, 49, 50, 51] Both the CSC and the entire phase diagram are temperature dependent,[33] leading to phenomena such as critical solution temperatures for (micro)phase separation.[14, 52, 53, 54]

Figure 2 depicts photographs of PECs PMA/PQVP-C1 to -C4 with increasing $[NaCl]_s$. At lower $[NaCl]_s$ the volume of the PEC remains roughly constant. As $[NaCl]_s$ approaches the CSC, the PECs inflate rapidly then dissolve. Again, it was surprising to observe the CSC *decreases* with increasing alkane chain length, indicating lower stabilities, although the results were consistent with the increase in endothermicity seen in Table 1. CSC values are given in Table 1.

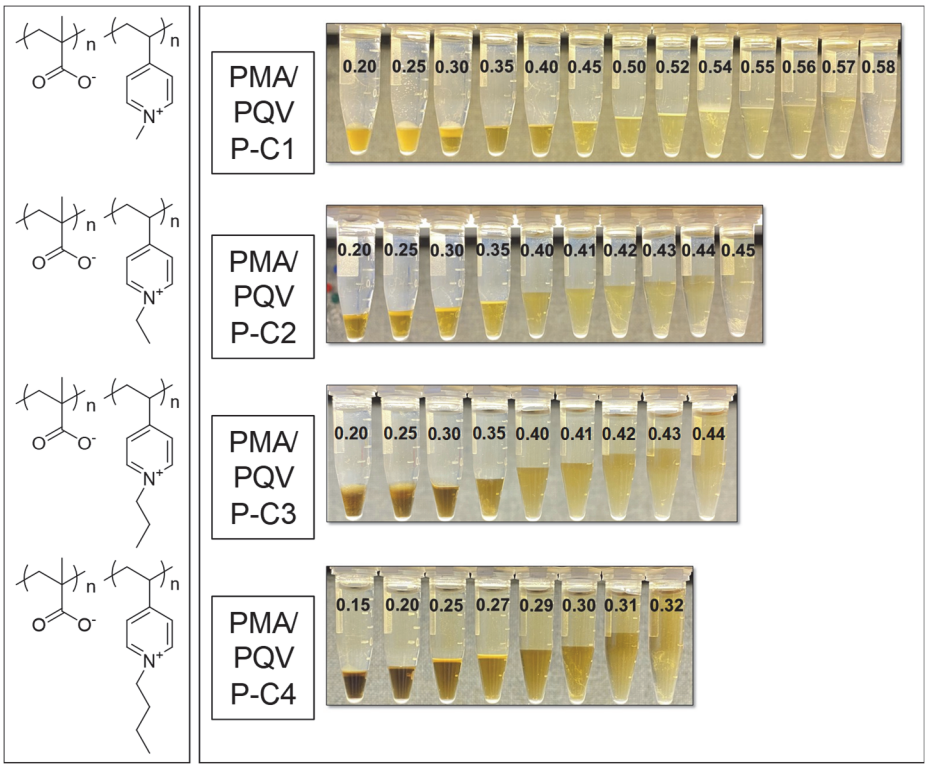

Figure 2. PMA/PQVP-C1 to -C4 PECs in solutions of increasing $[NaCl]_s$. The number on the 1.5 mL centrifuge tube represents $[NaCl]_s$. The lower phase is the polymer-rich complex/coacervate, whereas the upper phase is the "dilute" phase.

The use of radiolabeled NaCl permitted precise tracking of the all salt content within a PEC, $[NaCl]_{PEC}$ in response to the external (solution) salt concentration, $[NaCl]_s$.[20] In Figure 3 the salt content of PECs as a function of $[NaCl]_s$ is presented in two ways: first, as $[NaCl]_{PEC}$, also as *r*, the molar ratio of salt in PEC to polyelectrolyte repeat units, PE.

$$r = \frac{[MA]_{PEC}}{[PE]_{PEC}} \qquad [2]$$

For example, if r = 1.0 there is one salt ion for each Pol^+ or Pol^- polyelectrolyte repeat unit. In theory, for a site-specific model, a minimum of r = 1.0 is needed to break all Pol^+Pol^- charge pairs. In practice, not all salt in the PEC breaks charge pairs, so at the CSC and elsewhere, r > 1.0.

In the case where polyelectrolyte interactions are coupled to ions, if ΔH_{PEC} is endothermic,[20] $[NaCl]_{PEC} > [NaCl]_s$ (if interactions such as hydrogen bonding are not coupled to ions, this condition may not apply). This is clearly seen in Figure 3B.

