Chemorheology of Phenylboronate−Salicylhydroxamate Cross-Linked Hydrogel Networks with a Sulfonated Polymer Backbone

Meredith C. Roberts, Alamelu Mahalingam, Melissa C. Hanson, and Patrick F. Kiser*

Department of Bioengineering and Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, 20 S 2030 E Room 108, Salt Lake City, Utah 84112

Received June 5, 2008; Revised Manuscript Received August 7, 2008

ABSTRACT: Hydrogel networks cross-linked with polymer-bound phenylboronic acid (PBA) and salicylhydroxamic acid (SHA) demonstrate pH-reversible gel behavior due to the pH-dependent equilibrium of the cross-linking moieties that form the gel network. Furthermore, the pH at which gels behave dynamically can be controlled by use of a polyelectrolyte backbone. Here we report on the frequency-dependent chemorheological characterization of PBA−SHA cross-linked hydrogel networks with a sulfonated polymer backbone. Our results suggest that the anionic nature of the polymers allows reversible cross-linking at neutral pH that an otherwise neutral-backboned PBA−SHA cross-linked network cannot and that these charge-induced dynamics can be effectively screened by ions in solution. Moreover, moduli−frequency data can effectively be reduced into a single master curve with a neutral-backboned PBA−SHA gel data set as the reference condition.

Introduction

Understanding composition−structure−property relationships of macromolecular systems engineered with specific and tunable intermolecular interactions is paramount for the design and development of "smart", bioresponsive materials with tailored performance. For example, when the structural cross-links of a hydrogel network are reversible and therefore transient, the resulting network structure is also transient. Consequently, the gel's elasticity diminishes in time to a more liquidlike viscous property—by time-dependent changes in cross-link association and dissociation states—yielding variable degrees of a viscoelastic behavior. Though most reversibly cross-linked hydrogels under investigation utilize hydrogen-bonding or other noncovalent interactions,[1−6] there is a growing interest in developing reversible covalent chemistries as cross-linking technologies.[7−9]

Specifically, hydrogel networks cross-linked with borate esters or boronate esters expose the pH-sensitive, labile molecular nature of the coordinate covalent cross-links through the pH-dependent and self-healing chemorheological behavior of the resultant gels. Boric acid ($B(OH)_3$) commonly functions as a divalent cross-linker to macromolecular polyols, such as guar gum[10−12] and poly(vinyl alcohol),[13−15] to generate such self-healing networks. These borate−polyol gels demonstrate frequency-dependent rheological properties that translate into measures of the short time scales at which the network cross-links can relax and subsequently reorganize.[16−18] Because of the pH-sensitive binding equilibrium of the coordinate covalent bonds, the rheological properties of these cross-linked networks are pH-dependent. The sol−gel transition typically occurs at pH ~8−9, near the pK_a of the boron compound,[12] and the self-healing nature of these gels is evident by the viscous-dominant rheological response (i.e., loss modulus, G'', dominates storage modulus, G') at low angular frequencies, where the time scale of oscillation is long enough to allow network cross-link reorganization. Further increases in pH, which shifts the cross-link binding equilibrium toward a more associative, or bound, state, results in a shift in this frequency-dependent viscoelastic response toward a more elastic-dominant one (i.e., $G' > G''$) at all angular frequencies and thus experimentally probed time scales.[12]

Similarly, coordinate covalent cross-linking between multivalent, polymer-bound boronic acids ($RB(OH)_2$) and vicinal diols[8,9] or acids[19,20] also reveals pH-dependent and dynamically restructuring networks, though the pH sensitivity can be further adjusted by the selection of the non-boronate cross-linking moiety. For example, salicylhydroxamic acid (SHA) has a higher affinity for phenylboronic acid (PBA) at lower pHs than known diols and thus forms stable complexes at as low as pH 5, nearly 4 pH units below the pK_a of the phenylboronic acid used.[21,22] Furthermore, this PBA−SHA cross-linking chemistry demonstrates a pH-dependent binding equilibrium in which complex formation is almost entirely shifted toward the bound state at neutral pH (to the right in Figure 1) yet shifted more toward the unbound state at mildly acidic pH (to the left in Figure 1).[21] We have previously synthesized poly(2-hydroxypropylmethacrylamide) (pHPMAm)-based hydrogel networks cross-linked with polymer-bound PBA and SHA that demonstrate frequency-dependent rheological properties that change with pH just as the cross-link binding equilibrium shifts with pH—varying from dynamically restructuring and self-healing (i.e., $G'' > G'$ at low angular frequencies) at mildly acidic pH (4.2) to brittle and more permanently cross-linked (i.e., $G' > G''$ across the same angular frequency range) at neutral pH (7.6).[23]

Our laboratory is interested in synthetically controlling the frequency-dependent viscoelastic response of these PBA−SHA cross-linked hydrogel networks at different pHs such that we may be able to engineer the material's performance using a bottom-up approach. Two methods of synthetic manipulation

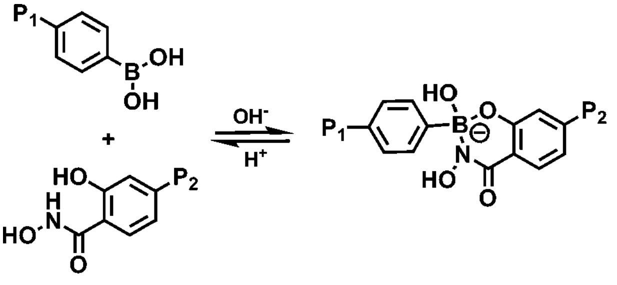

Figure 1. Scheme of reversible coordinate covalent phenylboronate−salicylhydroxamate (PBA−SHA) chemistry. When the PBA (upper left) and SHA (lower left) moieties are bound to water-soluble polymers (P_1 and P_2), the multivalent, binary polymer system will cross-link when mixed to form a hydrogel network under a range of pH conditions.

---

* Corresponding author: e-mail patrick.kiser@utah.edu; Tel (801) 587-3681; Fax (801) 585-5151.

10.1021/ma8012674 CCC: $40.75

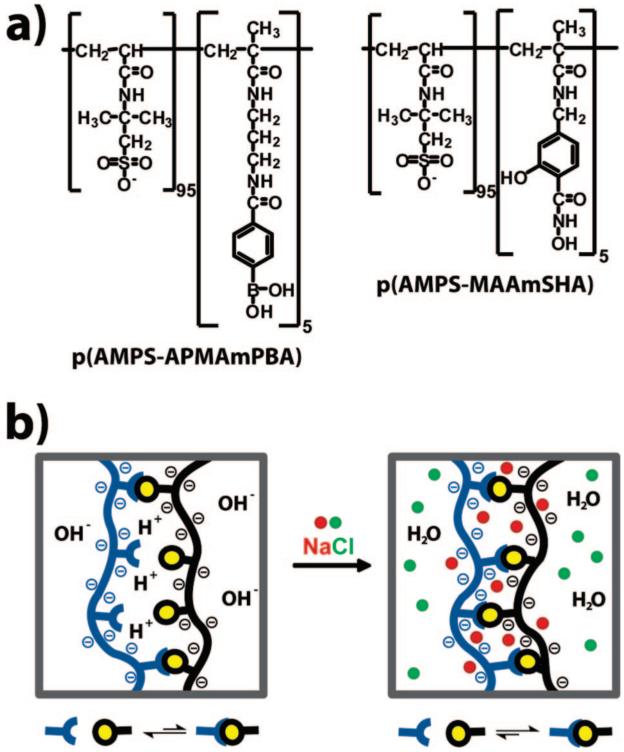

Figure 2. Reversibility of phenylboronate−salicylhydroxamate cross-linked hydrogel networks can be manipulated using an anionic polymer backbone and changes in ionic strength. (a) Linear water-soluble polymers containing either phenylboronic acid (p(AMPS−APMAmPBA), left) or salicylhydroxamic acid (p(AMPS−MAAmSHA), right) were synthesized with a polysulfonate (poly(2-acrylamido-2-methyl-1-propanesulfonic acid), or pAMPS) backbone. The polymers were free radical polymerized at a 95:5 molar feed ratio (AMPS:APMAmPBA or AMPS:MAAmSHA). (b) When the two different polymers (each represented in either blue or black with yellow) are mixed under aqueous, low salt conditions at neutral pH (left panel), dynamically restructuring hydrogel networks form. These gel dynamics occur because the anionic polymer backbone induces a locally acidic microenvironment due to the Donnan effect, thereby shifting the cross-linking reaction between PBA and SHA moieties toward the unbound state (see Figure 1). When the polymers are mixed in the presence of salt (right panel), Na^+ ions (red circles) competitively displace the protons and subsequently increase the pH adjacent to the negatively charged polymer backbone, causing the pH experienced by the cross-links to be closer to the bulk solution pH. This generates more permanent PBA−SHA cross-links and subsequently nonreversible networks.

