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Synthesis and solution properties of a new sulfobetaine/sulfur dioxide copolymer and its use in aqueous two-phase polymer systems

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Abstract

The zwitterionic monomer, 3-(*N*,*N*-diallylammonio)propanesulfonate, was copolymerized with sulfur dioxide in dimethyl sulfoxide using azo-bis-isobutyronitrile (AIBN) to afford the polysulfobetaine (PSB) copolymer in excellent yields. The PSB was converted into the corresponding anionic polyelectrolyte (APE) by treatment with 1 equiv. of sodium hydroxide. The solution properties of PSB and APE were investigated by potentiometric and viscometric techniques. The critical (minimum) salt concentrations (CSC) required to promote water solubility for the PSB at 23 °C have been measured. Basicity constant of the amine functionality in APE is found to be 'apparent' and as such follow the modified Henderson–Hasselbalch equation. The composition and phase diagram of the aqueous two-phase polymer systems of PSB (treated with 0.60 equiv. sodium hydroxide) and poly(ethylene glycol) (PEG) in 0.1N KCl has been studied for the first time for this class of PSB copolymer.

Keywords: Polysulfobetaine; Ionic polymer; Phase diagram

1. Introduction

The discovery of the cyclopolymerization of *N*,*N*-diallyl quaternary ammonium salts **1a** to **2a** by Butler [1], and subsequently, their copolymerizations with sulfur dioxide [2–4] led to the synthesis of an array of water-soluble cationic polyelectrolytes [5–9] of tremendous scientific and technological interest (Scheme 1). In recent times, the zwitterionic monomers **1b**,**c** have provided entries [10–17] into the polycarbo- and sulfobetaines **2b**,**c**. Polyzwitterions, unlike polyelectrolytes, can exhibit 'anti-polyelectrolyte behavior' [11,18–22] i.e. enhancement in viscosity and solubility in the presence of added electrolytes (e.g. NaCl) due to the neutralization of the ionically cross-linked network in a collapsed coil conformation of the polyzwitterions.

Very recently, the cyclopolymerization process has entered a new stage that deals with synthesis of pH-responsive ionic polymers. Quaternary ammonium salts-derived cyclopolymers **2** [13,15–17,23] having carboxy group in the pendant can demonstrate pH responsive solution behavior due to the incorporation of the pH-triggerable carboxybetaine functionality. The use of amine salt monomers **1b**,**c** ($R^1 = H$), however, permitted us to synthesize polymers **3**–**5** having two pH-responsive groups (a trivalent nitrogen as well as a carboxylate or sulfonate) [24–27]. The anionic polyelectrolyte (APE) **4** ($m = 1$) is currently being investigated for applications in protein purification in aqueous two-phase polymer systems,[1] and the results are indeed very promising. One of the most gratifying aspect of the polymer **4** ($m = 1$) is its almost zero solubility [24] in water below pH ~ 7 in the presence or absence of salt thus, making it a suitable candidate for aqueous two-phase polymer systems [28] since it will permit its effective removal from solution by precipitation. The incorporation of SO_2 drastically changes various aspects of solution chemistry of the copolymer **4** ($m = 1$) [24] when compared to the corresponding homopolymer **3** [26]; for instance while the former is insoluble below pH 7, the latter is soluble in the entire pH range 0–14. We anticipate interesting differences in the solution properties of the homopolymer **5** [27] and the corresponding copolymer containing SO_2 moiety. To our knowledge, the

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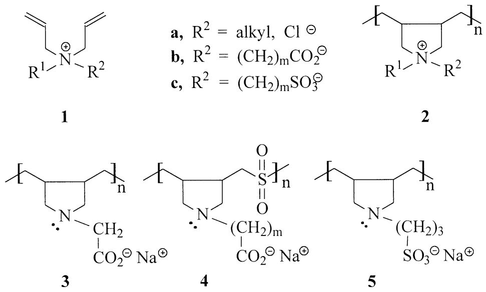


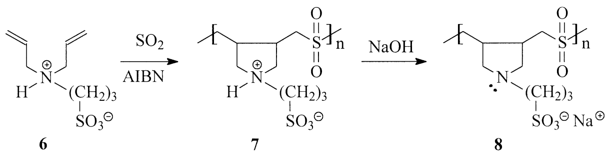
Scheme 1.