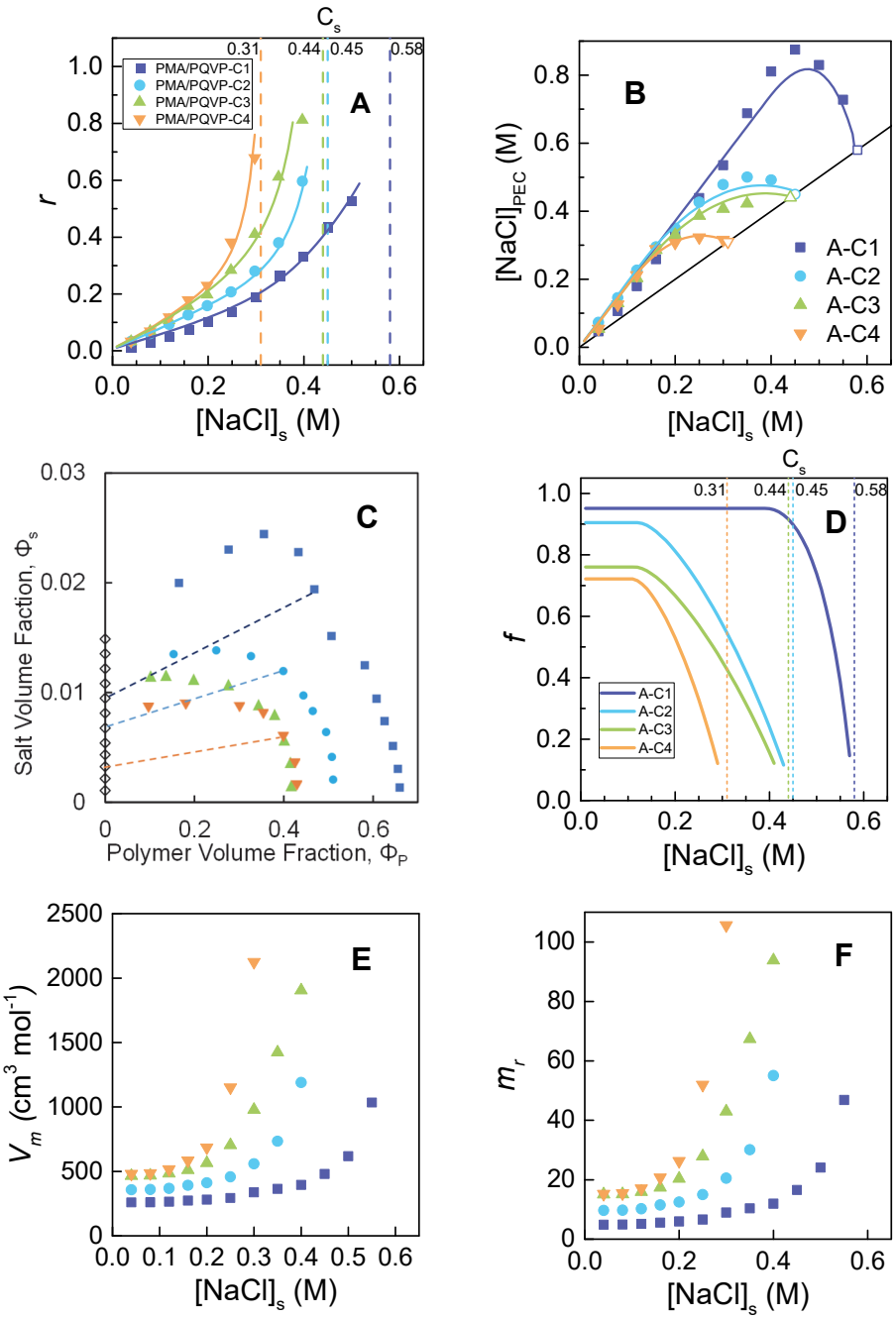

Figure 3. Composition of PMA/PQVP-C1 to -C4 PECs. **A**, *r* vs. $[NaCl]_s$; **B**, $[NaCl]_{PEC}$ vs. $[NaCl]_s$ the solid lines are a fit to Equation 4 using the *f* values in **D**; **C**, phase diagram representation of

volume fraction NaCl, Φ_s, versus volume fraction polymer in the PEC phase, Φ_P. Open diamonds on the left are for the dilute phase (where Φ_P remains less than 0.03 at all $[NaCl]_s$). Dotted lines are "tie lines." Open symbols show the dilute phase composition. **D,** values of *f* selected for best fit of Equation 4 in Panel **B**; **E**, molar volume of PEC vs. $[NaCl]_s$; **F**, ratio of water molecules to polyelectrolyte pairs Pol^+Pol^- vs. $[NaCl]_s$.