have been previously applied to modify the pH sensitivity of boronate−diol complexation: (1) adding electron-withdrawing substitutions on the phenyl group to shift the pK_a of the PBA moiety[24,25] and (2) locally adjusting the pH of macromolecular boronate systems via the Donnan effect.[24,26,27] Here, we applied the latter method for manipulating the pH responsiveness of PBA−SHA cross-linked gel viscoelasticity by utilizing an anionic polyelectrolyte backbone. Specifically, polymers comprised of a polysulfonic acid backbone with either PBA or SHA functionality were synthesized and used to evaluate whether the anionic microenvironment would mask the effect of a neutral pH on the PBA−SHA cross-link binding equilibrium and thus frequency-dependent rheological properties (Figure 2). Moreover, salt may be added to counteract the negative charge effect of the anionic polyectrolyte backbone (Figure 2b).[28,29] Dynamic moduli were measured as functions of angular frequency for varying pHs, ionic strengths, and temperatures, and the dynamic gel rigidity (G'_{plateau}), a measure of the material's overall elasticity or strength, and characteristic relaxation time (τ), a chemorheological measure of the lifetime of the dynamic network cross-links,[30] were quantified from the frequency-dependent rheological data. Furthermore, a master curve analysis of all frequency-dependent moduli collected was performed to confirm that the molecular dynamics of polysulfonate-backboned PBA−SHA cross-linked hydrogel networks are in fact the same in origin as those observed for neutral-backboned gels with similar PBA−SHA cross-links.

Experimental Section

Materials. *N*-(3-Aminopropyl)methacrylamide hydrochloride (APMAm) was purchased from Polysciences, Inc. (Warrington, PA). 4-Carboxyphenylboronic acid pinacol ester was purchased from Frontier Scientific, Inc. (Logan, UT). 2-Acrylamido-2-methyl-1-propanesulfonic acid (AMPS) was recrystallized from methanol (dissolved at 60 °C, recrystallized at −20 °C) prior to use in free radical polymerizations. All other chemicals and reagents were purchased from Aldrich (St. Louis, MO) or Acros (Morris Plains, NJ) and used without further purification unless otherwise noted. Dichloromethane (DCM), dimethylformamide (DMF), ethyl acetate, and methanol (MeOH) were dried over 4 Å molecular sieves. Thin layer chromatography was carried out on silica gel plates (Whatman, Florham Park, NJ). Flash chromatography was performed using silica gel (60 Å, 200−400 mesh size, Aldrich, St. Louis, MO). All ^{1}H and ^{13}C NMR spectra were acquired on a Varian Mercury 400 MHz spectrometer. ^{1}H chemical shifts are reported as δ referenced to solvent, and coupling constants (J) are reported in hertz. Polymer molecular mass distributions were determined in DMF using GPC (GPC 1100, Agilent Technologies, Santa Clara, CA) equipped with an organic column (PLgel mixed-B, Polymer Laboratories, Amherst, MA), a differential refractive index detector (BI-DNDC, Brookhaven Instruments, Holtsville, NY), and a multiangle light scattering detector (BI-MwA, Brookhaven Instruments, Holtsville, NY). 2-Hydroxypropylmethacrylamide (HPMAm),[31] 4-carboxyphenylboronic acid ethyl ester,[32] and methyl 4-(aminomethyl)salicylate hydrochloride[21] have been previously reported.

Chemical Synthesis. *N*-[3-(2-Methylacryloylamino)propyl]-4-amidophenylboronic acid (APMAmPBA) was synthesized using either 4-carboxyphenylboronic acid ethyl ester or 4-carboxyphenylboronic acid pinacol ester. As an example, the following describes the synthesis of the APMAmPBA pinacol ester: diisopropylcarbodiimide (3.67 g, 29.1 mmol) was added by syringe to a 0 °C chilled solution of 4-carboxyphenylboronic acid pinacol ester (6.86 g, 27.70 mmol) and *N*-hydroxysuccinimide (3.18 g, 27.7 mmol) in dry ethyl acetate (70 mL) under N_2(g). The reaction was stirred for 30 min at 0 °C and then 3 h at 25 °C. The solution was filtered to remove white urea precipitate, concentrated, and redissolved in dry DCM (40 mL). A solution of APMAm (3.96 g, 22.16 mmol) and diisopropylethylamine (DIPEA, 7.23 mL, 44.32 mmol) in dry isopropanol (50 mL) was then added to the DCM solution, and the reaction was allowed to stir under N_2(g) at 25 °C overnight. The solution was then concentrated and purified by silica gel chromatography (99:1 $CHCl_3$:MeOH; R_f (90:8:2 $CHCl_3$:MeOH:CH_3COOH) = 0.5 to yield a white solid (7.56 g, 20.32 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (m, 4 H), 7.24 (br, 1 H), 6.80 (br, 1 H), 5.77 (s, 1 H), 5.33 (s, 1 H), 3.47 (m, 2 H), 3.37 (m, 2 H), 1.97 (s, 3 H), 1.73 (m, 2 H), 1.32 (s, 12 H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.09, 168.00, 157.04, 139.69, 136.46, 134.92, 126.09, 120.01, 84.07, 36.17, 35.88, 29.76, 24.84, 18.65. FAB HRMS calcd for $C_{20}H_{30}N_2O_4B_1[M + H]^+$ 373.2299; found 373.2328.

4-[(2-Methylacryloylamino)methyl]salicylhydroxamic acid (MAAmSHA) was synthesized by the following: DIPEA (2.21 mL, 12.63 mmol) and *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 4.79 g, 12.63 mmol) were added to a solution of methacrylic acid (1.10 g, 12.79 mmol) in dried 10:1 DCM:DMF (44 mL) and stirred under N_2(g) at 25 °C for 3 h. This reaction solution was then added dropwise to a 0 °C chilled solution of methyl 4-(aminomethyl)salicylate hydrochloride (2.40 g, 13.25 mmol) in dried DCM (16 mL), followed by the addition of DIPEA (4.8 mL, 27.50 mmol). The reaction was stirred at 25 °C overnight before being washed with water (3×) and concentrated in vacuo. This crude reaction (3.2 g) was then dissolved in DMF (12 mL),

Table 1. Polymer Compositions and Molecular Weight Distributions[a]

	theoretical mole ratio (actual mole ratio[b]) (mol %)			molecular weight[c] (kDa)	
polymer	AMPS	APMAmPBA	MAAmSHA	M_w	M_n
p(AMPS$_{95}$−APMAmPBA$_5$)	95 (95.0)	5 (5.0)		339	272
p(AMPS$_{95}$−MAAmSHA$_5$)	95 (96.6)		5 (3.4)	391	330

[a] AMPS: 2-acrylamido-2-methyl-1-propanesulfonic acid; APMAmPBA: *N*-[3-(2-methylacryloylamino)propyl]-4-amidophenylboronic acid; MAAmSHA: 4-[(2-methylacryloylamino)methyl]salicylhydroxamic acid. [b] Actual molar ratio was determined by ^{1}H NMR. [c] M_w and M_n were determined by GPC-MALS with refractive index detection and are represented as means of triplicate experiments.

and DBU (4.1 g, 26.97 mmol) and 50% aqueous hydroxylamine (4.56 mL, 69.09 mmol) were added. The reaction was stirred under N_2(g) at 25 °C for 24 h. The solution was then concentrated under high vacuum and purified by silica gel chromatography (95:5 $CHCl_3$:MeOH; R_f (90:10 $CHCl_3$:MeOH) = 0.2 to yield a pale orange solid (3.06 g, 12.24 mmol, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.51 (m, 1 H), 7.61 (d, 1 H, $J = 8.2$ Hz), 6.76 (s, 1 H), 6.72 (d, 1 H, $J = 7.8$ Hz), 5.72 (s, 1 H), 5.37 (s, 1 H), 4.27 (d, 2 H, $J = 5.9$ Hz), 1.88 (s, 3 H), 1.59 (br, 1 H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 167.57, 166.19, 159.49 145.72, 139.76, 126.98, 119.50, 117.45, 115.44, 112.41, 41.98, 18.69. FAB HRMS calcd for $C_{12}H_{15}N_2O_4[M + H]^+$ 251.1032; found 251.1025.

