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Solution Properties of Polyvinylamine Derivatized with Phenylboronic Acid

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ABSTRACT: The phase behavior and surface tension of aqueous, linear polyvinylamine (PVAm) bearing pendant phenylboronic acid moieties (PBA) were measured as functions of pH and the degree of PBA substitution. Coupling of PBA with 4% of the nitrogen on low molecular weight (15 kDa) PVAm gave water-soluble copolymer from pH 1 to 12. By contrast, derivatives of 150 kDa PVAm phased separated at pH 9 with as little as 4% derivatization. The pH ranges over which phase separation occurred increased with PBA substitution. The copolymer based on 51% substitution was insoluble over most of the pH range. Electrophoresis and potentiometric titration confirmed that the copolymers were amphoteric with isoelectric points around pH 9.5. The hydrophobicity of the copolymers was reflected in the significant lowering of surface tension, particularly at high pH. Finally fructose, which binds to borate, influenced the titration curves but did not influence surface tension.

Introduction

The borate ion, $B(OH)_4^-$, and alkyl-substituted borates (i.e., boronates) are known to condense with carbohydrates[1] to give usually 5- or 6-member rings (see Scheme 1). The condensation occurs in water under mild alkaline conditions and is characterized by low binding constants that are specific to the carbohydrate.[2] Reversible, selective borate−carbohydrate binding has stimulated much literature describing the development of affinity columns[3,4] based on immobilized borate ions and a number of glucose detection strategies based on fluorescent boronates[5−7] and boronate hydrogels.[8−11] Furthermore, many of these publications describe the preparation of synthetic, water-borne polymers bearing boronate groups. More specifically, the boronate moieties are usually a derivative of phenylboronic acid (PBA). The PBA-containing polymers can be prepared either by free radical copolymerization with a vinyl-PBA monomer[5,12,13] or by covalent coupling of activated PBA derivatives to a polymer.[14−17]

Our interest in PBA-containing polymers arose from the discovery that PBA derivatives of polyvinylamine (PVAm) display remarkable instantaneous adhesion to wet cellulose.[18] In working with polyvinylamine−boronate (PVAm−PBA, see Scheme 2) polymers, we observed a strong tendency of the copolymers to phase separate depending upon pH and the degree of boronate substitution. In spite of the large number of publications involving water-borne boronate polymers, we found little information about the strong influence of PBA on solubility and surface tension. The phenylboronic acid group is hydrophobic, greatly impacting phase behavior in water. For example, Kuzimenkova et al. reported limited water solubility for PBA copolymers with *N*-isopropylacrylamide and with acrylamide when the boronic monomer contents were 16% and 14%, respectively.[13] Herein we present the solution properties of PVAm−PBA as functions of pH, ionic strength, the level of boronate substitution, and molecular weight. Although we present results for only one type of boronate, the PVAm derivatives, we believe that our results have implications for the wide range of linear polymers and gels that have been of interest in many applications.

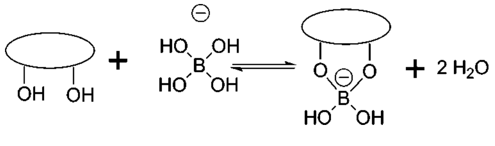
Experimental Section

Polyvinylamine samples with molecular weights of 15 and 150 kDa were obtained from BASF. To ensure complete hydrolysis from poly(*N*-vinylformamide), the polymers were treated further under nitrogen purge with 5% NaOH at 70 °C for 48 h to remove residual formamide groups. The polymers were dialyzed against water for 10 days using regenerated cellulose dialysis tubing (Spectra/Por 12 or 3.5 kDa molecular weight cutoff, Spectrum Laboratories, Inc.) and were subsequently freeze-dried. 4-Carboxyphenylboronic acid, *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 2-(*N*-morpholino)ethanesulfonic acid (MES), and fructose were purchased from Sigma-Aldrich and used as received. All experiments were performed with water from a Millipore Milli-Q system.

A series of PVAm pendant phenylboronic acid groups (PVAm−PBA) were synthesized using the method described in our previous publication (see Scheme 2).[18] In a typical experiment, 0.2 g of PVAm was dissolved in 10 mL of 0.1 M MES buffer at pH 6.1 and 90 mL of 3 g/L 4-carboxyphenylboronic acid in the same buffer; 8 g of EDC was added, and the mixture was stirred for 120 min at 25 °C. The product was dialyzed against water for 2 weeks. Proton NMR was used to characterize the degree of substitution (DS). The properties of the PVAm−PBA copolymers are summarized Table 1. ^{1}H NMR experiments were performed at using an AVANCE200 NMR instrument (Bruker) with 200 MHz at room temperature. A small amount of DCl in D_2O was used to dissolve the PVAm−PBA samples with high DS.