Figure 3C displays the salt and polymer content in a typical phase diagram format for PECs. Tie lines increase from left to right showing $[NaCl]_{PEC} > [NaCl]_s$, expected for endothermic ΔH_{PEC}s.[20] The shape of the phase diagram appears unusual[11] because of the broadly peaked nature of the data due to excess salt in the PEC at all compositions. Note that the CSC is usually depicted in the literature at the maximum of the phase diagram, although this is not necessarily the case.[11] In Figure 3C the CSC is to the left of the maximum (see Table 1 for CSC). It is universally true that $[NaCl]_{PEC}$ is close to $[NaCl]_s$ at the CSC. Near the CSC, polyelectrolyte was observed in the "dilute" (supernatant) phase (see Supporting Information Figure S6). The polyelectrolyte in the dilute phase was determined up to a salt concentration of about 0.04 M less than the CSC and was always less than about 3 mM which corresponded to a polymer volume fraction of about 0.01. The measured dilute phase concentration was used to construct the tie lines shown in Figure 3C. They have a positive slope because ΔH_{PEC} is endothermic.[20]

The content of water and polyelectrolyte, measured by weight, provide the material balance for the complete compositions of each PEC at each $[NaCl]_s$. These were used to calculate the water content in terms of water molecules per Pol^+Pol^- pair, m_r, and the volume containing one mole of PMA/PQVP, V_m[20]

$$V_m\ (cm^3 mol^{-1}) = \frac{1000}{[PE]_{PEC}} = \frac{M_{Pol^+Pol^-}}{\rho_{PEC}} + 18m_r + r\frac{M_{MA}}{\rho_{MA}} \qquad [3]$$

Where $M_{Pol+Pol-}$ and M_{MA} are the respective molar masses of (dry) Pol^+Pol^- and MA and ρ_{PE}, ρ_{MA} are their densities. Figures 3E and 3F indicate that the water contents, and thus the volume of

the PEC, do not change significantly for low $[NaCl]_s$ (here, < 0.2 M), a typical result for stoichiometric PECs at low doping. Counterintuitively, the water contents of undoped PECs *increase* with the (supposedly) more hydrophobic polycation (Figure 3E & F).

Focusing on the disposition of salt ions in the PEC, there are at least two environments in which ions may be found: ions located next to polyelectrolyte charges act as *counterions*, breaking Pol^+Pol^- pairs; ions within the PEC that do not break pairs are termed *co-ions*.[20, 33] Of course, there is a dynamic exchange between ions and locations, but the instantaneous fraction of ions acting as counterions is termed *f*. If f = 1 all ions in the PEC break Pol^+Pol^- pairs.

The balance of salt inside versus outside a PEC is given by a Donnan equilibrium (entropic) modified by an enthalpic term[20]

$$[NaCl]_{PEC} = [NaCl]_s e^{\frac{f\Delta H_{PEC}}{2RT}} \qquad [4]$$

The sign of the enthalpic terms causes $[NaCl]_{PEC}$ to be more or less than $[NaCl]_s$. The actual value of *f* is difficult to measure, but its influence on the composition of a PEC has been computed by Ghasemi et al.[55] If ΔH_{PEC} is endothermic, salt prefers to be associated with Pol^+ or Pol^- and *f* approaches 1.

The efficiency of breaking or unpairing Pol^+Pol^- pairs is given by an equilibrium constant K_{unp}. Equation 5 was derived by us[20] and by Ghasemi et al.[55]

$$K_{unp} = \frac{(fr)^2[PE]_{pec}}{(1-fr)[MA]_S^2} \qquad [5]$$

Combining this equation with Equations 2,3 & 4 yields

$$K_{unp} = \frac{f^2 V_m e^{\frac{f\Delta H_{pec}}{RT}}}{(1-fr)} \qquad [6]$$

As [NaCl] → 0, r → 0 and

$$K_{unp} = f_0^2 V_{m,0} e^{\frac{f_0 \Delta H_{pec}}{RT}} \quad [7]$$

where $V_{m,0}$ is the molar volume for undoped PEC and f_0 is the initial f at low doping levels. All terms in Equations 4 thru 7 are known except for f. Thus, values for f that fit the data in Figure 3B have been plotted in Figure 3D. From Figure 3D, f_0 decreases from 0.95 to 0.7 from C1 to C4 PECs. The corresponding fits are shown as solid lines on the plots in Figure 3A & B.