Polymer Synthesis. Polymers were synthesized using methods similar to those previously described.[23] Briefly, free radical polymerizations were performed using 95:5 molar ratios of AMPS with APMAmPBA or MAAmSHA. Polymerizations were performed using 25 wt % monomers with 2,2′-azobis(isobutyronitrile) (AIBN, 0.6 mol %) in DMF (75 wt %) at 65 °C for 24 h under a nitrogen atmosphere. Polymers were precipitated in acetone before being dissolved in water, centrifuged (3 times at 3000 rpm for 90 min) in ultrafiltration tubes (Amicon Ultra, 10 000 MWCO, Millipore), and lyophilized. Actual molar feed ratios of polymers were determined in DMSO-d_6 by ^{1}H NMR (Mercury 400 MHz spectrometer, Varian). Molecular weight distributions were measured by GPC in HPLC-grade DMF.

Gel Preparation. Polymers were individually dissolved in 25 mM phosphate buffer of varying pH (pH 7.2−8.0) at 50 mg/mL or in 25 mM pH 4.2 acetate buffer at 100 mg/mL. To vary ionic strength, different volumes of 5 M NaCl in water were added to dissolved polymers prior to pH and final concentration adjustments. The pH of each solution was carefully adjusted using tracked volumes of 0.1−5 M NaOH before final concentrations were determined. Ionic strength was calculated as

$$I_s = {}^1/_2 \sum c_i z_i^2 \tag{1}$$

where c_i is the concentration and z_i the charge number of ionic species i.

Gels were formed by simultaneously pipetting equal volumes of the prepared PBA- and SHA-functionalized polymer solutions at equal polymer concentrations and ionic strengths directly onto the rheometer's Peltier plate.

Dynamic Rheology. Dynamic rheology was performed using methods similar to previously described.[23] Briefly, a steel 20 mm diameter, 4° cone-shaped geometry on a stress-controlled rheometer (AR550, TA Instruments) was utilized for all experiments. First, oscillatory time sweeps at small-amplitude oscillatory stress (1−5 Pa), small angular frequency (0.1 rad/s) conditions were performed at 25 °C until gel formation was stabilized and/or complete. Second, on the first of each new gel condition (i.e., pH, ionic strength), oscillatory stress sweeps (1−100 Pa oscillatory stress, 0.1 rad/s angular frequency) were performed in order to determine the linear viscoelastic range from which an oscillatory stress was chosen and applied to subsequent experiments. Finally, oscillatory frequency sweeps were performed at a controlled, small-amplitude oscillatory stress (ranging from 1 to 5 Pa) over a multilog range of angular frequencies (as wide as 0.001−100 rad/s, depending on the gel's crossover frequency characteristics) at 25 °C and then 37 °C following a 2 min temperature equilibration. Dynamic gel rigidity (G'_{plateau}) was calculated as the average G' from the plateau region of each oscillatory frequency sweep. The characteristic relaxation time, τ, was then calculated as

$$\tau = 2\pi/\omega_c \tag{2}$$

where ω_c is the crossover frequency and is determined as the angular frequency at which $G' = G''$. All experiments were performed on triplicate gel samples unless otherwise stated.

Data are represented as the mean (±sem). For evaluating the effects of temperature on the rheological properties of repeatedly tested samples, paired two-tailed *t* tests were performed. All other statistical analyses were performed by either two-tailed unpaired, unequal variance *t* tests (for comparison of two data sets) or one-way ANOVA (for comparison of more than two data sets).

Master Curve Analysis. Master curve analysis was performed on the mean values of all $G'(\omega)$ and $G''(\omega)$ data sets with reference to the mean $G'(\omega)$ and $G''(\omega)$ curves for the neutral backboned pHPMAm-based PBA−SHA gels of similar composition (50 mg/mL, 5 mol % functionality) at pH 7.6, 25 °C, and $I_s = 57$ mM. Superpositioning was performed using Matlab. Specifically, a user-defined function (see Supporting Information) was written to find the horizontal (a) and vertical (b) shift factors that minimized the sum of the mean-squared residuals between the reference and shifted data curves. Interpolation, as well as extrapolation, was performed on the reference curve to provide enhanced curve fitting. The function simultaneously fits both the $G'(\omega)$ and $G''(\omega)$ curves and concurrently calculates a and b for the best fit. Furthermore, a weighting factor was applied such that the G'' residuals were of similar magnitude as the G' residuals, giving each curve equal importance in determining the optimal superimposition.

Results and Discussion

Free radical polymerization of AMPS monomer with 5 mol % APMAmPBA or MAAmSHA monomer generated highly water-soluble multivalent PBA- and SHA-functionalized anionic polymers of $M_w \sim 300-400$ kDa (Figure 2a, Table 1). When these polymers are individually dissolved (at 50 mg/mL) in weakly buffered (25 mM) phosphate solutions, carefully titrated to pH 7.6 (final $I_s = 102$ mM) and then mixed 1:1, self-healing gels that dynamically restructure result. This observation is dramatically different from our previous observations of neutral pHPMAm-based PBA−SHA networks, which demonstrated brittle, more permanently cross-linked network behavior at similar pH yet dynamic restructuring capabilities at pH < 6. However, when 100−300 mM NaCl (final $I_s = 208-415$ mM) is added to the pAMPS-based polymer solutions prior to mixing, networks that are more densely and permanently cross-linked result, much like the pHPMAm-backboned PBA−SHA networks at pH 7.6. These qualitative observations suggest that the negative charge of the pAMPS polymer backbone at low ionic strength is generating an acidic microenvironment in the vicinity of the PBA−SHA moieties due to a large Donnan ratio (i.e., $[H^+]_{\text{inside}}/[H^+]_{\text{outside}}$). This shifts the binding equilibrium to the left, causing the PBA−SHA cross-links to function dynamically at pH 7.6 much like our previously reported neutral-backboned PBA−SHA networks at pH ∼ 4.[23] When Na^+ is added, the Donnan ratio decreases, and therefore the pH in the vicinity of the PBA−SHA cross-links is closer to the bulk solution's neutral pH; at this pH, the equilibrium constant for