The charge−pH properties of PVAm−PBA copolymers were probed by electrophoresis measurements. To facilitate the measurements, the copolymers were adsorbed onto 200 nm anionic polystyrene latex (Bangs Laboratories, Inc.). The samples were prepared with 0.1 g/L copolymer mixed with 0.01 g/L latex. All measurements were made in 5 mM NaCl aqueous solution at 25 °C. The electrophoretic mobility measurements were made at 25 °C using a ZetaPlus from Brookhaven Instruments Corp. using PALS (phase analysis light scattering) Software Version 2.5. The reported values were based on five measurements with 25 cycles for each.

Scheme 1



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Scheme 2. Derivatization of PVAm

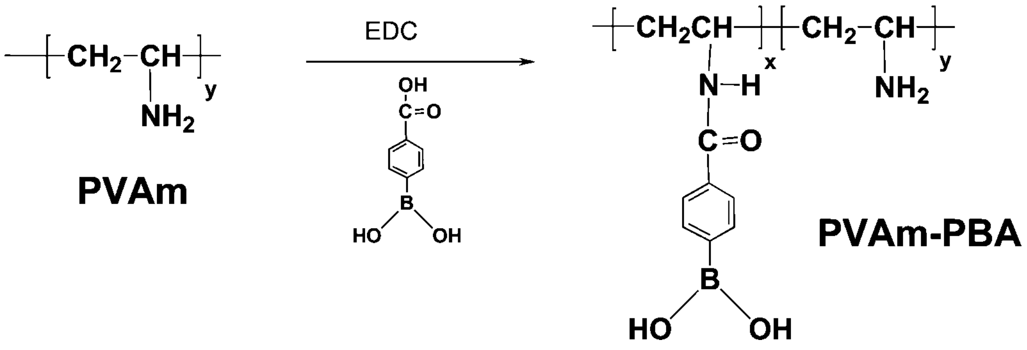


Table 1. Polymer Compositions and Molecular Weight[a]

sample name	degree of substitution (%)	molecular weight (kDa)
PVAm−PBA-1	4	15
PVAm−PBA-2	19	15
PVAm−PBA-3	30	15
PVAm−PBA-4	4	150
PVAm−PBA-5	16	150
PVAm−PBA-6	29	150
PVAm−PBA-7	51	150

[a] The degree of substitution is the number of substituted function groups per nitrogen atom, and the maximum possible value is 200% corresponding to tertiary amines.

The phase behaviors of the polymer solutions were determined by optical transmittance using a Beckman DU800 UV−vis spectrophotometer. The solutions were considered single phase if the transmittance at 600 nm was higher than 99%. The colloidally dispersed regions corresponded to transmittance values between 90% and 99% whereas more turbid solutions contained macroscopic precipitates which settled quickly.

Potentiometric and conductometric titrations were performed simultaneously with a PC-Titration Plus (ManTech Associates) at 25 °C. In a typical experiment, 50 mL of 0.01% PVAm−PBA was prepared in a 5 mM KCl solution, and the pH was adjusted to 3.0. The solution obtained was titrated with 0.1 M NaOH to pH 11. The addition rate was 1 drop per 30 s, using increments between 0.0001 and 0.04 mL. The conductometric titration was used to determine the total content of titratable acid. The degree of neutralization at intermediate pH values was calculated as the difference between the base consumed in a blank titration and the polymer titration divided by the total titratable acid.

Intrinsic viscosity measurements were made to identify changes in PVAm−PBA configuration with pH change. Polymers were dissolved into a 0.3 M salt solution, and the pH was adjusted to required values using 1 M NaOH and HCl. An Ubbelohde capillary viscometer (size 75, Cannon Instrument Co.) was used to measure the viscosity of PVAm−PBA solutions at 25 °C.

Surface tension was measured by the pendant drop method using a Kruss V1.80 drop shape analyzer. The temperature and humidity were kept at 22 °C and 100%. In a typical experiment, 0.5 wt % PVAm−PBA was dissolved in a 5 mM NaCl solution, and the pH values were adjusted by 0.1 M HCl or NaOH. Drops were formed using a 1 mL syringe with a 1.43 mm flat needle, and the volumes of drop were between 14 and 22 μL. Surface tension values were recorded as functions of drop lifetime.

