

Synthesis and Solution Properties of a New pH-Responsive Polymer Containing Amino Propanesulfonic Acid Residues

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Received 11 June 2002; accepted 15 October 2002

ABSTRACT: The reaction of diallyl amine with 1,3-propane sultone led to the synthesis of the zwitterionic monomer 3-(*N,N*-diallylammonio)propanesulfonate. The sulfobetaine was cyclopolymerized in water in the presence of sodium chloride with *t*-butylhydroperoxide as an initiator to afford a polysulfobetaine (PSB) in very good yield. PSB, upon treatment with sodium hydroxide, was converted into an anionic polyelectrolyte (APE). Although APE was readily soluble in salt-free water, PSB needed the presence of low-molecular-weight salts (e.g., NaCl, KI, etc., in the range of 0.135–1.04 N) for its dissolution. The solution properties of PSB and APE were investigated with potentiometric and viscometric techniques. The basicity constant of the amine was apparent and followed the modified Henderson–Hasselbalch equation; as the degree of protonation (α) of the whole macromolecule increases, the protonation of the amine nitrogens becomes increasingly more difficult. The composition and phase diagram of the aqueous two-phase systems of APE/PSB and poly(ethylene glycol) were also explored. © 2002 Wiley Periodicals, Inc. *J Polym Sci Part A: Polym Chem* 41: 172–184, 2003

Keywords: phase diagrams; polyelectrolytes; polysulfobetaines; polyzwitterions; viscosity

INTRODUCTION

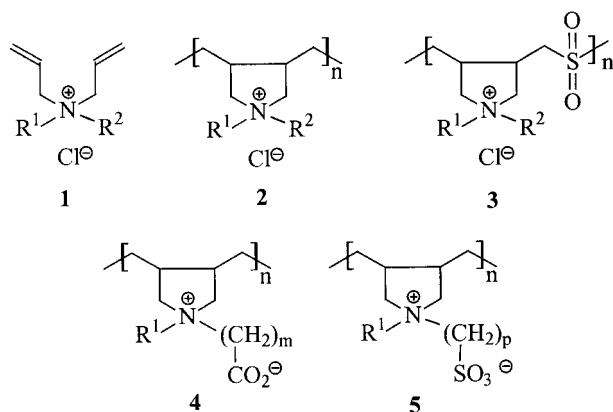
Butler's discovery¹ of the cyclopolymerization of *N,N*-diallyl quaternary ammonium salts (**1**) followed by their copolymerizations with sulfur dioxide^{2–4} led to the synthesis of an array of water-soluble cationic polyelectrolytes **2** and **3**^{5–11} (Scheme 1) of tremendous scientific and technological interest. Poly(diallyldimethylammonium chloride) alone accounts for over 1000 patents and publications. Both the homo- and co-cyclopolymerizations have been shown to proceed via alternating intra- and intermolecular chain propagation through the less-stable five-membered cyclic structure rather than the thermodynamically favorable six-membered one.^{12–17} Since Butler's

pioneering work, the cyclopolymerization process has traversed a long way of half a century, and along its way the modifications of Butler's monomers **1** have provided entries^{18–27} into the amphoteric polymers containing zwitterions on the same monomer (i.e., betaines). Amphoteric polymers containing zwitterions along the same polymeric backbone have also found many applications in various fields^{28,29} including their use as a simple model^{30,31} for understanding the complex behavior of proteins. Amphoteric polymers, unlike polyelectrolytes, can exhibit antipolyelectrolyte behavior,^{19,32–36} that is, enhancements in the viscosity and solubility in the presence of added electrolytes (e.g., NaCl) because of the neutralization of the ionically crosslinked network in a collapsed coil conformation of polyampholytes.

The development in the cyclopolymerization process has entered a new stage that deals with the synthesis of pH-responsive ionic polymers.

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Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 41, 172–184 (2003)
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Scheme 1

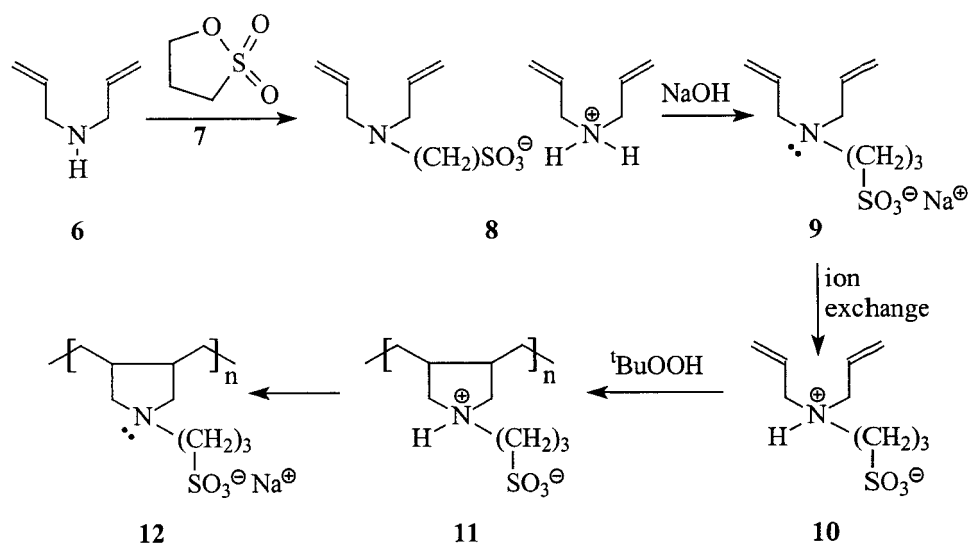
Quaternary ammonium salts-derived cyclopolymer **3–5** ($R^1 \neq H$)^{21,23–25,37} with an anionic pendant show pH-responsive solution behavior because of the incorporation of the pH-triggerable betaine functionality. However, amine salt-derived polymers **3** and **4** ($R^1 = H$) permitted us to generate two pH-responsive groups (a trivalent nitrogen as well as a carboxy) in the resultant polymer.^{38–40} The presence of strongly basic trivalent nitrogen as well as weakly basic carboxy functionalities has demonstrated interesting pH-responsive solution behavior in a wider spectrum of the pH scale. One of the most gratifying aspects of polymer **3** ($R^1 = H$, $R^2 = CH_2CO_2^-$) is its almost zero solubility³⁸ in water below pH ~ 7 in the presence or absence of salt, therefore making it a suitable candidate⁴¹ for applications in protein

purification in two-phase aqueous polymer systems⁴² because it will permit its effective removal from solution by precipitation. The homopolymers from amine salt of the type **10** containing sulfo-betaine moiety (Scheme 2) have not been reported. In our continuing studies of zwitterionic polymers, we report the synthesis, solution properties, and two-phase aqueous polymer systems of the homopolymers derived from the zwitterionic monomer **10**.