Viscoelasticity of PECs

Variable temperature rheology measurements were undertaken to investigate trends in viscoelastic response with hydrophobicity. All the hydrated C1-C5 PECs studied here are assumed to be above their glass transition temperature[56] since the modulus remained relatively low and no T_g could be detected (see Supporting Information Figure S7). The LVR was determined while PECs were immersed in 0.01 M NaCl, which is a sufficiently low salt concentration to cause minimal doping (see Figure 3A) but provide enough osmotic pressure to prevent spontaneous inflation and pore formation induced by the residual osmotic pressure in the PEC.[57] Keeping the PECs essentially undoped allows full pairing between Pol^+ and Pol^-. The small reservoir containing the 0.01 M NaCl also offered temperature control. Thus, frequency sweeps were performed from 0.1 to 100 rad s^{-1} at eight temperatures ranging from -5 °C to 65 °C. Frequency sweeps were then stitched together using time-temperature superposition,[58, 59] TTS, and the shift factors, a_T, in Supporting Information Figure S8. The upper temperature of PMA/PQVP-C4 was limited to 55 °C due to evidence of thermal instability (possibly de-alkylation) at higher temperatures. Shifts in the vertical axis ("b_T") were small and presumed to account for slight changes in volume. In our experience, the composition of a PEC does not change substantially over the limited temperature ranges employed.[60]

Figure 4 presents G' and G" for all four complexes. Characteristic crossing points indicate the reptation rate, ω_{rep}, at lowest frequencies;[61] the entanglement rate, ω_e, at intermediate frequency, and the rate for a certain number of Pol^+Pol^- units to exchange places, ω_b, at the highest frequency (only measurable in PMA/PQVP-C4).[62] These frequencies are presented in Table 2. The area between ω_{rep} and ω_e is the rubbery region where the rubbery plateau modulus, G_0, was taken to be G' at the point where tanδ (= G"/G') was at a minimum (see Supporting Information Figure S9). The LVR of PMA/PQVPC1-C4 did not depend on pH from pH 6 to 10, indicating little change in the state of protonation of the PMA (Supporting Information Figure S10).[51]