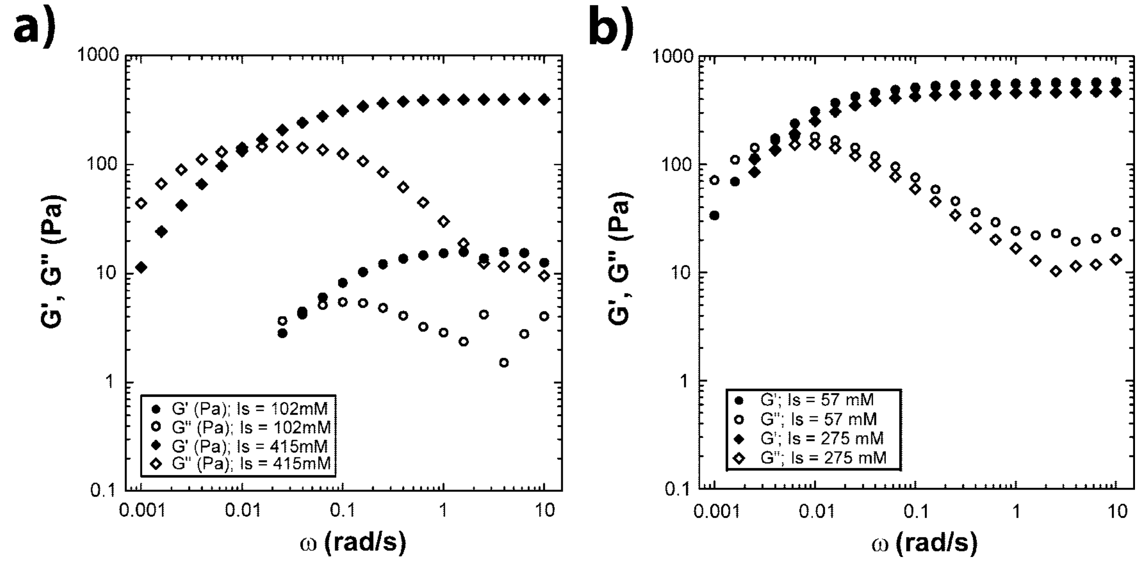

Figure 3. Frequency-dependent viscoelastic behavior of PBA−SHA cross-linked hydrogel networks formed at pH 7.6 and varying ionic strengths. Storage (G') and loss (G'') moduli vs angular frequency for PBA−SHA cross-linked polymer networks with either an anionic (pAMPS, a) or neutral (pHPMAm, b) polymer backbone. Note the leftward and upward shift in frequency-dependent viscoelasticity of the polysulfonate-backboned gel networks by increasing ionic strength (I_s, a), whereas the frequency-dependent viscoelasticity of neutral-backboned gels was minimally affected by ionic strength (b). Mean ($n = 3$).

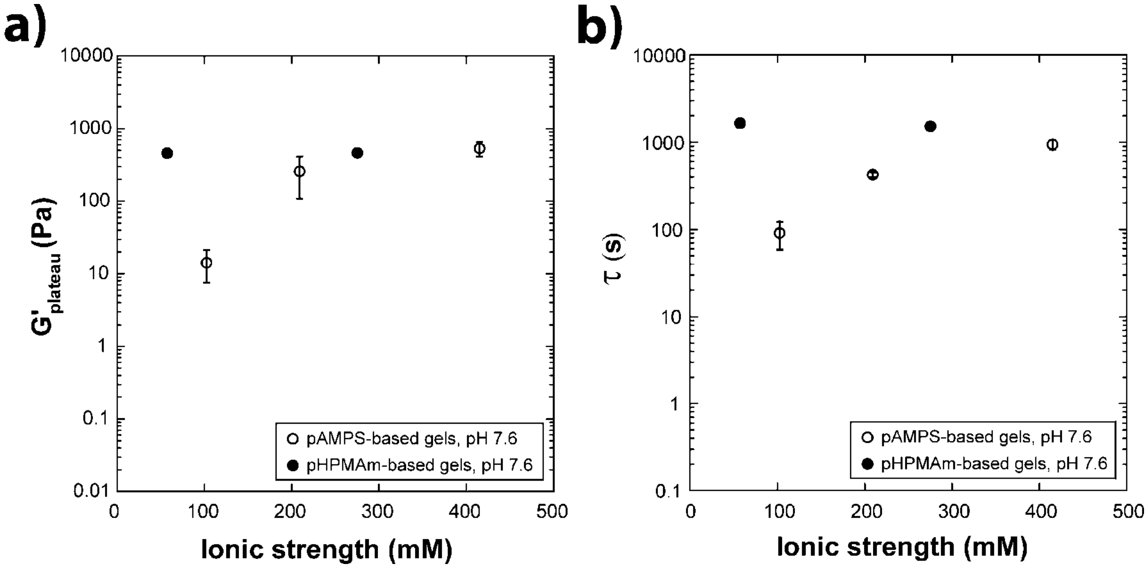

Figure 4. Effect of ionic strength on viscoelastic gel behavior. Dynamic gel rigidity (G'_{plateau}, a) and characteristic relaxation time (τ, b) vs ionic strength for PBA−SHA cross-linked gels comprised of either anionic/polysulfonate (pAMPS-based gels, open symbols) or neutral (pHPMAm-based gels, closed symbols) polymer backbones at pH 7.6. Both G'_{plateau} and τ significantly increased with ionic strength for polysulfonate-backboned PBA−SHA polymer networks ($p < 0.05$), whereas G'_{plateau} and τ for neutral-backboned PBA−SHA polymer networks were unaffected by ionic strength. Mean ± sem ($n = 3$).

the association of PBA and SHA is large, and the gel becomes more highly cross-linked (Figure 2b).

Effect of Ionic Strength on Frequency-Dependent Moduli. Quantitative evaluation of the polysulfonate-backboned PBA−SHA cross-linked gels at varying ionic strengths by dynamic rheology further supports the hypothesis that the negatively charged polysulfonate backbone affects the dynamics of the viscoelastic PBA−SHA cross-linked networks at neutral pH. As can be seen in Figure 3a, the moduli−frequency curves for polysulfonate-backboned PBA−SHA gels (50 mg/mL) at pH 7.6 are highly dependent on the ionic strength of the gel composition. Whereas polysulfonate-backboned gels at low ionic strength ($I_s \sim 100$ mM) revealed viscous-dominant gel behavior at $\omega < 0.09$ rad/s (i.e., $G'' > G'$), gels with a 1−4-fold increase in ionic strength demonstrated a dramatic shift in the frequency response such that viscous-dominant gel behavior occurs at ~1 order of magnitude lower angular frequencies (i.e., $\omega < 0.006$ and 0.01 rad/s for I_s of 415 and 208 mM, respectively). Alternatively, PBA−SHA gels comprised of a neutral pHPMAm polymer backbone were insensitive to changes in ionic strength, demonstrating minimal shifts in their frequency-dependent moduli with $G'' > G'$ at $\omega < 0.005$ rad/s for both $I_s = 57$ or 275 mM conditions (Figure 3b).

When we consider the dynamic gel rigidity, G'_{plateau} (Figure 4a), and characteristic relaxation time, τ (Figure 4b), for these networks, we find that both G'_{plateau} and τ significantly increase by at least 1 order of magnitude with a 1−4-fold increase in ionic strength ($p = 0.044$ and 0.006, respectively). When a similar comparison of ionic strength was performed on neutral pHPMAm-backboned PBA−SHA gels at similar pH and

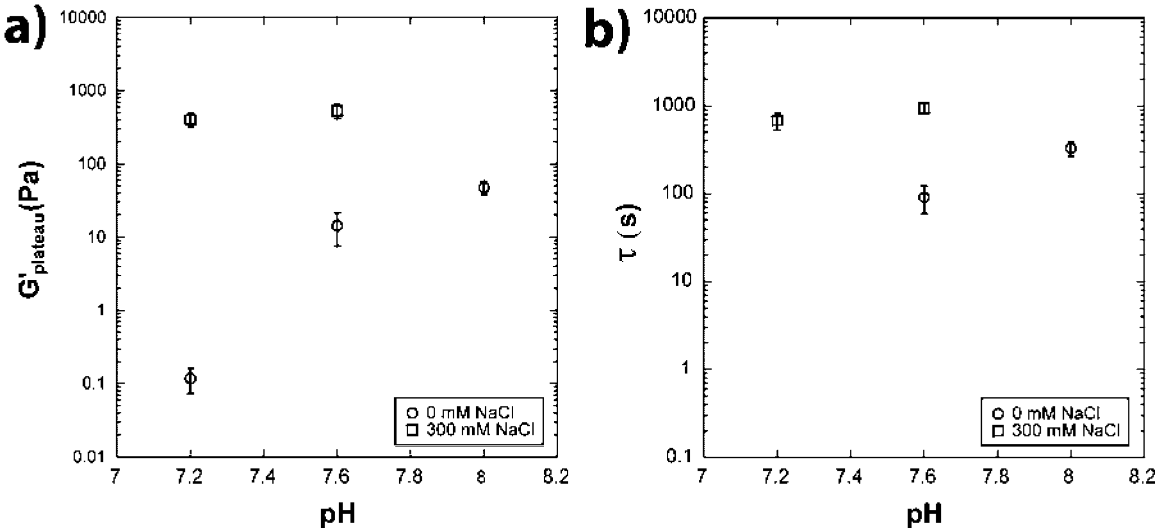

Figure 5. Effect of small pH changes on viscoelastic gel behavior. Dynamic gel rigidity (G'_{plateau}, a) and characteristic relaxation time (τ, b) vs pH for polysulfonate-backboned PBA−SHA cross-linked gels with 0 mM (I_s = 102−127 mM) or 300 mM (I_s = 415−456 mM) NaCl added. Both G'_{plateau} and τ significantly increased with small increases in pH (pH 7.2−8.0) for polysulfonate-backboned PBA−SHA networks at low salt conditions (0 mM NaCl added; $p < 0.05$), whereas those for similar gels with 300 mM NaCl added were unaffected by pH in this neutral pH range. Note samples at pH 7.2 with no salt added did not form gels. Mean ± sem ($n = 3$).