EXPERIMENTAL

Physical Methods

Melting points were recorded in a calibrated Electrothermal IA9100 Digital melting-point apparatus at heating rates of 1 °C/min in the vicinity of the melting points. Elemental analyses were carried out in a Carlo Erba model 1102 elemental analyzer. IR spectra were recorded on a PerkinElmer 16F PC Fourier transform infrared spectrometer (spectral resolution: 4 cm^{-1} , number of scans: 19). 1H and ^{13}C NMR spectra of the polymers were measured in D_2O with dioxane as an internal standard on a JEOL LA 500-MHz spectrometer. Viscosity measurements were made with an Ubbelohde viscometer (viscometer constant: 0.005718 cSt/s at all temperatures) with CO_2 -free water under N_2 to avoid CO_2 absorption that could affect the viscosity data.



Scheme 2

Materials

t-Butylhydroperoxide (80% in ditertiarybutylperoxide), diallyl amine, and 1,3-propane sultone from Fluka Chemie AG (Buchs, Switzerland) were used as received. All glassware was cleaned with deionized water. For dialysis, a Spectra/Por membrane with a molecular weight cutoff value of 6–8000 was purchased from Spectrum Laboratories, Inc. Poly(ethylene glycol) (PEG) with a molecular weight of 35,000 was purchased from Merck-Schuchardt.

Sodium 3-(*N,N*-Diallylamino)propanesulfonate (9)

A solution of diallylamine (**6**) (49.6 g, 98% pure, 0.500 mol) and 1,3-propanesultone (**7**) (28.3 g, 98% pure, 0.227 mol) in acetonitrile (65 cm³) was stirred under N₂ at 40 °C for 24 h. The solvent and the excess diallylamine were removed by a gentle stream of N₂ at 60 °C. The residual mixture was treated with 1 N NaOH solution (235 cm³, 0.235 mol) and was extracted with ether (3 × 100 cm³) to remove the excess diallyl amine. The sodium salt of monomer **9** was recovered from the aqueous layer by freeze-drying followed by crystallization from a methanol/acetone mixture as white crystals (49.4 g, 90.2%); mp: 125–127 °C (closed capillary).

ELEM. ANAL. Calcd. for C₉H₁₆NO₃SNa: C, 44.80%; H, 6.68%; N, 5.81%; S, 13.29%. Found: C, 44.5%; H, 7.09%; N, 5.67%; S, 13.09%. ν_{\max} (KBr): 3625 (w), 3452 (w), 3078, 3006, 2976, 2932, 2806, 1644, 1450, 1420, 1354, 1262, 1194 (br), 1148, 1110, 1056, 994, 950, 918, 842, 792, 736, 620 cm⁻¹. δ_{H} (D₂O): 1.79 (2H, quint, *J* = 7.9 Hz), 2.47 (2H, t, *J* = 7.9 Hz), 2.73 (2H, t, *J* = 7.9 Hz), 3.02 (4H, d, *J* = 6.8 Hz), 5.13 (4H, m), 5.75 (2H, m) (HOD: 4.65). δ_{C} (D₂O): 21.51, 49.93, 51.66, 56.58, 120.16, 134.44 (dioxane, 67.40 ppm).

3-(*N,N*-Diallylammonio)propanesulfonate (10)

A solution of the sodium salt (44.6 g, 0.185 mol) in water (55 cm³) was stirred with Dowex 50Wx8 (78 g) at room temperature for 1 h. The aqueous solution became acidic as indicated by a litmus test. The solution was filtered through a cotton bed in a column, and the resin was thoroughly washed by passing deionized water (150 cm³) through it. The zwitterionic monomer was recovered by freeze-drying. The thick liquid was then dried to a constant weight *in vacuo* at 65 °C to obtain 38.5 g

(95%) of the sulfobetaine monomer **10** as a light yellow thick liquid.

ν_{\max} (neat): 3448, 3000, 2672 (br), 1462, 1212 (br), 1164, 1036, 952, 784, 730 cm⁻¹. δ_{H} (D₂O): 2.10 (2H, quint, *J* = 8.0 Hz), 2.89 (2H, t, *J* = 7.3 Hz), 3.23 (2H, t, *J* = 8.1 Hz), 3.73 (4H, d, *J* = 7.3 Hz), 5.53 (4H, m), 5.85 (2H, m) (HOD: 4.65). δ_{C} (D₂O): 20.17, 48.45, 51.38, 55.70, 126.18, 127.61 (dioxane, 67.40 ppm).

General Procedure for the Polymerization of 10

All the polymerizations were carried out under the conditions described in Table 1. The experiment under entry 16 was repeated on a large scale as described subsequently. A solution of monomer **10** (10.5 g, 47.9 mmol) and NaCl (72 mg) in CO₂- and O₂-free deionized water (3.5 g) in a 10-cm³ round-bottom flask was purged with N₂, and after adding the required amount of the initiator (22.5 mg), the mixture was stirred in the closed flask at 80 °C for 24 h followed by 90 °C for another 24 h. The reaction mixture remained transparent throughout the process. At the end of the specified time, the ¹H NMR spectra of the crude reaction mixture revealed the composition of the polysulfobetaine (PSB) **11** and unreacted monomer **10**. The reaction mixture was cooled, dissolved in a minimum amount of 1 N NaCl, transferred to a dialysis bag, and dialyzed against deionized water for 24 h for the removal of the unreacted monomer and the added salt (NaCl) (checked with a AgNO₃ test). Within 1 h, the polymer started separating out of the solution. The dialysis was continued for an additional 72 h. The separated polymer was then dried at 70 °C *in vacuo* and then crushed into powder and redried to a constant weight at 70 °C to obtain PSB **11** (7.95 g, 75.7%) as a white polymer.

Onset of thermal decomposition (closed capillary): the color changed to brownish black at 275 °C, but the polymer did not melt or char up to 400 °C. ν_{\max} (KBr): 3458, 2932, 2742, 1654, 1466, 1204 (br), 1038, 734 cm⁻¹. ELEM. ANAL. Calcd. for C₉H₁₇NO₃S: C, 49.29%; H, 7.81%; N, 6.39%; S, 14.62%. Found: C, 48.7%; H, 8.25%; N, 6.25%; S, 14.6%.