Results

Two PVAm homopolymers, 15 and 150 kDa, were derivatized with 4-carboxyphenylboronic acids to give the seven copolymers summarized in Table 1. The structures and proton NMR spectra for PVAm−PBA-4 are shown in Figure 1. The PBA contents of the copolymers are expressed in Table 1 as a degree of substitution we defined as the number of PBA moieties per PVAm nitrogen. These values were determined from the relative areas of the aliphatic and aromatic peaks in the proton NMR spectra.

PVAm−PBA Phase Behavior. Substitution of PVAm with phenylboronic acid lowered the water solubility of the polymer. Figure 2 shows the phase boundary diagrams for the higher molecular weight (150 kDa) copolymers in Table 1. All the 150 kDa copolymers showed both colloidal phase and macroscopic precipitate separation regions. In colloidal phase separation domains, the copolymers were present as a slightly turbid (90−99% transmittance at 600 nm) suspension, whereas the polymer formed macroscopic precipitates in the macro-phase domain. The most striking observation from the results in Figure 2 is that the pH range over which PVAm−PBA was insoluble increased with the degree of PBA substitution. The 4% copolymer displayed a narrow range, ~1.5 pH units, whereas the 51% copolymer was insoluble virtually over the whole pH range.

The phase separation domains for the PVAm−PBA copolymers are centered around pH 9, which approximately corresponds to the pH giving zero electrophoretic mobility (see below). We propose that the colloidally stable, phase-separated copolymers were electrostatically stabilized. Thus, colloidal stability was lost when the pH approached pH 9.

The phase behavior was dependent upon PVAm molecular weight. The PVAm−PBA (DS = 4%) based on 15 kDa did not phase separate between pH 1 and 12 in 5 mM NaCl.

PBA substitution also caused soluble copolymer chains to contract slightly compared to the parent PVAm. Figure 3 compares the intrinsic viscosity of PVAm−PBA-4 with the parent PVAm at three pH values. For all pH values, PBA substitution lowered the intrinsic viscosity, suggesting a more

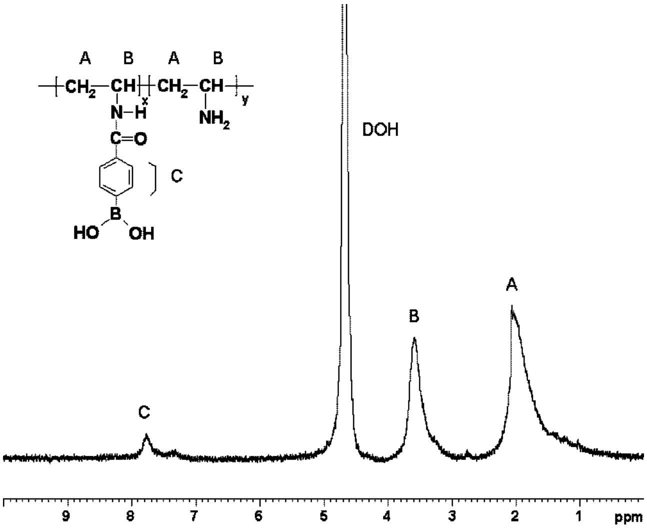


Figure 1. ^{1}H NMR spectra of PVAm−PBA-4 (150 kDa).