polymer concentration, we found that both G'_{plateau} and τ were unaffected by ionic strength ($p = 0.112$ and 0.401, respectively; Figure 4). These results suggest that the negatively charged polymer backbone, in the absence of salt (i.e., at low ionic strength), dramatically alters the network cross-link binding equilibrium, thereby generating minimally cross-linked networks that are capable of reorganizing on short time scales. In the presence of salt (i.e., at increased ionic strength), the effect of the anionic polymers on the cross-link binding equilibrium is nullified, rendering more densely cross-linked and much less transient networks at this neutral pH. Moreover, these chemorheological properties are very similar to those of salt-insensitive, neutral pHPMAm-based gels of otherwise comparable composition. We hypothesize that these effects result from a Donnan equilibrium where the polyanionic backbone concentrates protons near the PBA−SHA cross-links under low salt conditions; under high salt, sodium ions replace the protons, resulting in a locally increased pH.

It should be noted that we attempted to compare these experimental determinations of G'_{plateau} and τ with those mathematically determined using a best-fit Maxwell model with one relaxation time and plateau modulus. This Maxwell model is a commonly used tool for describing many viscoelastic materials, including borate ester cross-linked gels,[18,33] and predicting their relaxation behavior. However, despite the qualitatively model-consistent rheological behavior of the gels described here (i.e., a single G'_{plateau} and a single inflection in G''), the simple Maxwell model provided poor fits, particularly in terms of ω_c, for all gel compositions tested and thus was found to be insufficient to describe the relaxation dynamics of these polysulfonate-backboned PBA−SHA cross-linked hydrogel networks (data not shown). Until a more sophisticated model can be determined, the presentation of the mean (with statistical error) plateau modulus and characteristic relaxation time for triplicate samples that were experimentally tested was deemed a better method of evaluating the gel's chemorheological properties.

Effect of pH. Given the strong dependence of these polysulfonate-backboned PBA−SHA cross-linked gels on ionic strength at pH 7.6, it is likely that similar gels may also show finely pH-dependent rheological properties (i.e., in the neutral pH range) depending on the ionic strength. In fact, we found that at low ionic strength (final I_s = 102−127 mM), 50 mg/mL of mixed polymer solutions do not gel at pH 7.2, form slowly self-healing gels at pH 7.6, and generate more brittle, permanently cross-linked networks at pH 8.0. However, when the polysulfonate-backboned polymers were exposed to higher ionic strengths (final I_s = 415−456 mM), strong gels now formed at pH 7.2, and no difference was observed in the gel behaviors at pH 7.2 and pH 7.6 (pH 8.0 was not tested at higher ionic strengths), similar to pHPMAm-based gels at pH > 6 (data not shown). Quantitatively, both G'_{plateau} and τ significantly increased with pH at low ionic strength ($p = 0.007$ and 0.041, respectively) but were unaffected by pH at high ionic strength ($p = 0.440$ and 0.252, respectively; Figure 5). These results suggest that the polysulfonate-backboned PBA−SHA networks under high salt conditions behave more like pHPMAm-backboned networks at neutral pH, whereas the polysulfonate-backboned PBA−SHA networks under low salt conditions are more sensitive to pH in the neutral pH range and thus behave more like the dynamic pHPMAm-backboned networks at mildly acidic pH. Moreover, it should be noted that polysulfonate-backboned PBA−SHA samples tested at pH 4.2 did not gel at any salt concentration tested ([NaCl] ≤ 1 M, corresponding to final I_s ≤ 1.2 M), whereas pHPMAm-backboned samples formed weakly cross-linked gels at similar pH regardless of ionic strength (data not shown). Note that at this low pH the PBA−SHA cross-links are highly transient, and so even with the p(HPMAm)-backboned PBA−SHA networks, the gels that formed were just above the critical gel point. Thus, it is likely that the polysulfonated PBA−SHA system is still below this gel point at pH 4.2, potentially due to differences in chain conformation, and may require a slight increase in pH at high ionic strength to reach the gel point.

Effect of Temperature. Up to now, we have discussed the chemorheological properties of the polysulfonate-backboned PBA−SHA cross-linked networks at ambient temperature (i.e., 25 °C). However, due to the exothermic nature of the coordinate covalent PBA−SHA cross-links, we wanted to evaluate the effect of a slight increase in temperature from ambient to body temperature on both dynamic gel rigidity and the characteristic relaxation time. As shown in Figure 6a, G'_{plateau} tended to decrease at the slightly elevated temperature for polysulfonate-backboned PBA−SHA networks at low ionic strength but was

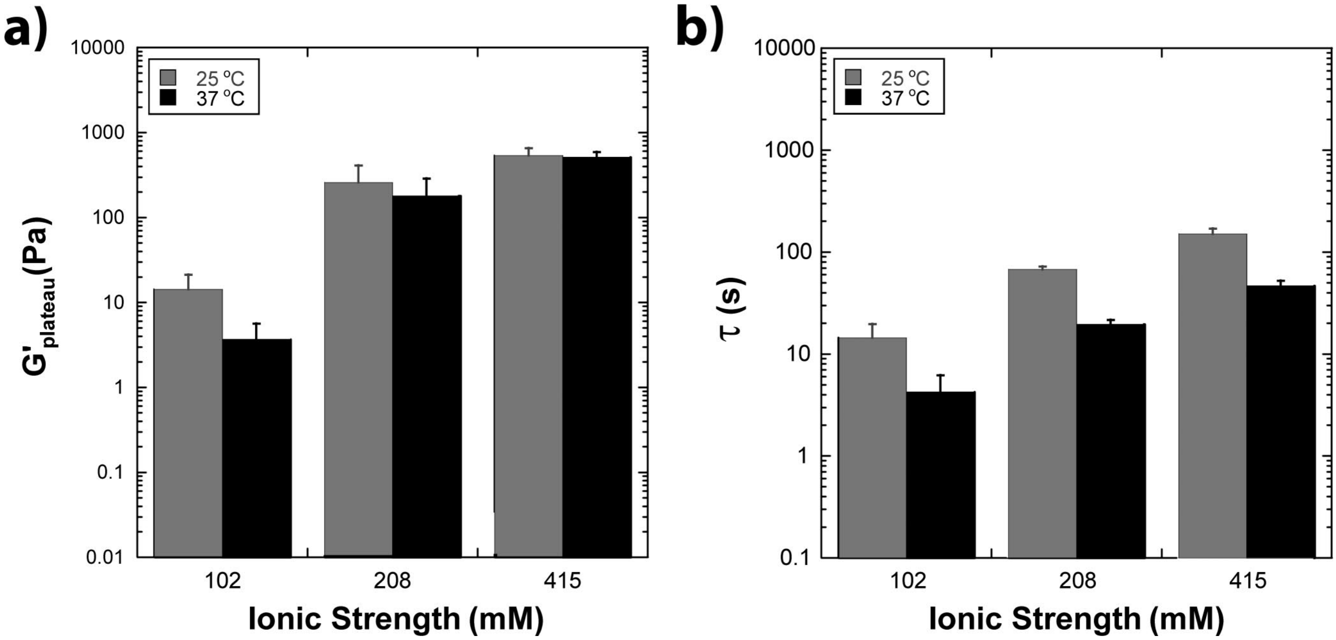

Figure 6. Effect of temperature on viscoelastic gel behavior. Dynamic gel rigidity (G'_{plateau}, a) and characteristic relaxation time (τ, b) for pH 7.6 polysulfonate-backboned PBA−SHA cross-linked gels of varying ionic strengths at 25 °C (gray bars) and 37 °C (black bars). Gel rigidity tended to decrease with increased temperature at lower ionic strengths, whereas characteristic relaxation time tended to decrease at all ionic strengths tested. Mean + sem ($n = 3$).

less impacted by temperature when exposed to higher ionic strengths. Because of relatively large sample variance, this trend at low ionic strength was not always statistically significant, as paired *t* tests only determined a statistically significant decrease in G'_{plateau} for the pH 8.0, $I_s = 127$ mM samples ($p = 0.033$, data not shown). However, this temperature dependence of G'_{plateau} is consistent with our previous findings,[23] is related to the bond enthalpy[34] and initial degree of cross-linking, and is indicative of a dynamically cross-linked network that transitions from cross-links that are few and transient (i.e., at low ionic strength) to those that are more dense and permanent (i.e., at high ionic strength). Furthermore, τ also tended to decrease with increased temperature at all pHs and ionic strengths tested (Figure 6b, pH 7.6 data shown); this decrease in τ is due to the temperature-induced shift in the binding equilibrium of the PBA−SHA cross-links. However, this trend was also found to be statistically significant for only some data sets—*p* values for pH 7.6, $I_s = 102$, 208, and 415 mM were 0.087, 0.002, and 0.054, respectively; *p* values for pH 7.2, $I_s = 456$ mM and pH 8.0, $I_s = 127$ mM were both 0.033.