Conversion of PSB 11 to Anionic Polyelectrolyte (APE) 12

PSB **11** (0.877 g, 4.0 mmol) was treated with 2 N NaOH (3.0 cm³) at 20 °C for 5 min, and the resulting APE **12** was precipitated into acetone (25

Table 1. Effect of Concentration of Monomer and Initiator [Tertiary Butylhydroperoxide, (TBHP)], Sodium Chloride, and Hydrogen Chloride on Cylopolymerization of Monomer **10**

| Entry | Monomer ^a Concentration (w/w %) | NaCl (M) | HCl (mmol) | TBHP (mg) | Yield ^{b,c} (%) | Intrinsic Viscosity ^d (dL g ⁻¹) |
|-----------------|--|----------|------------|-----------|--------------------------|--|
| 1 | 60 | 3 | 0 | 10 | 78 (55) | 0.228 |
| 2 | 60 | 7 | 0.5 | 50 | 44 (35) | 0.0750 |
| 3 | 65 | 3 | 0 | 10 | 80 (61) | 0.270 |
| 4 | 65 | 7 | 0.5 | 50 | 30 (19) | 0.0576 |
| 5 | 65 | 7 | 0.5 | 85 | 52 (40) | 0.0590 |
| 6 | 75 | 0 | 0.5 | 85 | 85 (65) | 0.117 |
| 7 | 75 | 3 | 0.5 | 85 | 72 (57) | 0.115 |
| 8 | 75 | 3 | 0 | 85 | 95 (75) | 0.174 |
| 9 | 75 | 7 | 0 | 10 | 35 (24) | 0.117 |
| 10 | 75 | 3 | 0 | 50 | 93 (74) | 0.222 |
| 11 | 75 | 3 | 0 | 10 | 86 (72) | 0.338 |
| 12 ^c | 75 | 1.5 | 0 | 10 | 86 (69) | 0.333 |
| 13 | 75 | 0.7 | 0 | 10 | 88 (73) | 0.378 |
| 14 | 75 | 0.7 | 0 | 5 | 77 (65) | 0.521 |
| 15 | 75 | 0.35 | 0 | 5 | 87 (71) | 0.510 |
| 16 | 75 | 0.35 | 0 | 3.5 | 83 (76) | 0.570 |
| 17 | 75 | 0 | 0 | 85 | 74 (61) | 0.214 |

^a Polymerization reactions were carried out with 7.5 mmol of the monomer in aqueous medium at 80 °C for 24 h followed by 90 °C for 24 h.

^b Determined by ¹H NMR analysis. Isolated yields are written in parentheses.

^c The yield after 24 h at 80 °C was 76% (by ¹H NMR), which was changed to 86% after additional heating at 90 °C for 24 h. Further heating did not increase the yield.

^d Viscosity of 1–0.125% polymer (**11**) solution in the presence of 1 equi of NaOH in 0.1 N NaCl at 30 °C as measured with an Ubbelohde viscometer ($K = 0.005718$).

cm³). The polymer was redissolved in water (1 cm³) and reprecipitated in acetone (25 cm³). The polymer was then soaked in methanol for 12 h and then dried at 70 °C *in vacuo* to a constant weight (0.782 g, 81%).

Onset of thermal decomposition (closed capillary): the color changed to blackish brown at 350 °C, but the polymer did not melt or char up to 400 °C. ν_{max} (KBr): 3456, 2926, 2854, 2800, 1656, 1458, 1200 (br), 1050, 798, 738 cm⁻¹. ELEM. ANAL. Calcd. for C₉H₁₆NNaO₃S · H₂O: C, 41.69%; H, 7.00%; N, 5.40%; S, 12.36%. Found: C, 41.5%; H, 7.3%; N, 5.25%; S, 12.0%.

Solubility Measurements and Cloud Point Titrations in Aqueous Salt Solutions

The solubility of PSB **11** and APE **12** in organic solvents at room temperature was established for 2 wt %/wt solutions, after preheating at 70 °C for 1 h. The critical (minimum) salt concentration (CSC) promoted water solubility at 23 °C that was measured by titration of 1% w/w polymer solution at a sufficiently high salt concentration with

deionized water. The accuracy of the CSC values, obtained by visual determination of the first cloud point, was approximately ±1–2%. The results of the solubility and CSC are given in Tables 2 and 3, respectively.

Table 2. Solubility^{a,b} of Polysulfobetaine **11** and Corresponding Anionic Polyelectrolyte **12**

| | ϵ | PSB 11 | APE 12 |
|------------------------|------------|---------------|---------------|
| Water | 78.4 | — | + |
| Methanol | 32.3 | — | — |
| Formic acid | 58.5 | ± | ± |
| Formamide ^c | 111.0 | + | + |
| Ethylene glycol | 37.3 | — | + |
| Triethylene glycol | 23.7 | — | + |
| Acetic acid | 6.15 | — | — |

^a 2% (w/w) of polymer-water mixture (solution) was made after heating the mixture at 70 °C for 1 h and then cooling to 23 °C.

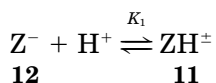
^b ‘+’ indicates soluble, ‘—’ indicates insoluble, and ‘±’ indicates partially soluble.

Table 3. Critical Salt Concentration for Aqueous Solutions of Polysulfobetaine **11** at 23 °C

| Salt | CSC (M) |
|--------------------|---------|
| LiCl | 1.04 |
| NaCl | 0.670 |
| KCl | 0.638 |
| KBr | 0.331 |
| KI | 0.135 |
| NH ₄ Cl | 0.718 |
| CaCl ₂ | 0.541 |
| HCl | 0.940 |

Potentiometric Titrations

The potentiometric titrations were carried out at 25 °C. A gentle stream of N₂ was passed through distilled and deionized water at 90 °C for 15 min for the removal of dissolved gases. This water was used for titration in salt-free and salt (NaCl, 99.9% purity) solutions and for viscosity measurements. For each titration, 200 cm³ of a salt-free or 0.1 N NaCl solution containing a weighed amount (usually ca. 0.40–0.63 mmol) of the anionic monomer **9**, PSB **11** (or APE **12**) were used. In the case of PSB, the polymer was first dissolved in 1 equiv of 0.1046 N NaOH and then diluted to the required volume. The solution was titrated at 25 °C with 0.1015 M HCl delivered by a buret under N₂. After each addition of the titrant (0.25 cm³), the solution was stirred briefly with a magnetic stir bar under N₂. The pH of the solution was recorded with a Corning 220 pH meter. Under the conditions (including a large volume of 0.1 N NaCl), the small amount of polymers or the small volume of the titrant used did not contribute much to the ionic strength; the data were not corrected to zero ionic strength, and the activity coefficient corrections were not applied