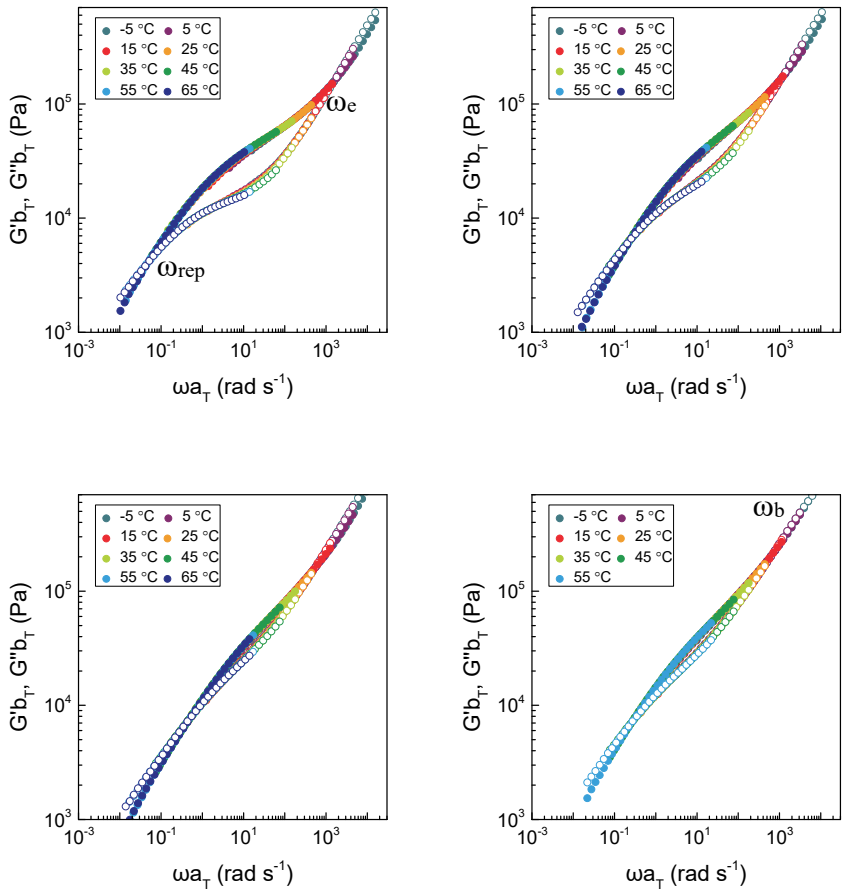

Figure 4. Linear viscoelastic response for hydrated PMA/PQVP-C1 to -C4 PECs in 0.01 M NaCl. Upper left: PMA/PQVP-C1, upper right: PMA/PQVP-C2, lower left: PMA/PQVP-C3, lower right:

PMA/PQVP-C4. Filled symbols are for G' and open symbols refer to G". Reference temperature is 25 °C.

Table 2. Relaxation rates and rubbery plateau modulus for hydrated PMA/PQVP-C1 through -C4 PECs.

PEC	ω_{rep} (s^{-1})	ω_e (s^{-1})	ω_b (s^{-1})	G_0 (Pa)
PMA/PQVP-C1	0.0013	38	-	4.4×10^4
PMA/PQVP-C2	0.0056	29	-	4.7×10^4
PMA/PQVP-C3	0.011	13	-	4.6×10^4
PMA/PQVP-C4	0.0070	17	133	4.5×10^4

There are trends, but not stark differences, comparing the LVR of PECs in Figure 4. All complexes appear to be entangled, the C3 and C4 less so. It is to be expected that the dilution of polymer volume fraction by water (Figure 3E) increases the entanglement molecular weight and only allows longer chains of this wide molecular weight distribution material to remain entangled. In a general trend, reptation times decrease and entanglement times increase (as would be expected for longer entanglement lengths) but the trends are not strong. No difference, within experimental error, was observed for plateau modulus. Given that water is a strong plasticizer for PECs,[1, 63, 64] one might have expected much softer materials going from undoped PMA/PQVP-C1 to C4 (5 to 15 water molecules per Pol^+Pol^- pair). This is another example of the importance of the location of the water for influencing LVR.[52, 56] To accelerate relaxation dynamics and decrease all modulii, water should probably surround the Pol^+Pol^- pair in a "relaxation shell." Referring to Figure 3, if it is assumed that PMA/PQVP-C1 allows ≤ 5 water molecules in the hydration shell around the Pol^+Pol^- pair, the balance of water in the other PECs must be beyond this hydration shell. Table 1 shows only small increases in endothermicity consistent with the discussions above that the enthalpies of hydration of short hydrocarbon chains reveal no

particular “phobicity” against water. Thus, it would be reasonable to find water molecules around these alkane chains.

PMA/PQPV-C5 & -C6 PECs

As mentioned above, some experiments, notably the calorimetry studies, on the formation of PECs from the most hydrophobic polycations (PQVP-C5 & -C6) could not be performed in water.. Conditions for PEC formation with the two polymers were eventually identified. The properties of these PECs contrasted to those from PQVP-C1 thru -C4. PMA/PQVP-C5 was soft in 0.01 M NaCl and expanded drastically in 0.1 M NaCl. PMA/PQVP-C6 was more than 100 times stiffer in 0.01 M NaCl and did not swell in solutions of higher salt concentration (up to 2.5 M).

The viscoelastic properties of these PECs in 0.01 M NaCl are shown in Figure 5. G’ and G” in PMA/PQVP-C6 were well separated and exhibited little dependence of modulus on frequency or temperature - a response reminiscent of a crosslinked gel. The LVR of PMA/PQVP-C5 was between those of PMA/PQVP-C6 and the other PECs. TTS for PMA/PQVP-C5 was somewhat satisfactory (Figure 5C, and Supporting Information Figure S11) but not for PMA/PQVP-C6 (see individual LVR plots in Figure S12 Supporting Information).