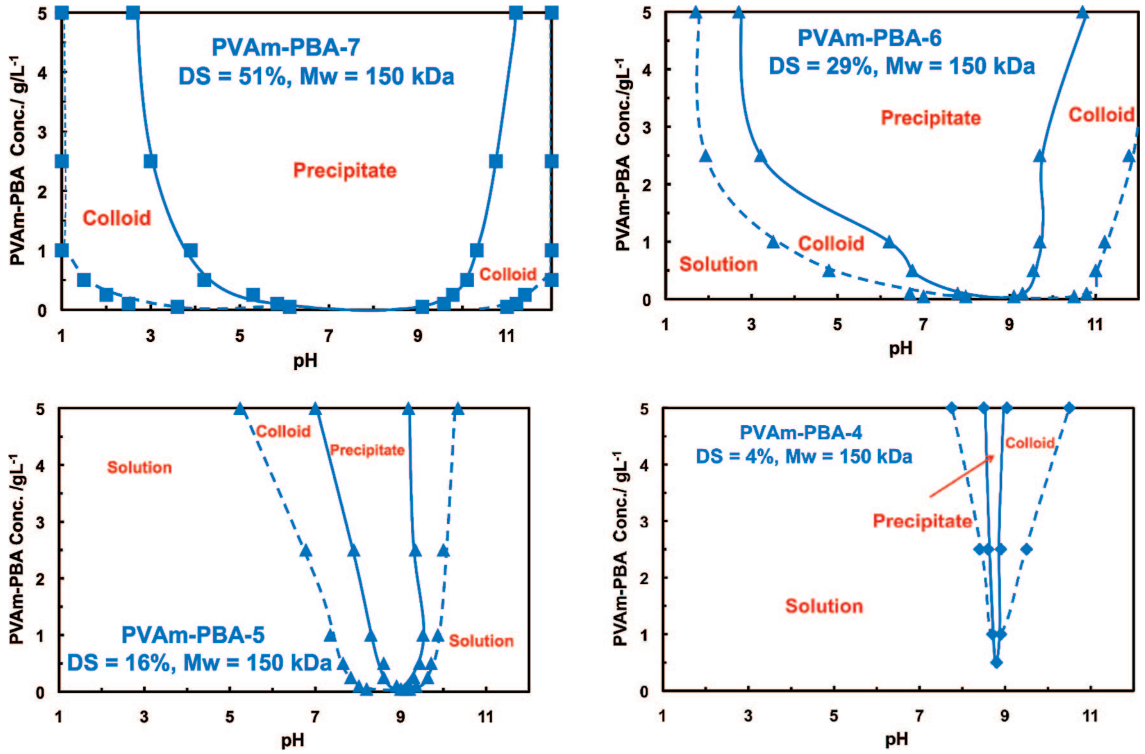


Figure 2. Phase boundaries of PVAm−PBA as functions of pH, polymer concentration, and the degree of substitution. All measurements were made in 5 mM NaCl at 25 °C.

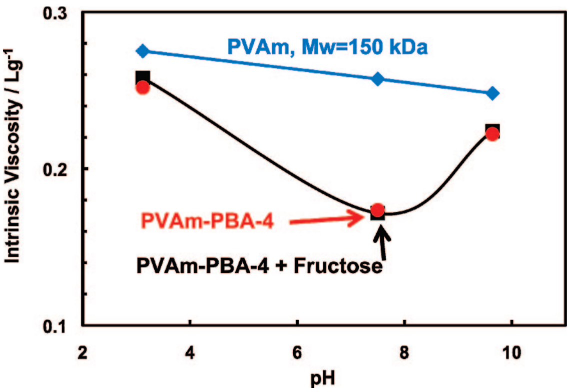


Figure 3. Intrinsic viscosity of PVAm, PVAm−PBA-4, and PVAm−PBA-4 plus fructose in 0.3 M NaCl at 25 °C (molar ratio of boronic acid to fructose was 1:10).

compact configuration in solution. Fructose addition had no effect on intrinsic viscosity.

Charge−pH Behavior of PVAm−PBA. The charge−pH behaviors of the PVAm−PBA copolymers were probed by potentiometric titration and by microelectrophoresis. Figure 4 shows the potentiometric titration behavior of the three 15 kDa copolymers and the parent PVAm—the data points are experimental, whereas the solids lines were calculated with a model described below. The results are displayed as $(1 - \beta)$, where β is the degree of neutralization, defined as the titrated acid divided by the total titratable acid. Note that for PVAm homopolymer $(1 - \beta)$ is the degree of ionization.

The PVAm curve in Figure 4 is approximately linear over the whole pH range, reflecting an extreme polyelectrolyte effect, first reported by Katchalsky many years ago.[19] The extent of deviation from the PVAm curve displayed by the PVAm−PBA copolymers increased with PVAm substitution. The main consequence of adding phenylboronate groups was to retard the removal of protons.