$$\log K_i = \text{pH} - \log [(1 - \alpha)/\alpha] \quad (1)$$

$$\text{pH} = \log K_i^0 + n \log [(1 - \alpha)/\alpha] \quad (2)$$

$$\log K_i = \log K_i^0 + (n - 1) \log [(1 - \alpha)/\alpha] \quad (3)$$

The protonation constant of the amine nitrogen, $\log K$, was calculated at each pH value by the well-known Henderson–Hasselbalch equation (eq 1) where the degree of protonation (α) is the ratio $[\text{ZH}^+]_{\text{eq}}/[\text{Z}]_0$. $[\text{Z}]_0$ is the initial analytical concentration of the monomeric units in APE **12** (or PSB **11**), and $[\text{ZH}^+]_{\text{eq}}$ is the concentration of the protonated species at the equilibrium given by $[\text{ZH}^+]_{\text{eq}} = C_{\text{H}}^+ - [\text{H}^+] + [\text{OH}^-]$, where C_{H}^+ is the concentration of the added HCl; $[\text{H}^+]$ and $[\text{OH}^-]$ at equilibrium were calculated from the pH value.⁴³ The typical electrolytes having apparent basicity constants can be described by eq 2, where $\log K^0$ is the pH at $\alpha = 0.5$ and $n = 1$ in the case of sharp basicity constants. The linear regression fit of pH versus $\log[(1 - \alpha)/\alpha]$ gave $\log K^0$ and n as the intercept and slope, respectively. Simultaneous protonation of the two basic sites was least likely because the basicity constant for the SO₃[−] group was less than that of the amine group by at least eight orders of magnitude. In the case of **9**, the second-step protonation constant ($\log K_2$) involving the protonation of SO₃[−] was not determined because it would require a large amount of HCl to protonate the very weakly basic sulfonate group to a meaningful extent. In any case, the determination of $\log K_2$ was not possible because APE **12** started to precipitate in 0.1 N NaCl and salt-free water after the addition of around 0.80 and 0.97 equiv of HCl, respectively.

The experimental details of the potentiometric titrations are summarized in Table 4. Inserting the value of pH from eq 2 into eq 1 leads to a modified Henderson–Hasselbalch equation (eq 3),^{44,45} where $n - 1$ gives a measure of the deviation of the studied polymers from the behavior of small molecules showing sharp basicity constants (for molecules having sharp basicity constants, n becomes 1) (Fig. 1).

Viscometric Titration

The viscometric titration was performed at 25 °C in the presence of various amounts of HCl, but the concentration of the polymer was kept constant throughout. Two-stock solutions of 0.02280 M (#A) and 2×0.02280 M (#B) polymer APE **12** were prepared in 0.1 and 0.2 N NaCl, respectively (the stock #A contained 0.5 g/dL of PSB **11** neutralized with 1 equiv of NaOH). The reduced viscosity and pH of the 0.02280 M stock solution were measured. A known volume (usually ca. 0.4–0.7 mL) each of 0.1015 N HCl and stock

Table 4. Experimental Details for the Protonation of Monomer **9** and Polymers APE **12** (Z^-) at 25 °C in Salt-Free Water and 0.1 N NaCl with 0.1015 N HCl

| Run | ZH ^{±a} or Z ⁻ (mmol) | α Range | pH Range | Points ^b | Log K^0 ^c | n^c | R^2 ^d |
|---|---|----------------|------------|---------------------|------------------------|----------|--------------------|
| Polymers in salt-free water | | | | | | | |
| 1 | 0.4008 (ZH [±]) | 0.61–0.95 | 10.66–8.62 | 10 | 10.90 | 1.84 | 0.9864 |
| 2 | 0.4970 (ZH [±]) | 0.59–0.97 | 10.65–8.20 | 12 | 10.79 | 1.76 | 0.9905 |
| 3 | 0.6293 (ZH [±]) | 0.56–0.97 | 10.78–8.24 | 16 | 10.83 | 1.79 | 0.9825 |
| 4 | 0.4636 (Z ⁻) | 0.57–0.98 | 10.74–7.35 | 13 | 10.78 | 1.85 | 0.9853 |
| Average | | | | | 10.82 (5) | 1.81 (4) | |
| Log $K^e = 10.82 + 0.81 \log [(1 - \alpha)/\alpha]$; for the reaction: $Z^- + H^+ \rightleftharpoons ZH^+$ | | | | | | | |
| Monomer in salt-free water | | | | | | | |
| 1 | 0.3028 (Z ⁻) | 0.099–0.89 | 9.43–7.49 | 11 | 8.48 | 1.03 | 0.9973 |
| 2 | 0.4129 (Z ⁻) | 0.034–0.79 | 9.82–7.78 | 14 | 8.46 | 1.02 | 0.9889 |
| 3 | 0.5224 (Z ⁻) | 0.15–0.92 | 9.32–7.46 | 18 | 8.58 | 0.97 | 0.9901 |
| Average | | | | | 8.51 (6) | 1.01 (3) | |
| Log $K^e = 8.49$; for the reaction: $Z^- + H^+ \rightleftharpoons ZH^+$ | | | | | | | |
| Polymers in 0.1 N NaCl | | | | | | | |
| 1 | 0.3990 (ZH [±]) | 0.25–0.83 | 10.69–8.94 | 14 | 9.90 | 1.53 | 0.9951 |
| 2 | 0.5144 (ZH [±]) | 0.22–0.84 | 10.76–8.90 | 18 | 9.88 | 1.55 | 0.9936 |
| 3 | 0.6197 (ZH [±]) | 0.17–0.82 | 10.72–8.91 | 21 | 9.78 | 1.42 | 0.9982 |
| 4 | 0.5168 (Z ⁻) | 0.23–0.74 | 10.77–9.27 | 16 | 9.95 | 1.55 | 0.9992 |
| Average | | | | | 9.88 (7) | 1.51 (6) | |
| Log $K^e = 9.88 + 0.51 \log [(1-\alpha)/\alpha]$; for the reaction: $Z^- + H^+ \rightleftharpoons ZH^+$ | | | | | | | |
| Monomer in 0.1 N NaCl | | | | | | | |
| 1 | 0.2944 (Z ⁻) | 0.091–0.89 | 9.16–7.11 | 11 | 8.30 | 1.03 | 0.9850 |
| 2 | 0.4144 (Z ⁻) | 0.072–0.86 | 9.33–7.48 | 14 | 8.36 | 0.98 | 0.9881 |
| 3 | 0.5141 (Z ⁻) | 0.059–0.84 | 9.42–7.52 | 17 | 8.33 | 1.02 | 0.9914 |
| Average | | | | | 8.33 (3) | 1.01 (3) | |
| Log $K^e = 8.33$; for the reaction: $Z^- + H^+ \rightleftharpoons ZH^+$ | | | | | | | |

^a Polysulfobetaine **11** is treated with one 1 equiv of a 0.1046 N NaOH, and the resultant APE **12** is then titrated with 0.1015 N HCl.

^b Number of data points from the titration curve.

^c Values in the parentheses are standard deviations in the last digit.

^d R = correlation coefficient.

^e $\log K = \log K^0 + (n - 1) \log [(1 - \alpha)/\alpha]$.

solution #B was added to a known weight or volume (~20 mL) of stock solution #A in an Erlenmeyer flask. Equal volumes of HCl solution and stock #B (having twice the molarity of stock #A) were added to stock #A to keep the concentrations of NaCl and the polymer unchanged. The reduced viscosity and pH of the new solution A₁ were measured. The solution was then returned to the original Erlenmeyer flask, and its weight was noted. Again, an equal volume of #B and 0.1015 N HCl were added to the known weight of A₁. The pH and viscosity of the new solution A₂ were measured. The dilution changes the molar equiv-

alent of the added HCl, and its amount should be recalculated. Each time the viscometer is cleaned and dried. Similar dilutions were done to obtain pH and reduced viscosity values of the 0.02280 M polymer solution in the presence of different amounts of added HCl, the range of which is shown in Figure 2. Pure CO₂-free water was used throughout, and the solutions were used immediately after preparation. A gentle stream of N₂ was passed over the open arms of the viscometer to prevent carbon dioxide absorption. The percentage of different ionic species in the solutions is calculated in eq 2.