Master Curve Analysis. Thus far, we have considered the influence of a negatively charged polymer backbone, as well as the specific effects of further manipulations in ionic strength, pH, and temperature, on the dynamic chemorheological properties of polysulfonate-backboned PBA−SHA cross-linked hydrogels. To better understand these effects, we have considered the data from each experimental condition separately. However, given that the dynamics of these hydrogel networks are a direct result of the condition-specific shifts in the relaxation characteristics of the transient cross-links or their density, it is likely that we can compile all data sets into a single master curve of the relaxation response of PBA−SHA cross-linked gels in general. By reducing variables using vertical and horizontal shift factors, we may superimpose frequency−moduli data. This principle of reducing variables to describe a common relaxation mechanism in viscoelastic polymer systems originated with Leaderman,[35] Tobolsky and Andrews,[36] and Williams, Landel, and Ferry[37] with the use of time−temperature superimposition. Since then, master curve analysis by superimposition has been applied to temperature[2,15,17,18,38] as well as other relationships, including pH,[17,18] polymer concentration,[2,38] molecular weight,[39] ionic strength,[38] competition experiments,[2] and various combinations of these parameters.[38,39]

We thus performed a master curve analysis on the mean values of all data sets collected from polysulfonate-backboned PBA−SHA cross-linked gels under varying conditions (i.e., ionic strength, pH, temperature) after choosing a neutral pHPMAm-backboned PBA−SHA cross-linked gel of similar composition (50 mg/mL, 5 mol % functionality, pH 7.6, $I_s = 57$ mM, 25 °C) as the reference condition. Horizontal, *a*, and vertical, *b*, shift factors were determined for each condition utilizing a Matlab-based, user-defined function that minimized the sum of the mean-squared residuals between the reference and shifted $G'(\omega)$ and $G''(\omega)$ curves simultaneously. One set of shift factors (*a* and *b*) was then determined for each gel condition, such that

$$a = a_I a_{\text{pH}} a_T \tag{3}$$

$$b = b_I b_{\text{pH}} b_T \tag{4}$$

where a_I and b_I, a_{pH} and b_{pH}, and a_T and b_T are undetermined shift factor components corresponding to ionic strength, pH, and temperature, respectively. Superimposition of polysulfonate-backbboned PBA−SHA cross-linked gels to a reference poly-neutral backboned PBA−SHA gel was successful for all data sets, including those which varied in ionic strength ($I_s = 102−456$ mM), temperature (25 and 37 °C), and pH (7.2−8.0). For clarity sake, the following figures demonstrate our master curve analysis for gels that varied in ionic strength and temperature at pH 7.6 only (the same pH as the reference gel). For a table of all determined shift factors, see the Supporting Information.

As can be seen in Figure 7, raw $G'(\omega)$ data (Figure 7a) can effectively be shifted into a single master $G'(\omega)$ curve (Figure 7b). Furthermore, the same shift factors effectively superimpose the terminal region of the corresponding raw $G''(\omega)$ curves (Figure 8); this good superpositioning in the terminal region of the master $G''(\omega)$ curve corresponds to the longest cross-link-

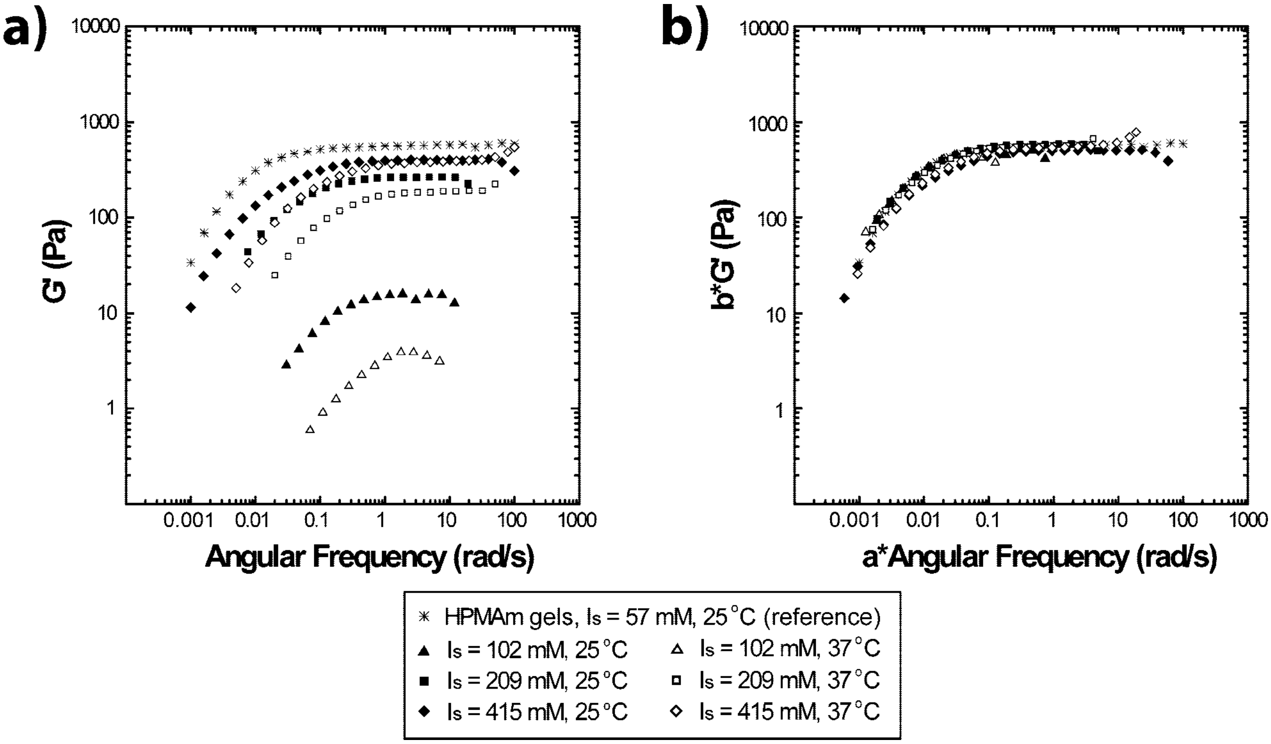

Figure 7. Master curve analysis of the elastic modulus, G', for polysulfonate-backboned PBA−SHA cross-linked gels. Raw (a) and reduced (b) $G'(\omega)$ curves for polysulfonate-backboned PBA−SHA cross-linked gels at pH 7.6 and varying ionic strength (I_s = 102−415 mM) and temperature (25−37 °C). Reduced curves were obtained using horizontal and vertical shift factors plotted in Figures 9 and 10, respectively. Neutral pHPMAm-backboned PBA−SHA cross-linked gels of similar polymer concentration (50 mg/mL), degree of functionality (5 mol %), and pH (pH 7.6) at I_s = 57 mM and 25 °C were used as the reference condition. The mean moduli of triplicate samples were used for both the reference curve and shifted curves.

associated relaxation time, whereas poor superpositioning at higher frequencies is due to interference by short-time relaxation processes that correlate to a global motion in the polymer chains.[2,17,18]