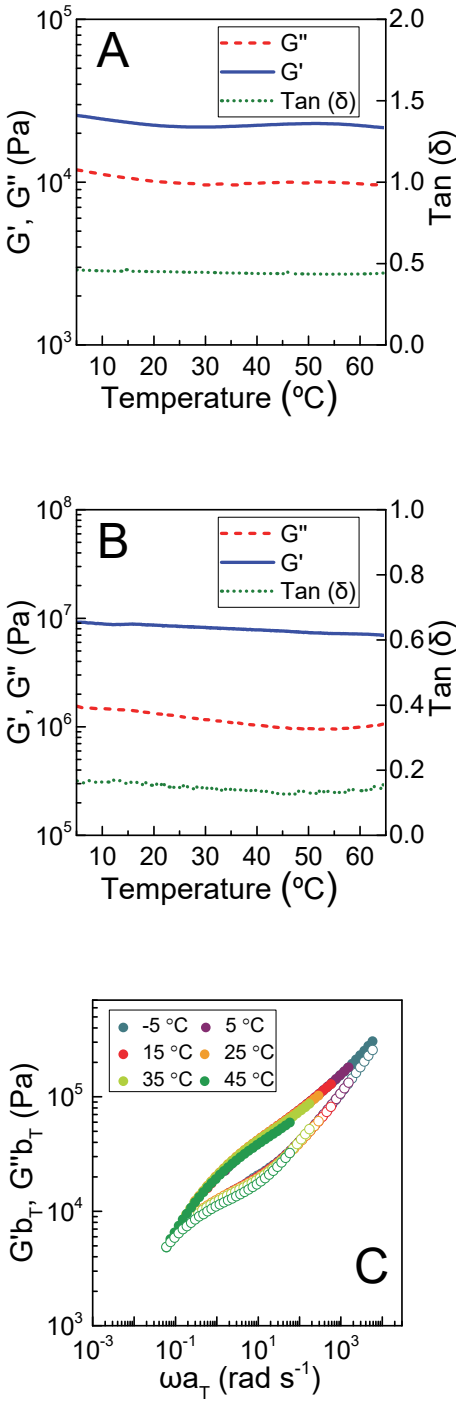

Figure 5. Viscoelastic response as a function of temperature for **A**, PMA/PQVP-C5; and **B**, PMA/PQVP-C6 in 0.01 M NaCl. Ramp rate = 1 °C min^{-1}. **C**, TTS attempt for PMA/PQVP-C5, reference temperature = 25 ºC; filled symbols refer to G' and open symbols to G".

The much higher modulus of PMA/PQVP-C6 is an expected behavior for a complex that is held together more strongly by hydrophobic interactions, as observed in other studies. Together with the lack of temperature response, the PMA/PQVP-C6 results are consistent with a class of material that contains additional non-electrostatic interactions between polymer chains, specifically, aggregates of a hydrophobic phase containing the alkane chains of the quaternized PVP.

X-ray scattering was performed on PMA/PQVP-C4, -C5, and -C6 at 15 ^{o}C (Figure 6). A weak scattering peak at Q ≈ 0.2 A^{-1} is seen for PMA/PQVP-C4. PMA/PQVP-C6 shows strong scattering at Q ≈ 0.3 A^{-1}, while PMA/PQVP-C5 displays transitional behavior with scattering at both 0.2 and 0.3 A^{-1}. The PMA/PQVP-C6 feature may be due to aggregation of -C6 chains and may be compared to the X-ray scattering of poly(acrylate) with C8-trimethylammonium counterions in water, reported by Svensson et al.,[65] which was interpreted to stem from a disordered micellar phase. The PMA/PQVP-C5 peak at 0.2 A^{-1} has disappeared at 45 ^{o}C (Figure S13 Supporting Information) indicating possible reorganization of the C5 chains. PQVP-C5 by itself was found to dissolve in 0.1 M NaCl at 45 ^{o}C, an observation consistent with reorganization of hydrophobic units in the corresponding PEC. A modest increase in the modulus of PMA/PQVP-C5 for temperatures greater than 35 ^{o}C (Figure 5A) may be related to reorganization of the hydrophobic content. The steep upturn at low Q for small angles (Figure S13, Supporting Information) where the intensity scales with Q^{-4} is consistent with bulk aggregation on a variety of length scales. Lamellar structures seen in polyelectrolyte/surfactant coacervates[66] are unlikely here due to interchain mixing between the two polyelectrolytes imposed by charge pairing.