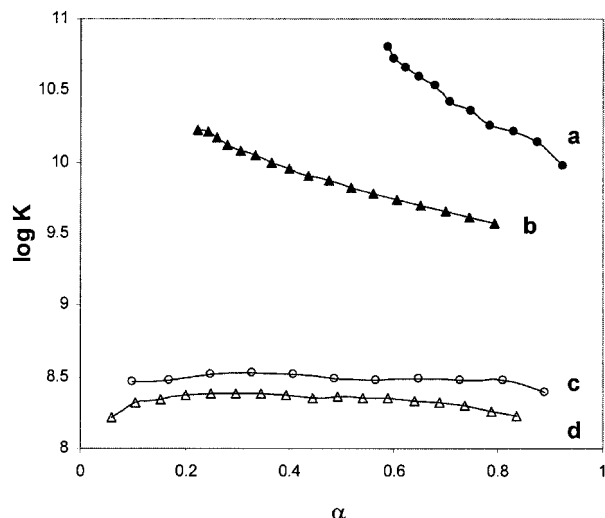


Figure 1. Plot of the apparent $\log K$ versus α for APE **12** (a) in salt-free water and (b) in 0.1 N NaCl and that of precursor molecule **9** (c) in salt-free water and (d) in 0.1 N NaCl.

Phase Compositions and Phase Diagram

NMR Method

Several systems of known compositions of PEG-35,000 and PSB **11** in 0.1 N KCl (as represented by points A–D and E in Fig. 3) were made. The PSB stock solution was treated with 0.60 equiv of NaOH. The phase separation happened within

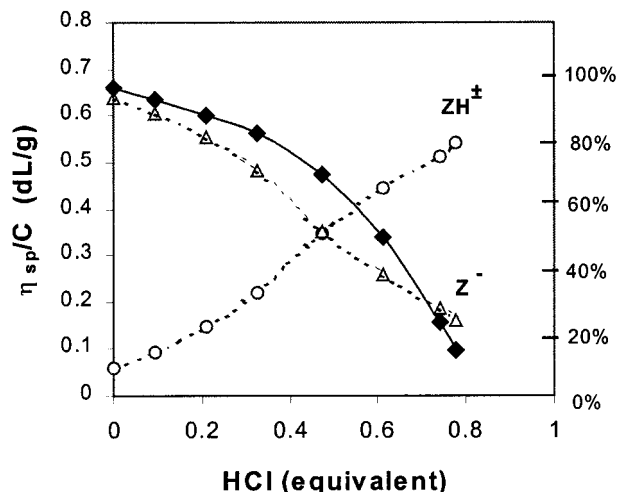


Figure 2. Reduced viscosity (η_{sp}/C) of a 0.0228 M (i.e., 0.50 g/dL) solution of polymer PSB **11** (+1 equiv NaOH) in 0.1 N NaCl (\blacklozenge) versus equivalent of added HCl at 25 °C. Distribution curves (dashed lines) of the various ionized species calculated from the basicity constants $\log K$ in 0.1 N NaCl at 25 °C.

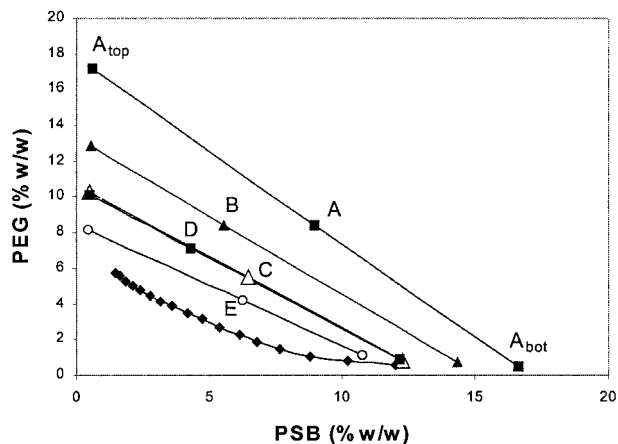


Figure 3. Phase diagram of the system containing PSB **11** (treated with 0.60 equiv NaOH) and PEG-35000 in 0.1 N KCl at 23 °C.

minutes after thorough shaking, and the mixture was kept at 23 °C for 24 h. The weight percentage of the polymers in each phase was determined with the ^1H NMR technique as described elsewhere.^{4,38} The top and bottom layers were rich in PEG and PSB, respectively. In the presence of PSB and APE (because the mixture contained a 40:60 mixture of the ionic polymers), the determination of the molar ratios was difficult because the singlet for PEG at $\delta 3.58$ overlapped with the signals for the PSB protons that were shifted downfield. However, basification (K_2CO_3) followed by ^1H NMR measurements allowed us to determine the molar ratios. The ^1H NMR signals for the polymers were well separated in the absence of HCl, and the molar ratios of the repeating units of the polymers were easily calculated with the integration of the four-proton singlet at $\delta 3.58$ for PEG and the 16-proton complex signals in the range $\delta 0.75$ – 3.10 for polymer APE. The tie lines A–E were constructed with the weight percentage of the polymers in the top, bottom, and total systems.

Turbidity Method

The binodal curve was constructed with a procedure as described elsewhere.³⁸ About 1.5 g of a concentrated solution (25% w/w) of PSB (treated with 0.60 equiv NaOH) in 0.1 N KCl was titrated with a concentrated solution (25 % w/w) of the PEG-35,000 (in 0.1 N KCl). Points obtained by the turbidity method are joined together in Figure 3. The experiments were carried out at 23 °C.