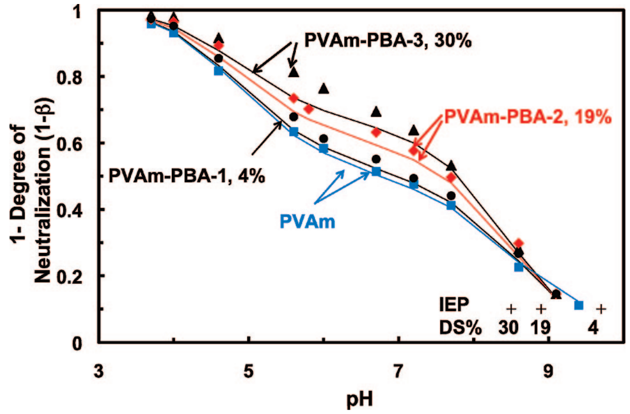


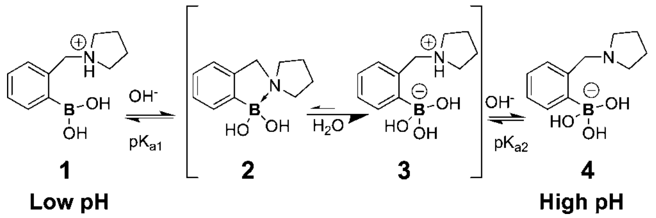
Figure 4. Potentiometric titration of three PVAm−PBA copolymers and the parent PVAm homopolymer. All measurements were performed in 5 mM NaCl at 25 °C. The solid lines and the isoelectric points (IEP) were calculated from the model.

In an effort to understand the shapes of the titration curves in Figure 4, we modeled the copolymer titrations based on two assumptions: (1) the ionization behavior of the vinylamine moieties was given by Katchalsky's model[19] as implemented by Feng[20] (see below), and (2) the ionization behavior of the PBA moieties was described by an equilibrium constant which we assigned to equal 5.01×10^{-9} M, the value for 4-carboxyphenylboronic acid.[4] Thus, we ignored interactions between the phenylboronic groups and neighboring primary amines or ammonium groups. Specific details of the model follow.

In the absence of a tractable mechanistic model, we extended Katchalsky's theory by including the following empirical function in which the dissociation constant, pK_{PVAm}, is a function of ionic strength, I.

$$pK_{\mathrm{PVAm}} = 8.4 + \frac{3.5I}{0.8 + 2I} \qquad (1)$$

Scheme 3. Anslyn's Analysis of Amino Borate Species Equilibria[21]



The remainder of the Katchalsky equations are given below where α is the degree of PVAm ionization and A (= 47) is the nearest-neighbor interaction parameter.

$$X = \frac{A(2\alpha - 1) - 2\alpha + \sqrt{A^2(2\alpha - 1)^2 + 4A\alpha(1-\alpha)}}{2(A-1)} \qquad (2)$$

$$\mathrm{p}H_{\mathrm{PVAm}} = \mathrm{p}K_{\mathrm{PVAm}} + \log\left[\frac{\alpha}{1-\alpha}\,\frac{(1-2\alpha+X)^2}{(\alpha - X)^2}\right] \qquad (3)$$

The corresponding degree of PBA ionization, α_{PBA}, was given by

$$\mathrm{pH} = \mathrm{p}K_{\mathrm{PBA}} + \frac{\alpha_{\mathrm{PBA}}}{1-\alpha_{\mathrm{PBA}}} \qquad (4)$$

The overall degree of neutralization, β, is given by the following where DS is the fraction of primary amine groups substituted with PBA moieties.

$$(1-\beta) = \mathrm{DS}(1-\alpha_{\mathrm{PBA}}) + (1-\mathrm{DS})\alpha \qquad (5)$$

The calculated curves, shown as solid lines in Figure 4, show the same general trends as the experimental data. However, the difference between the model and the data was greatest for the polymer with the highest boronate substitution. Therefore, in analogy with small molecule behavior summarized in Scheme 3, we propose that electrostatic interactions between the alkylboronic groups and the ammonium groups inhibit deprotonation at low pH.

We also used the model to estimate the isoelectric points (IEP) for the three PVAm−PBA, and the results are also displayed in Figure 4. The calculated values are compared to experimental estimates (see below).

Figure 5 illustrates the influence of fructose on the potentiometric titration of PVAm−PBA-3. The presence of fructose facilitated the release of protons from the copolymer at low pH.

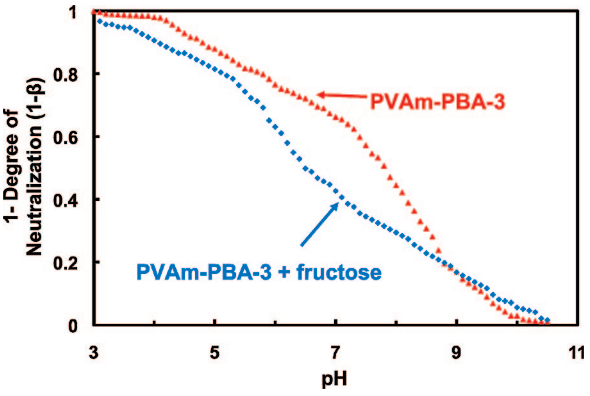


Figure 5. Influence of 0.017 M fructose on the ionization behavior of PVAm−PBA-3 (the molar ratio of boronic acid to fructose was 1:10).