Not only are we successful in generating a master curve of the relaxation response of polysulfonate-backboned PBA−SHA cross-linked gels relative to a neutral pHPMAm-backboned gel reference condition, but we can elicit remarkable trends in the condition-specific shift factors determined as a function of ionic strength and temperature. As can be seen in Figures 9 and 10, both horizontal (*a*) and vertical (*b*) shift factors of data sets at 25 °C asymptotically approach the value of 1, demonstrating that moduli−frequency curves from polysulfonate-backboned PBA−SHA cross-linked gels at elevated ionic strength required minimal shifting to superimpose with the pHPMAm-based gel reference curve. However, as ionic strength decreased, the extent of the leftward horizontal shift (indicated by $a < 1$) and upward vertical shift (indicated by $b > 1$) dramatically increased, thus describing the extensive requirements of superimposing curves from gels that are increasingly dynamic and weak with the highly brittle and stronger reference gel. Moreover, moduli−frequency data sets at 37 °C required even more exaggerated horizontal shift factors than those at 25 °C for all ionic strengths tested. This result was expected given the exothermic nature of the PBA−SHA cross-linking chemistry and the chosen pHPMAm-based gel reference at 25 °C. If the reference condition were chosen at 37 °C, we would expect the polysulfonate-backboned PBA−SHA cross-linked gel curves at 37 °C to asymptote at 1 and corresponding curves at 25 °C to require rightward shifting (i.e., $a > 1$) for high ionic strength conditions. As for the vertical shift factors at the elevated temperature, more extensive shifting was required at low ionic strength due to a reduced cross-link density and subsequently lower G'_{plateau} of the weak gels, yet a minimal effect was observed at higher ionic strength, where G'_{plateau} was also found to be unaffected. This master curve analysis leads us to the conclusion that it is not the mechanism of relaxation in these polysulfonate-backboned PBA−SHA cross-linked gels that is affected by the presence of a negatively charged polymer backbone, as superimposition would not be possible with reference to the neutral pHPMAm-based gels. Rather, it is the relaxation rate of the PBA−SHA cross-links comprising the gel networks that is affected by the anionic backbone of the polysulfonate-backboned gels as well as ionic strength, temperature, and pH. This interpretation is consistent with other research groups performing similar master curve analyses on other reversibly cross-linked polymeric networks, including noncovalent hyaluronic acid hydrogels[38] and metal−ligand associatively cross-linked organogels.[40]

Conclusion

To the best of our knowledge, this is the first quantitative rheological study of reversible gels with specialized, pH-dependent coordinate covalent cross-links manipulated by the Donnan effect. Specifically, we have demonstrated the ability to manipulate the pH dependence of dynamically cross-linked PBA−SHA hydrogel networks by the incorporation of a negatively charged polymer backbone. At low ionic strength, these polysulfonate-backboned networks reveal frequency-dependent chemorheological properties that are highly sensitive to pH changes in the neutral pH range. However, at higher ionic strengths, the PBA−SHA networks become more permanently cross-linked and pH independent in this neutral pH range. Moreover, the relaxation response of polysulfonate-backboned gels is similar to that of neutral-backboned gels, as was demonstrated by the effective reduction of all moduli−frequency data into a single master curve with reference to a pHPMAm-backboned gel of comparable composition. This particular reversible gel system has the advantages that it is synthetically flexible compared to the borate−polyol systems and compositional changes in the polymers can readily provide a wide range

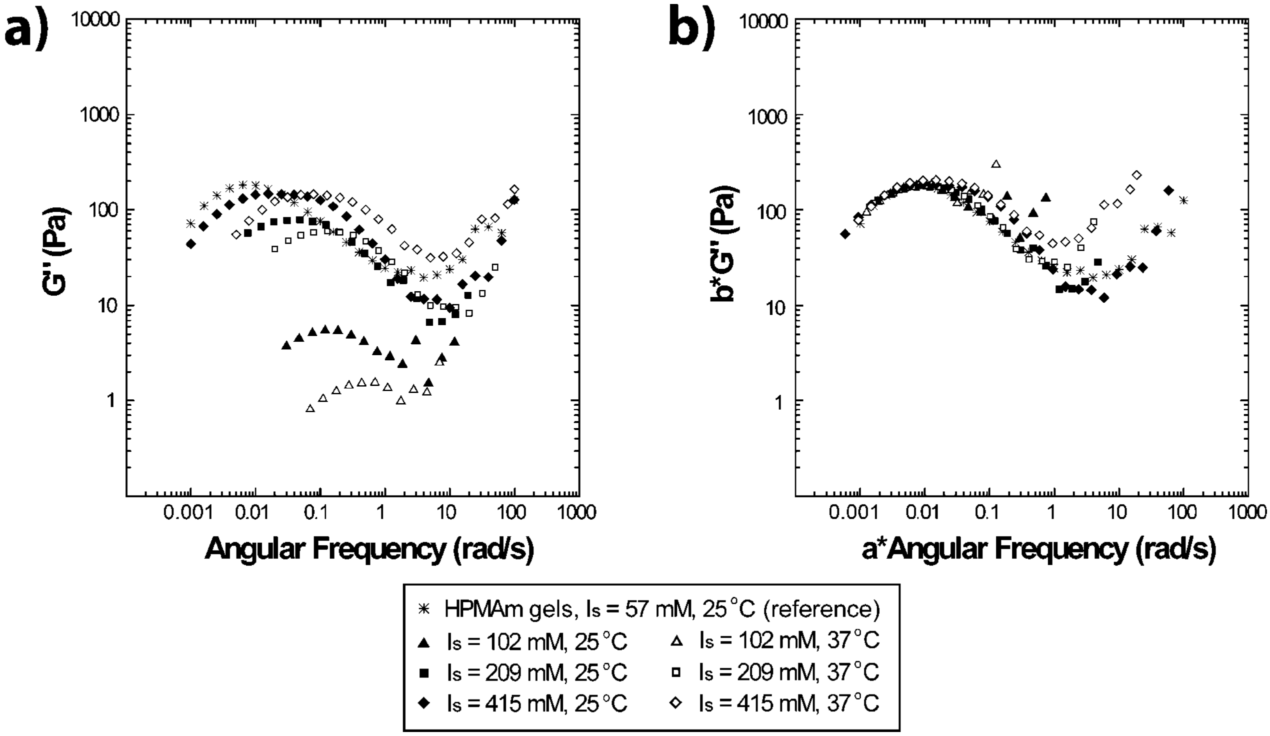

Figure 8. Master curve analysis of the viscous modulus, G'', for polysulfonate-backboned PBA−SHA cross-linked gels. Raw (a) and reduced (b) $G''(\omega)$ curves for polysulfonate-backboned PBA−SHA cross-linked gels at pH 7.6 and varying ionic strength (I_s, 102−415 mM) and temperature (25−37 °C). Reduced curves were obtained using horizontal and vertical shift factors plotted in Figures 9 and 10, respectively. Neutral pHPMAm-backboned gels of similar polymer concentration (50 mg/mL), degree of functionality (5 mol %), and pH (pH 7.6) at I_s = 57 mM and 25 °C were used as the reference condition. The mean moduli of triplicate samples were used for both the reference curve and shifted curves. Note that good superpositioning can be observed in the terminal region, corresponding to the cross-link-associated relaxation time, whereas the poor superpositioning at higher frequencies is due to interference by short-time processes that correspond to polymer chain motion.