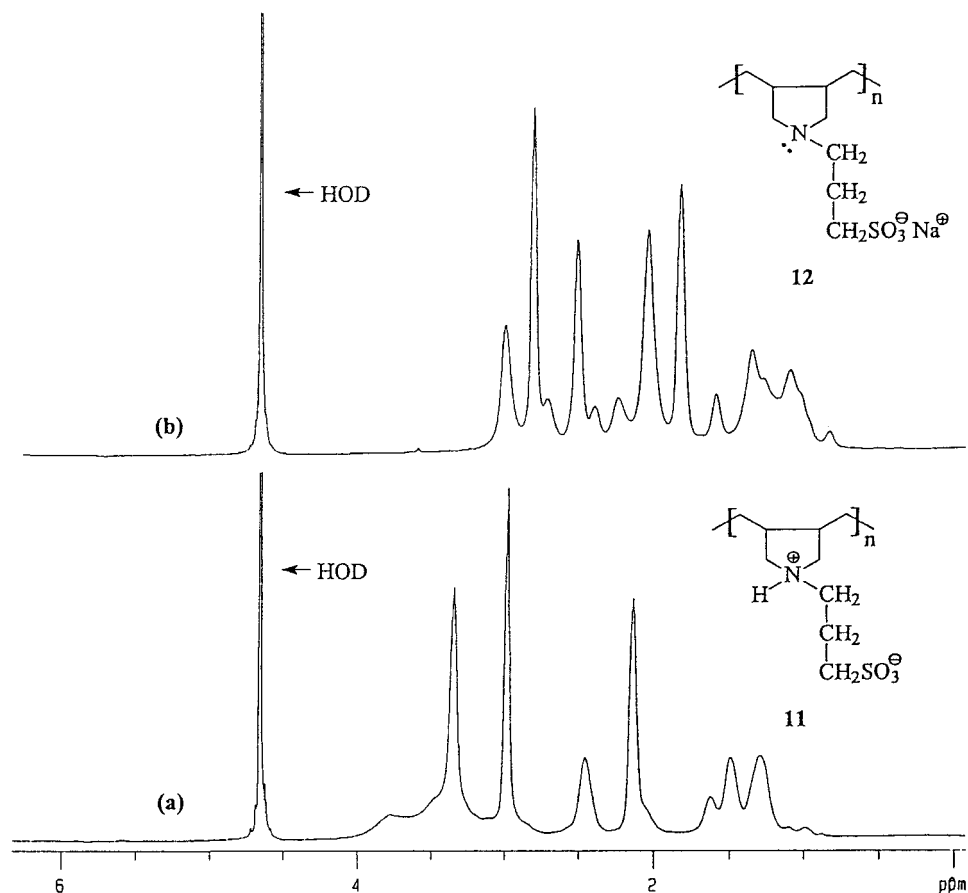


Figure 4. ^1H NMR spectrum of (a) PSB **11** (in the presence of sufficient concentration of NaCl) and (b) APE **12** in D_2O .

RESULTS AND DISCUSSION

6, on treatment with 2 equiv of 1,3-propane sulfone (**7**) followed by treatment with 1 equiv of sodium hydroxide, afforded **9** in excellent yield (90%) (Scheme 2). The precursor molecule **9**, upon cation exchange, gave the zwitterionic monomer, sulfobetaine **10** (95%). The monomer was subjected to a cyclopolymerization reaction with *t*-butylhydroperoxide as an initiator to give PSB **11** in very good yields. PSB **11**, upon neutralization with sodium hydroxide, was converted into the corresponding APE **12**. The results of the polymerizations under various conditions and the intrinsic viscosities of the resultant polymers **12** are given in Table 1. Most of the polymerizations were run in the presence of NaCl that prevents precipitation of the polymers during the reactions. As is evident from Table 1, the highest yield and viscosity value are obtained for the polymerization reaction under entry 11 with a monomer

concentration of 75% (w/w) (cf. entries 1, 3, and 11). The viscosity value increases slightly by keeping the initiator concentration constant and decreasing the molarity of the NaCl (cf. entries 9 and 11–13). In some polymerization reactions, HCl was added to ensure the complete protonation of the nitrogen; however, there was no improvement in the yields (cf. entries 7 and 8). Among all the polymerization reactions, the highest viscosity value was obtained under entry 16; the table suggests that the intrinsic values increase with decreasing concentrations of both the NaCl and initiator at a constant monomer concentration (cf. 9 and 11–16). In the absence of NaCl, however, the polymer yields are low unless a high concentration of the initiator is used (entry 17).

The IR spectrum of **9–12** indicates the presence of the sulfonate group by its characteristic bands at ~ 1200 and $\sim 1040\text{ cm}^{-1}$. The ^1H and ^{13}C NMR spectra of polymers **11** and **12** are displayed in Figures 4 and 5, respectively. The absence of any

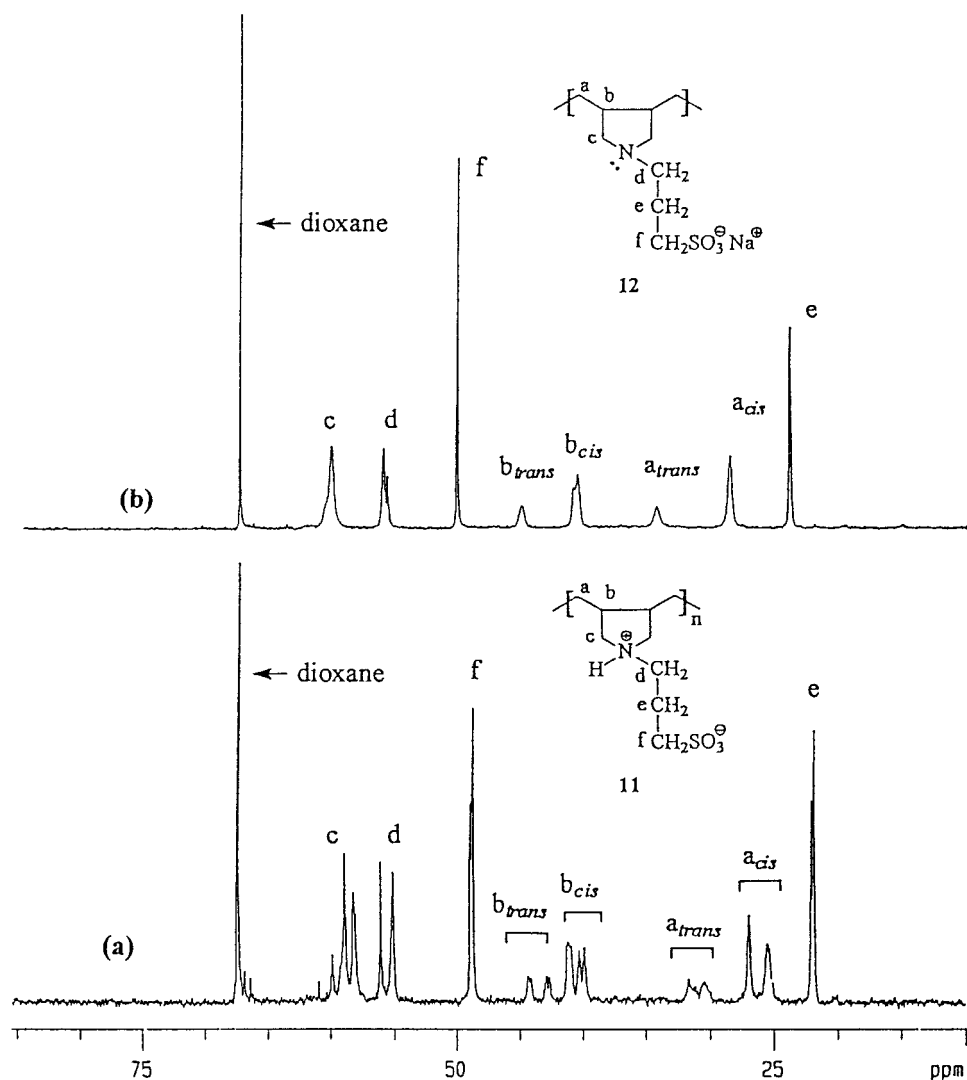


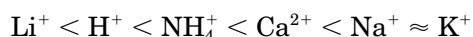
Figure 5. ^1H NMR decoupled ^{13}C NMR spectrum of (a) PSB **11** (in the presence of sufficient concentration of NaCl) and (b) APE **12** in D_2O .