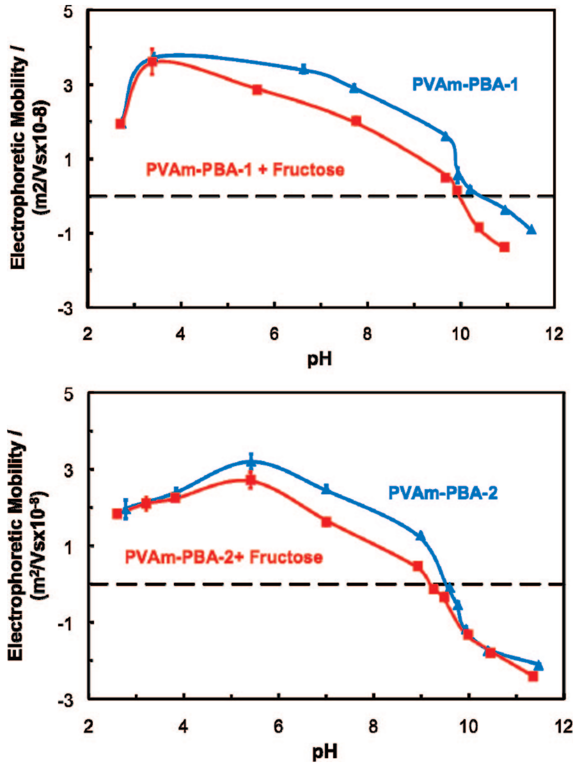


Figure 6. Electrophoretic mobility as functions of pH for 0.1 g/L PVAm−PBA adsorbed onto 0.01 g/L 200 nm diameter anionic polystyrene latex. All measurements were made in 5 mM NaCl aqueous solution at 25 °C. For the experiments with fructose the molar ratio of boronic acid to fructose was 1:10.

Microelectrophoresis was also used to probe the charge versus pH behavior of the copolymers. Figure 6 shows the electrophoretic mobility of two PVAm−PBA copolymers as functions of pH with and without fructose. The copolymers were adsorbed onto anionic polystyrene latex to facilitate measurements. At low and neutral pH the copolymers were positively charged, whereas at high pH the copolymers were negatively charged, reflecting the presence of the charged phenyl borate moieties. The point of zero electrophoretic mobility of PVAm−PBA-1 was 10.3 (model 9.7) whereas PVAm−PBA-2 was at pH 9.8 (model 8.9), reflecting the higher PBA content. The model values were calculated from the ionization model described above. The model predictions were systematically lower than the electrophoresis results, reflecting the approximate nature of our model. In addition, the ionization model does not account for the influence sulfate groups on the latex particle surfaces.

Finally, the presence of fructose shifted the mobility−pH curves toward lower pH values due to the formation of fructose boronate esters. Similar effects have been reported for small molecule boronates.[21]

Surface Activity of PVAm−PBA. PVAm is not surface active at low pH, where it is highly charged, whereas it is slightly surface active at high pH (56 mN/m at pH >9) where the degree of ionization is low.[22] Chen et al. showed that benzylic or aliphatic substituents on PVAm greatly increased surface activity, particularly at higher pH.[23] Figure 7 shows pendant drop surface tension data as functions of drop age and pH for PVAm−PBA-1, a 15 kDa copolymer. Preliminary experimentation with 150 kDa copolymers gave surface tension curves which continually drifted, never reaching a steady state—Chen et al. observed similar behaviors with the hydrophobically modified higher MW PVAm derivatives.[23]

Figure 8 shows the equilibrium surface tension values from Figure 7 as functions of pH. Also shown are the corresponding

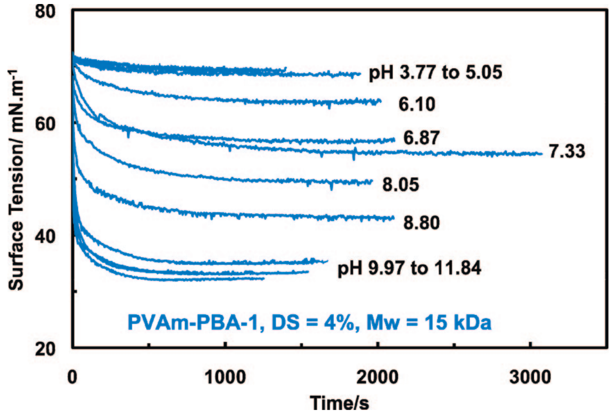


Figure 7. Surface tension versus drop age for 0.5 wt % PVAm−PBA-1 in 5 mM NaCl as functions of pendant drop age at 22 °C.