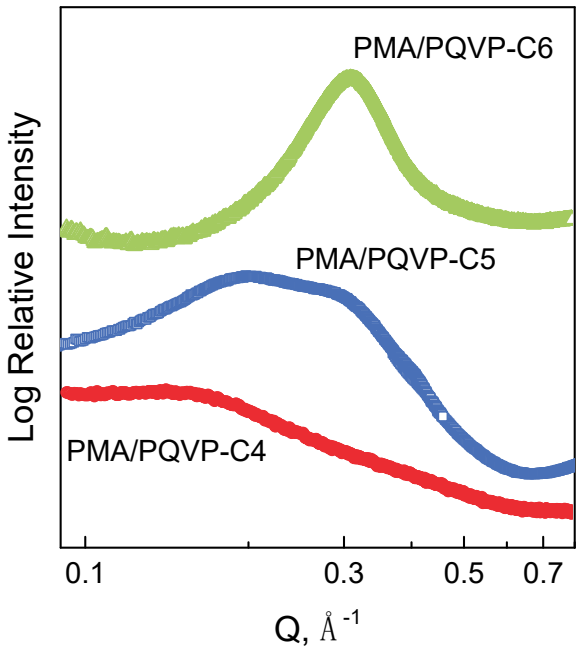

Figure 6. X-ray scattering data for PMA/PQVP-C4, -C5 and -C6 at 15 °C in 0.1 M NaCl. Intensities have been scaled and offset to show peak positions.

Reconciling an Increase in Hydrophobicity with a Decrease in PEC Stability

The counterintuitive results above emphasize the fact that hydrophobicity in PECs, as with hydrophobicity in neutral polymers, is not a straightforward concept and depends on how it is quantified, or at least "ranked." For example, Foster et al.[67] found, for aqueous solutions of neutral polymers, that water surface tensions scaled somewhat with Hildebrand solubility parameters (HSP) but not with solubility parameters described by Mathers and coworkers.[68] In contrast, the cloud temperatures of the same polymers were not correlated to the HSP but were correlated to the Mathers parameter.[67] Initial assumptions regarding the expected response of PECs may have been steered by classical arguments for the hydrophobic effect, or hydrophobic "bonding" used to account for protein folding.[24, 25, 26]

The question of hydrophobicity in PECs was recently explored by Huang and Laaser using a library of acrylamide copolymers modified with C2 - C12 alkanes.[18] In these polymers the hydrophobic side chains were located on neutral comonomers. Measurement of the CSC showed a complex dependence of stability on chain length and charge fraction. For a high charge fraction, there was no difference in the CSC up to C4 – unlike the results seen here. Longer alkane chains C6 – C12 yielded more salt-stable complexes.[18]

Sadman et al.[27], using PVP alkylated with one to three carbons, found the LVR of QPVP/PSS was relatively insensitive to hydrophobicity, in line with results seen here. However, in contrast to Figure 3A, they also found that more hydrophobic PECs were harder to dope.[27] Tabandeh and Leon,[28] using PECs from polypeptides, also concluded that hydrophobicity had a stabilizing influence against dissolution by salt. A similar conclusion was reached in a comparison of PECs made with backbones of different hydrophobicity.[29]

In the present study, PECs containing QPVP-C5 and -C6 could be prepared only with mixed solvents and the materials were strongly scattering, suggesting a secondary aggregation process other than $Pol^{+}Pol^{-}$ pairing such as the association of the -C5 or -C6 chains. For example, PMA/PQVP-C5 PECs prepared by mixing components in water:ethanol, then rinsing and storage in aqueous 0.1 M NaCl, became scattering/opaque over time (weeks) rather than clear, as was the case with the C1-C4 PECs. Such opacity suggests slow intra-PEC aggregation.

A switch from destabilizing to stabilizing against salt may occur if the hydrophobic moieties become long enough to form a distinct phase such as a micelle or even a continuous phase within the PEC. The (Connelly solvent-excluded) volume of a PMA repeat unit was estimated to be 82 $Å^3$ using ChemDraw 20, while the volumes of C4, C5 and C6 alkane units were 76, 93 and 110 $Å^3$ respectively. Consistent with these estimates, a transition from isolated to associated alkane units might occur when the volume of the alkane unit becomes larger than that of the PMA repeat.