residual alkene proton or carbon signal in the spectra suggested the degradative chain-transfer process⁴⁶ for the termination reaction. The proton signals of PSB **11** are, as expected, shifted downfield as compared with APE **12** because of the presence of positively charged nitrogen in the former. The assignments of the ^{13}C peaks are based on earlier works^{12,13,23,47} on quaternary ammonium salt monomers that undergo cyclopolymerization to afford kinetically favorable five-membered ring structures. The use of the amine salt monomer in this work, however, does not change the preference—the spectral data point to the dominating (if not exclusive) formation of the pyrrolidine rings. Integration of the relevant peaks in the ^{13}C NMR spectrum yielded a 76/24

cis–trans ratio of the ring substituents that is similar to that observed for the polymers derived from quaternary ammonium salts.^{23,24} The substituents at the C-b of polymers **11** and **12** can either be in the symmetrical cis or unsymmetrical trans dispositions (Fig. 5). The unequal splitting of the cis form can be attributed to the difference in the configuration at N^+ ; the cis substituents at C-b are cis or trans to the $\text{N}-(\text{CH}_2)_3\text{SO}_3^-$ in unequal proportion. Only one form is possible for the unsymmetrical minor trans isomer, and its C-b and C-b' signals show two weak lines of equal intensity [Fig. 5(a)]. However, polymer **12**, with trivalent nitrogen, undergoes a fast nitrogen lone-pair inversion in the NMR timescale, and the $\text{N}-(\text{CH}_2)_3\text{SO}_3$ group loses its stereochemical in-

tegrity; the splitting of the carbon signals in the cis or trans forms is not observed [Fig. 5(b)].

The solubility behavior of the polymers is shown in Table 2. APE **12** was very soluble in most of the protic solvents except methanol and acetic acid. Like the overwhelming majority of reported^{19,32,33} polybetaines, PSB **11** was insoluble in water as well as in most of the protic solvents except formic acid and formamide. In three separate solubility tests, a sample of polymer PSB **11** (80 mg) was soaked in 4 cm³ of deionized water, 0.1 N NaCl, and 0.1 N HCl at 25 °C for 48 h. In each case, the polymer was recovered almost quantitatively, thus indicating its virtual insolubility in these aqueous systems. However, as anticipated for zwitterionic polymers, PSB **11** was soluble in aqueous solutions of HCl and various salts of sufficient strengths including divalent cation such as Ca²⁺, which precipitates out polyelectrolytes. The low-molecular-weight anions and cations of the added electrolyte enter and partially neutralize a portion of the intrachain interactions in PSB, thus allowing the collapsed coil in pure water to expand in the presence of small ions. For various salts, the CSC required the promotion of water solubility of PSB **11** at 23 °C, which is shown in Table 3. For a common anion, Cl⁻, the sequence of increasing solubility power (decreasing order of CSC values) was



Ca²⁺ is placed before Na⁺ because each mole of the former is associated with 2 mol of the chloride ions; the anions play a more dominant role in deciding the solubility behavior. The hydration shell of the cations, especially H⁺, Li⁺, and Ca²⁺, in water is generally fairly large because of their large charge/radius ratio and presumably cannot approach close enough to effectively neutralize the charge on the sulfonate group.³² For a common cation, K⁺, the CSC values are very sensitive to the nature of the anions. The sequence of increasing solubilizing power was

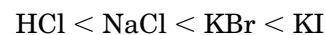


The concentration of KCl required the promotion of water solubility that was 4.7 times more than that of KI. The iodide anion is the most polarizable (soft); therefore, it is particularly effective in neutralizing ionic crosslinks, increasing the solubility of PSB. There is a considerable dif-

ference in the concentration of common electrolytes required to promote solubility of PSB **11**; amounts of the salts required were much higher than required in the cases involving polymers of quaternary ammonium sulfobetaines.^{32,33} The amine N in PSB **11** is less crowded because H instead of an alkyl group is attached to it, and as such the negatively charged SO₃⁻ is expected to have a closer approach to the positive nitrogen to enjoy greater electrostatic attraction. The increased internal neutralization of the charges would make the polymeric backbone tightly coiled and less hydrated. The corresponding polycarboxylate **4** (R¹ = H or Me; m = 1) is readily soluble in salt-free water, 0.1 N NaCl, or HCl solution.^{23,40}

Viscosity data for APE **12** (entry 16, Table 1, obtained by treating PSB **11** with 1 equiv of NaOH) are presented in Figures 6 and 7. In the absence of added salt (NaCl), the plot for APE **12** [Fig. 6(a)] is typical for a polyelectrolyte, that is, it is concave and upward in the concentrated solution. However, at a higher dilution the reduced viscosity tends to fall off, owing to the formation of the zwitterionic species (as in PSB **11**) by partial protonation of the basic amine nitrogen in APE; the relative proportion of the zwitterions as well as the compactness of the polymer chains are expected to increase with dilution. As expected, the viscosity values for APE decrease in the presence of added salt NaCl [Fig. 6(b)].

The reduced viscosities of PSB **11** in aqueous solution containing various added salts were examined and are illustrated in Figure 7. An increase in the intrinsic viscosity of PSB **11** with increasing NaCl concentration (0.7, 1.0, and 1.5 N) is a demonstration of the antipolyelectrolyte behavior of PSB **11**. The sequence of increasing intrinsic viscosity is



The salt effects on the viscosity and CSC values (discussed previously) are very similar.