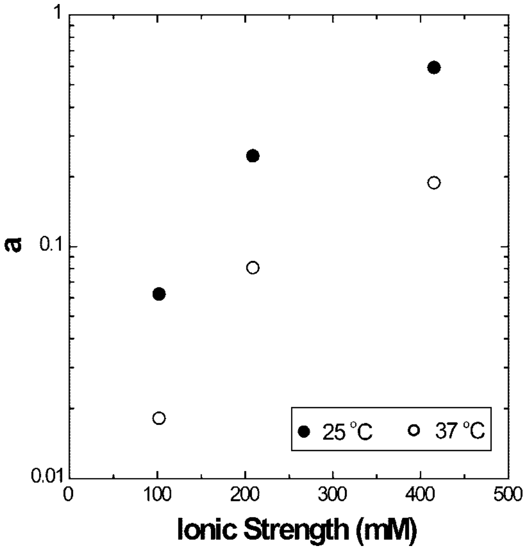

Figure 9. Effect of ionic strength on the horizontal shift factor, *a*, used to obtain the master curves for polysulfonate-backboned PBA−SHA cross-linked gels at pH 7.6 in Figures 7b and 8b. Neutral pHPMAm-backboned PBA−SHA cross-linked gels of similar polymer concentration (50 mg/mL), degree of functionality (5 mol %), and pH (pH 7.6) at I_s = 57 mM and 25 °C were used as the reference condition. Note that for data sets at 25 °C, in which $a = a_{pH}$, increasing ionic strength causes *a* to approach the minimally shifted value of 1.

of tuned properties. This work suggests that these materials may be engineered to fit the design needs of “smart”, bioresponsive biomedical applications in which specific responses to natural or unnatural cues are required.

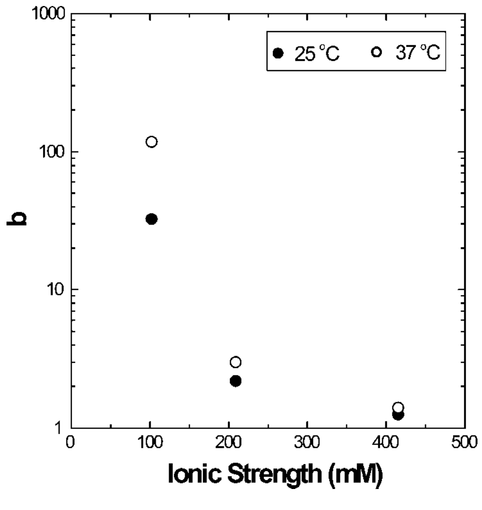

Figure 10. Effect of ionic strength on the vertical shift factor, *b*, used to obtain the master curves for polysulfonate-backboned PBA−SHA cross-linked gels at pH 7.6 in Figures 7b and 8b. Neutral pHPMAm-backboned PBA−SHA cross-linked gels of similar polymer concentration (50 mg/mL), degree of functionality (5 mol %), and pH (pH 7.6) at I_s = 57 mM and 25 °C were used as the reference condition. Note that as ionic strength increases, *b* approaches the minimally shifted value of 1 for both 25 and 37 °C data sets.

Acknowledgment. This work was supported by NIH-NIAID R21 AI62445-01.

Supporting Information Available: Matlab-based superpositioning function and table of all shift factors determined from the master curve analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Breedveld, V.; Nowak, A. P.; Sato, J.; Deming, T. J.; Pine, D. J. *Macromolecules* **2004**, *37*, 3943–3953.
(2) Charlot, A.; Auzely-Velty, R. *Macromolecules* **2007**, *40*, 9555–9563.
(3) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. *Nature (London)* **2002**, *417*, 424–428.
(4) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389–392.
(5) Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer, B. J. B.; Hirschberg, J. H. K. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. *Science* **1997**, *278*, 1601–1604.
(6) Yokoi, H.; Kinoshita, T.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8414–8419.
(7) Boeseken, J. *Adv. Carbohydr. Chem.* **1949**, *4*, 189–210.
(8) Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769–774.
(9) Sugihara, J. M.; Bowman, C. M. *J. Am. Chem. Soc.* **1958**, *80*, 2443–2446.
(10) Bucci, S.; Gallino, G.; Lockhart, T. P. *Polym. Prepr.* **1991**, *32*, 457–458.
(11) Pezron, E.; Ricard, A.; Lafuma, F.; Audebert, R. *Macromolecules* **1988**, *21*, 1121–1125.
(12) Power, D. J.; Rodd, A. B.; Paterson, L.; Boger, D. V. *J. Rheol.* **1998**, *42*, 1021–1037.
(13) Robb, I. D.; Smeulders, J. B. A. F. *Polymer* **1997**, *38*, 2165–2169.
(14) Lin, H. L.; Liu, Y. F.; Yu, T. L.; Liu, W. H.; Rwei, S. P. *Polymer* **2005**, *46*, 5541–5549.
(15) Schultz, R. K.; Myers, R. R. *Macromolecules* **1969**, *2*, 281–285.
(16) Goodwin, J. W.; Hughes, R. W. *Rheology for Chemists: An Introduction*; Royal Society of Chemistry: Cambridge, UK, 2000.
(17) Pezron, E.; Ricard, A.; Leibler, L. *J. Polym. Sci., Part B: Polym. Phys.* **1990**, *28*, 2445–2461.
(18) Kesavan, S.; Prud'homme, R. K. *Macromolecules* **1992**, *25*, 2026–2032.
(19) Djanashvili, K.; Frullano, L.; Peters, J. A. *Chemistry* **2005**, *11*, 4010–4018.
(20) Zhao, J.; Davidson, M. G.; Mahon, M. F.; Kociok-Kohn, G.; James, T. D. *J. Am. Chem. Soc.* **2004**, *126*, 16179–16186.
(21) Stolowitz, M. L.; Ahlem, C.; Hughes, K. A.; Kaiser, R. J.; Kesicki, E. A.; Li, G.; Lund, K. P.; Torkelson, S. M.; Wiley, J. P. *Bioconjugate Chem.* **2001**, *12*, 229–239.
(22) Wiley, J. P.; Hughes, K. A.; Kaiser, R. J.; Kesicki, E. A.; Lund, K. P.; Stolowitz, M. L. *Bioconjugate Chem.* **2001**, *12*, 240–250.
(23) Roberts, M. C.; Hanson, M. C.; Massey, A. P.; Karren, E. A.; Kiser, P. F. *Adv. Mater.* **2007**, *19*, 2503–2507.
(24) Winblade, N. D.; Nikolic, I. D.; Hoffman, A. S.; Hubbell, J. A. *Biomacromolecules* **2000**, *1*, 523–533.
(25) Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Tetrahedron* **2004**, *60*, 11205–11209.
(26) Hisamitsu, I.; Kataoka, K.; Okano, T.; Sakurai, Y. *Pharm. Res.* **1997**, *14*, 289–293.
(27) Matsumoto, A.; Yoshida, R.; Kataoka, K. *Biomacromolecules* **2004**, *5*, 1038–1045.
(28) Ricka, J.; Tanaka, T. *Macromolecules* **1984**, *17*, 2916–2921.
(29) Eichenbaum, G. M.; Kiser, P. F.; Simon, S. A.; Needham, D. *Macromolecules* **1998**, *31*, 5084–5093.
(30) Yanovsky, Y. G. *Polymer Rheology: Theory and Practice*; Chapman & Hall: London, UK, 1993.
(31) Kopecek, J.; Bazilova, H. *Eur. Polym. J.* **1973**, *9*, 7–14.
(32) Haber, S.; Meudt, A.; Scherer, S.; Vollmueller, F. Process for preparing substituted phenylboronic acids. 2000-107821, 1046640, 20000412, 2000.
(33) Koike, A.; Nemoto, N.; Inoue, T.; Osaki, K. *Macromolecules* **1995**, *28*, 2339–2344.
(34) Franse, M. W. C. P. A Rheological and Structural Study of Polymer Networks. Dissertation, Delft University of Technology, Delft, The Netherlands, 2002.
(35) Leaderman, H. *Elastic and Creep Properties of Filamentous Materials and Other High Polymers*; Textile Foundation: Washington, DC, 1943.
(36) Tobolsky, A. V.; Andrews, R. D. *J. Chem. Phys.* **1945**, *13*, 3–27.
(37) Williams, M. L.; Landel, R. F.; Ferry, J. D. *J. Am. Chem. Soc.* **1955**, *77*, 3701–3707.
(38) Gibbs, D. A.; Merrill, E. W.; Smith, K. A.; Balazs, E. A. *Biopolymers* **1968**, *6*, 777–791.
(39) Calciu-Rusu, D.; Rothfuss, E.; Eckelt, J.; Haase, T.; Dick, H. B.; Wolf, B. A. *Biomacromolecules* **2007**, *8*, 1287–1292.
(40) Yount, W. C.; Loveless, D. M.; Craig, S. L. *J. Am. Chem. Soc.* **2005**, *127*, 14488–14496.

MA8012674