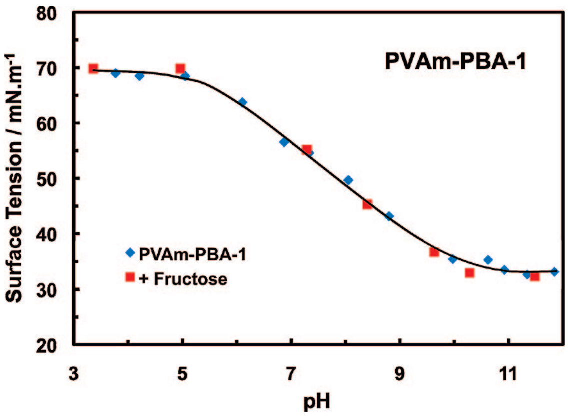


Figure 8. Equilibrium surface tension of PVAm−PBA-1 as functions of pH. Molar ratio of boronic acid to fructose was 1:10.

data measured in the presence of 1 M fructose. Fructose bonding to PVAm−PBA-1 did not influence the surface activity of the copolymer as a function of pH. Perhaps the PBA content was too low to see an effect.

Interestingly, there is no discontinuity in the surface tension versus pH curve around pH 10 corresponding to the isoelectric point of PVAm−PBA-1. Thus, the polymeric surfactant can be switched from being a negatively charged surfactant to a positive one simply by increasing pH.

Discussion

PVAm−PBA chains have a very high concentration of primary amine groups, which behave as weak bases, and a much lower content of boronic groups, which behave as Lewis acids. This combination gives complex pH-dependent behaviors which can be partially understood by considering the properties of analogous small molecules. Several publications have reported the properties of phenylboronic acid derivatives bearing amine groups. A very convincing recent paper from Anslyn's group proposes the structures shown in Scheme 3.[21] Structure **1** exists at low pH; the amine is protonated and boron is not charged. Adding base gives structures **2** and **3**. Structure **2**, which contains a B−N dative bond, has been proposed by a number of authors.[24] With our PVAm−PBA, dative B−N bond formation should give cross-linking, converting the copolymers to hydrogels. However, Anslyn argues that in polar solvents **3** is far more prevalent than **2**. Since we did not observe hydrogel formation, we do not believe that B−N bond formation is significant in our polymers. Finally, at high pH the model small molecule aminoboronate, **4** in Scheme 3, has negatively charged boron and a neutral amine.

For the small molecules in Scheme 3, pK_{a1} is ∼6.5 and pK_{a2} is in the range 9−10.[21] Thus, when titrating an acidic solution, the two base-consuming steps in Scheme 3 will occur nearly sequentially. By contrast, PVAm displays the archetypical polyelectrolyte effect, meaning the effective pK_{a2} value is a strong function of pH.[25] In this case, some of the most acidic primary amines may release protons before the boron becomes negatively charged. From titration data alone, it is not possible to determine the exact mix of ionized ammonium and boronate groups at a specific pH.

The PBA moieties seem to dominate the phase behavior. The phenylboronic groups display two influences on the phase behavior. The hydrophobic benzene rings tend to drive phase separation in water, whereas the ionized form of boron is hydrophilic. For example, PVAm−PBA-5 becomes soluble when the pH is raised above 10. Under these conditions the amines are mainly not ionized, whereas the borates are. However, the copolymers with high PBA contents, PVAm−PBA-6 and PVAm−PBA-7, are phase separated over most of the pH range. In these cases the hydrophobic benzene rings dominate over the influence of hydrophilic ionized borate groups.

Finally, the fructose results show contradictory behaviors. The electrophoresis and potentiometric results show a significant influence of fructose addition whereas neither the surface tension nor the intrinsic viscosities were sensitive to fructose addition. Fructose binds to phenyl boronate, shifting the boronic acid groups to a higher degree of ionization. Over most of the pH range the polymers are strongly cationic because of the many primary amine groups. Thus, the presence of fructose increases the fraction of negatively charged PBA groups, and the effect is measurable by titration and electrophoresis by a decrease in overall cationic charge. However, the configuration and thus the intrinsic viscosity were not influenced much by small changes in net charge density. We did expect a greater influence of fructose on the surface tension.

Conclusions

The major conclusions of this work are as follows:

1. PBA derivatization lowers the water solubility of PVAm. For example, PVAm−PBA-7 with 51% substitution is phase separated over most of the pH range whereas PVAm−PBA-4 with 4% substitution is only insoluble at pH 8−10.