To gain further insight on the conformational transitions, we determined the basicity constants, *K*^o, and the corresponding *n* values relative to the protonation of the tertiary amine of both the monomer precursor **9** and APE **12** in salt-free water and 0.1 N NaCl solution. Basicity constants and the corresponding *n* values as well as the experimental details of the potentiometric titrations are given in Table 4. The basicity constants

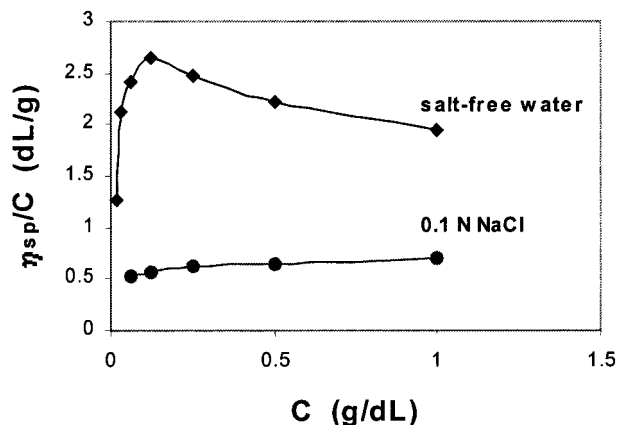


Figure 6. Viscosity behavior of APE **12** (obtained by the basification of PSB **11** from entry 16, Table 1) in (a) salt-free water and (b) 0.1 N NaCl at 30 °C with an Ubbelohde viscometer.

of this polymer are apparent^{44,45,48–50} because the n values were greater than 1, and as such they decreased with the degree of protonation (α) (Fig. 1). The magnitude of n reflects the polyelectrolyte effect and can be regarded as an index of accessibility of the proton to the amine nitrogen during the protonation reaction. The basicity constants for the corresponding monomer **9**, as expected, were “real” with an n value of 1. The basicity constant ($\log K^0$) of the amine group in APE **12** is higher by almost a unit in salt-free water as compared with 0.1 N NaCl (Fig. 1 and Table 4). The n values in salt-free water and 0.1 N NaCl were 1.81 and 1.51, respectively (Table 4). The n values greater than 1 indicated that the approach of the incoming protons to the amine nitrogen groups became more and more difficult as α for the whole macromolecule increased. The basicity constant ($\log K$) decreased progressively because of a decrease in the electrostatic field force as a result of a decreasing overall negative charge density in the macromolecule (Fig. 1).

Several studies⁴⁸ have indicated that the entropy effects involving the release of water molecules from the hydration shell of the repeating unit that is being protonated dictate the protonation of polymers. APE **12** in salt-free water is highly extended as indicated by higher viscosity values (Fig. 6) and are more hydrated (more water molecules in each hydration shell) than in salt solution (0.1 N NaCl) where the polymer chain adapts a compact conformation because of screening of the negative charges by the sodium ions. Therefore, with progressive protonation the num-

ber of water molecules released from the hydration shell of the repeating unit being protonated is greater in salt-free water than in 0.1 N NaCl. This is reflected by the higher n value (1.81), greater basicity constant ($\log K$: 10.82), and greater changes in the basicity constant in salt-free water [Fig. 1(a)] than in the 0.1 N NaCl where the basicity constant and the n values are 9.88 and 1.51, respectively. The $\log K$ values for the corresponding monomer precursor **9** were much less—8.51 (in salt-free water) and 8.33 (0.1 N NaCl).

A viscometric titration of a 0.0228 M (0.5 g/dL) solution of PSB **11** in the presence of 1 equiv of NaOH at 25 °C is depicted in Figure 2. This figure also includes the distribution curves of the different ionized species ZH^+ (PSB **11**) and Z^- (APE **12**) calculated from the basicity constants (discussed previously) and pH values. Reduced viscosity decreases continuously as the concentration of the zwitterionic species, ZH^+ , increases and that of the anionic species, Z^- , decreases. The preceding observations are supported by the viscometric studies (discussed previously).

The study of the phase diagram was an important step in this work. The phase diagram and the tie lines (A–E) for a system composed of PEG-35000 and PSB **11** (+0.60 equiv NaOH) in 0.1 N KCl are constructed by the turbidity method and ¹H NMR technique,^{4,38} respectively. The binodal curve (Fig. 3) distinguishes between single- and two-phase regions and provides information about the concentration of both polymers required

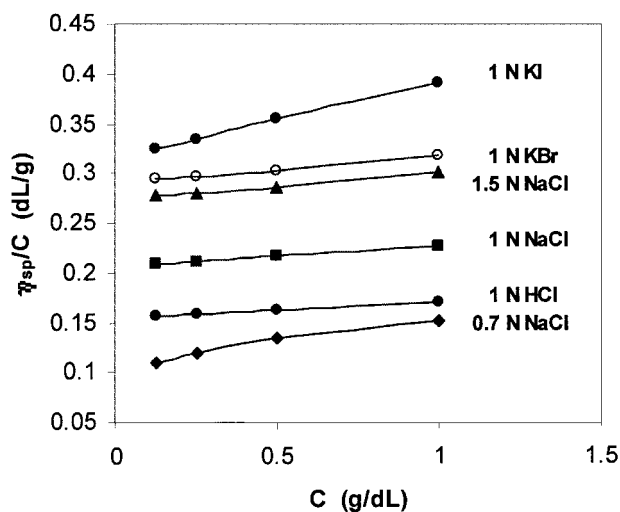


Figure 7. Effect of added salt on the viscosity behavior of PSB **11** (from entry 16, Table 1) at 30 °C with an Ubbelohde viscometer.

for protein separation work. The phase separation took place at relatively low total polymer concentrations, typically below 10%; phase separation in a low concentration could be useful from an industrial point of view. PEG and PSB displayed preference to remain in the top and bottom phases, respectively. The tie lines were helpful in constructing two phases with suitable volume ratios of the top and bottom phases. For instance, the total system represented by point A will have the volume or mass ratio of the top and bottom phases determined by the ratio of length $A - A_{\text{bot}}$ and $A - A_{\text{top}}$, respectively. Aqueous two-phase polymer systems as constructed previously may offer a friendly environment for the separation^{42,51–53} of labile proteins. Purified dextran, used in the most common dextran-PEG system for protein separation, is quite expensive and biodegradable.⁵⁴ The pH-dependent solubility behavior of the new polymer described in this work may allow its effective removal and recycling.

CONCLUSIONS

A very convenient synthetic route for obtaining the new ionic polymers **11** and **12** with inexpensive starting materials has been achieved. This work represents the first example of a cyclopolymerization involving amine salt containing sulfo-betaine moiety. The interesting pH-responsive solution properties associated with this structural feature enabled us to use PSB, for the first time, in a two-phase aqueous polymer system. Conformational behavior of the polymers is strongly influenced by the nature and the net charge on the chain. The protein's affinity toward a particular polymer phase in a two-phase system is influenced by the hydrophilicity and hydrodynamic volume of the polymer. As indicated by the viscosity and basicity constant data, the hydrophilicity and hydrodynamic volume of polymer APE can be controlled by the degree of protonation of the pH-responsive amine functionality. This new ionic polymer may have applications in protein purification; its almost-zero solubility in water in the absence of added salt below pH ~ 7 will permit its effective removal from solution by precipitation, thus leaving the protein in the solution. Additional studies on the hydrophobic modification of this polymer as well as its potential in protein separation are underway in our laboratory.

The facilities provided by the King Fahd University of Petroleum and Minerals (Dhahran) are gratefully ac-

knowledge. The authors thank M. Arab for recording the NMR measurements.

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