An alternative explanation draws from extensive work on the subtle balances of repulsive (entropic) and attractive (enthalpic) forces built into water mediated hydrophobic interactions.[69]

For individual small alkane chains, these two forces oppose and are almost perfectly balanced such that the free energy of hydration is slightly endothermic.[70] The poor solubility of, for example, hexane in water is due to a significant negative entropy term, traditionally associated with formation of ordered clathrate-like water structure around small nonpolar solutes, that overwhelms the favorable enthalpy of hydration. Macroscopic reporters of hydrophobicity, such as the immiscibility of oil in water, may be explained by efforts of the system to minimize interfacial free energy ΔG_A by reducing the area. However, it was found by Tanford,[71] and many others, that small hydrophobic molecules had an unusually small ΔG_A. Using the theory of Lum et al.,[72] Chandler[73] showed that the hydration enthalpy per unit area approached that expected from macroscopic surface tension measurements for objects greater than about 1 nm in size. Many have since described a length scale crossover, supported by theory and experiment, at this characteristic size.[69] The experimental outcome is that molecules (regions or side-chains) smaller than about 1 nm appear to be less hydrophobic when compared to the macroscopic scale.[69, 74]. Using hydrophobic polymers, Walker and coworkers also found that the length scale for water exposed surface area to become prominent in polymer hydrophobicity is about 1 nm.[75]

The energy of charge pairing between Pol^+ and Pol^- may also decrease slightly with increasing N-alkane chain length due to a combination of lower dielectric constant between the charges and steric hinderance (keeping them further apart). On the other hand, steric effects might decrease the access of counterions to the Pol^+Pol^- pair, yet Figure 2 clearly shows counterion access (doping) increases with increasing alkane chain length. Whatever the mechanism, the net result is reported by changes in ΔH_{PEC}.

The puzzling increase in water content with increasing alkane length for undoped PECs, shown in Figure 3F may be related to additional volume provided by the N-alkane unit, combined with a greater distance between Pol^+ and Pol^-. The additional water molecules as the alkane chain grows are probably located in a second solvation shell, farther from the Pol^+Pol^- pair. The net effect of additional hydration is to decrease the volume charge density of the PEC (the molar

volume increases), which allows more salt per Pol^+Pol^- to enter the PEC (K_{unp} increases, as in Equation 7).[20] In the absence of enthalpy changes, this explains why PECs bind more strongly if they have less water.

Conclusions

Poly(4-vinyl pyridine) was fully alkylated with haloalkanes from 1 to 6 carbons in length to explore the influence of what was assumed to be an increase in hydrophobicity on the phase compositions, doping, salt resistance and viscoelasticity of stoichiometric complexes made from these polycations and poly(methacrylate). Surprisingly, the strength of the -1C to -4C alkylated PECs, assessed by the ease of doping with salt and the critical salt concentration, weakened with increasing hydrophobic content. Complexation was endothermic and became slightly more so with increasing alkane chain length for these PECs. The weaker interactions are thought to be related to a decrease in volume charge density caused by the additional alkane chain volume and a substantial increase in water volume, a mainly entropic effect. Viscoelastic measurements on undoped hydrated PECs showed them to be entangled with characteristic reptation rates, entanglement rates, and plateau moduli that varied little. The association strength assessed by doping or salt resistance was not correlated to viscoelastic response in these doped PECs. PECs made from the -C5 and -C6 alkylated PVP exhibited a transition in behavior to stiffer materials with little temperature dependence in modulus. X-ray scattering results suggested a new morphology for the -C5 and -C6 materials with microphase separated alkane chain domains, and perhaps a continuous hydrophobic phase throughout the PEC, which could form additional physical interactions that dominate the viscoelasticity. This morphology and mechanical change may be related to a crossover in hydrophobic hydration, widely discussed for small molecules and in protein folding, on a length scale of about 1 nm.

Supporting Information

10.1021/acs.macromol.2c00267

FTIR spectra of PQVP-C1 to -C6 showing quantitative alkylation of the P4VP starting material; ^{1}H NMR spectra of dissolved PECs; SEC-MALLS trace of PMA; ITC thermograms for the aqueous complexation of PMA/PQVP-C1 – C4; UV-vis absorbance spectra of the dilute phase; modulus versus temperature of PMA/PQVP-C1 and PMA/PQVP-C4 PECs; modulus versus frequency using time-temperature superposition, TTS, of PMA/PQVP-C4 PECs at two pH values; solubilities of PMA-TEA and PQVP-C6 in water-ethanol mixtures; shift factors versus temperature used for TTS of PMA/PQVPC1-C4 PECs; tanδ versus frequency used to locate the plateau modulus; shift factors versus temperature for attempted TTS of PMA/PQVP-C5; viscoelastic response of PMA/PQVP-C6 as a function of frequency and temperature.; X-ray scattering over a broad Q-range for PMA/PQVP-C5. This information is available free of charge.

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Notes

The authors declare no competing financial interest

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