2. The presence of phenylboronic moieties retards the deprotonation of PVAm at low pH due to electrostatic interactions between neighboring boronate and ammonium ions.

3. Polyvinylamine is slightly surface active at high pH (56 mN/m[22]), whereas surface activity is substantially increased at high pH by adding as little as 4% PBA.

4. Fructose−boronate ester formation increased the PBA ionization at neutral pH values as evidence by potentiometric titration and the electrophoretic mobility of PVAm−PBA adsorbed onto polystyrene latex.

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References and Notes

(1) Deul, H.; Neukom, H.; Weber, F. *Nature (London)* **1948**, *161*, 96–7.
(2) Springsteen, G.; Wang, B. H. *Tetrahedron* **2002**, *58* (26), 5291–5300.
(3) Bergold, A.; Scouten, W. H. Borate Chromatography. In *Solid Phase Biochemistry: Analytical and Synthetic Methods*; Scouten, W. H., Ed.; Wiley: New York, 1983.
(4) Soundararajan, S.; Badawi, M.; Kohlrust, C. M.; Hageman, J. H. *Anal. Biochem.* **1989**, *178* (1), 125–134.

(5) Kanekiyo, Y.; Sato, H.; Tao, H. *Macromol. Rapid Commun.* **2005**, *26* (19), 1542–1546.

(6) Cordes, D. B.; Gamsey, S.; Sharrett, Z.; Miller, A.; Thoniyot, P.; Wessling, R. A.; Singaram, B. *Langmuir* **2005**, *21* (14), 6540–6547.

(7) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Nature (London)* **1995**, *374* (6520), 345–7.

(8) Hoare, T.; Pelton, R. *Macromolecules* **2007**, *40* (3), 670–678.

(9) Lapeyre, V.; Gosse, I.; Chevreux, S.; Ravaine, V. *Biomacromolecules* **2006**, *7* (12), 3356–3363.

(10) Nolan, C. M.; Serpe, M. J.; Lyon, L. A. *Biomacromolecules* **2004**, *5* (5), 1940–1946.

(11) Zhang, Y. J.; Guan, Y.; Zhou, S. Q. *Biomacromolecules* **2006**, *7* (11), 3196–3201.

(12) Ivanov, A. E.; Nilsson, L.; Galaev, I. Y.; Mattiasson, B. *Int. J. Pharm.* **2008**, *358* (1−2), 36–43.

(13) Kuzimenkova, M. V.; Ivanov, A. E.; Galaev, I. Y. *Macromol. Biosci.* **2006**, *6* (2), 170–178.

(14) Alexeev, V. L.; Sharma, A. C.; Goponenko, A. V.; Das, S.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N.; Asher, S. A. *Anal. Chem.* **2003**, *75* (10), 2316–2323.

(15) Ben-Moshe, M.; Alexeev, V. L.; Asher, S. A. *Anal. Chem.* **2006**, *78* (14), 5149–5157.

(16) Smoum, R.; Rubinstein, A.; Srebnik, M. *Bioconjugate Chem.* **2006**, *17* (4), 1000–1007.

(17) Winblade, N. D.; Nikolic, I. D.; Hoffman, A. S.; Hubbell, J. A. *Biomacromolecules* **2000**, *1* (4), 523–533.

(18) Chen, W.; Lu, C.; Pelton, R. *Biomacromolecules* **2006**, *7* (3), 701–702.

(19) Katchalsky, A.; Mazur, J.; Spitnik, P. *J. Polym. Sci.* **1957**, *23*, 513–30.

(20) Feng, X.; Pelton, R.; Leduc, M.; Champ, S. *Langmuir* **2007**, *23* (6), 2970–2976.

(21) Zhu, L.; Shabbir, S. H.; Gray, M.; Lynch, V. M.; Sorey, S.; Anslyn, E. V. *J. Am. Chem. Soc.* **2006**, *128* (4), 1222–1232.

(22) Hong, J.; Pelton, R. *Colloid Polym. Sci.* **2002**, *280* (2), 203–205.

(23) Chen, X. N.; Wang, Y.; Pelton, R. *Langmuir* **2005**, *21* (25), 11673–11677.

(24) Wulff, G. *Pure Appl. Chem.* **1982**, *54* (11), 2093–2102.

(25) Borkovec, M.; Jonsson, B.; Koper, G. J. M. *Surf. Colloid Sci.* **2001**, *16*.

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