copolymer from amine salt of the type **1c** ($R^1 = H$) containing sulfobetaine moiety has not been reported before. In our continuing study of the zwitterionic polymers, we now report the synthesis, solution properties and aqueous two-phase polymer systems of the zwitterionic monomer **6**/sulfur dioxide copolymer **7** (Scheme 2). The work will indeed provide an opportunity to study the effect of SO_2 spacer in the backbone of the new copolymer **7** on the critical solution concentration (CSC) required to promote water solubility, basicity constant (and its nature: 'real' or 'apparent') of the nitrogen, pH-responsive viscosity behavior, and phase diagram and phase composition in aqueous two-phase polymer systems.

2. Experimental

2.1. Physical methods

Melting points are recorded in a calibrated Electrothermal-IA9100- Digital Melting Point Apparatus using heating rates of 1 °C/min in the vicinity of the melting points. Elemental analyses were carried out in a Carlo-Erba elemental analyzer Model 1102. IR spectra were recorded on a Perkin–Elmer 16F PC FTIR spectrometer (spectral resolution, 4 cm^{-1}; number of scans, 19). ^1H and ^{13}C NMR spectra of the polymers were measured in D_2O using dioxane as internal standard on a JEOL LA 500 MHz spectrometer. Viscosity measurements were made by an Ubbelohde viscometer (having viscometer constant of 0.005718 cSt/s at all temperatures) using CO_2-free water under N_2 in order to avoid CO_2 absorption that may affect the viscosity data. A gentle stream of N_2 was passed through distilled deionized water at 90 °C for 15 min in order to remove dissolved gases. This water was used in the potentio- and viscometric titrations in salt-free and salt (NaCl, 99.9% purity) solutions and for viscosity measurements.



Scheme 2.

2.2. Materials

AIBN from Fluka Chemie AG (Buchs, Switzerland) was purified by crystallization from a chloroform–ethanol mixture. Poly(ethylene glycol) PEG of molecular weight of 35,000 was purchased from Merck-Schuchardt. Dimethylsulfoxide (DMSO) was dried over calcium hydride overnight and then distilled under reduced pressure at a bp of 64–65 °C (4 mmHg). All glassware were cleaned using deionized water. 3-(*N*,*N*-diallylammonio)propanesulfonate (**6**) was prepared as described in a previous report [27].

2.3. General procedure for the copolymerization of the monomer **6** with SO_2

All the polymerizations were carried out using conditions as described in Table 1. The experiment under entry no. 8 was repeated in a larger scale as described below. In a typical experiment, SO_2 (1.922 g, 30 mmol) was absorbed in a solution of the monomer **6** (6.58 g, 30 mmol) in DMSO (9.87 g). The required amount of the initiator (AIBN) (96 mg) was then added under N_2 and the closed flask was stirred using magnetic stir-bar at 60 °C for 24 h. Within hours, the magnetic bar stopped stirring and initial reaction mixture (slurry) became a solid mass of white polymer. At the end of the elapsed time, the hard polymeric mass was crushed to powder, soaked in methanol, filtered and washed with liberal excess of hot (50 °C) methanol to ensure the complete removal of the unreacted monomer (as indicated by ^1H NMR). The copolymer was then dried to a constant weight (7.46 g, 87.8%) at 60 °C under vacuum. The white polymer, polysulfobetaine (PSB) **7**, was stored in a desiccator. The ^1H and ^{13}C NMR spectra are displayed in Figs. 1 and 2, respectively; ν_{max} (KBr) 3454, 3028, 2974, 2724, 1638, 1464, 1420, 1306, 1208 (broad), 1125, 1038, 852, 788, and 734 cm^{-1}. The onset of thermal decomposition (closed capillary): the color changed to brown at 273 °C and brownish black at 290 °C; (found: C, 38.46; H, 6.65; N, 4.89; S, 22.12. $C_9H_{17}NO_5S_2$ requires C, 38.15; H, 6.05; N, 4.94; S, 22.63%).

2.4. Conversion of the polysulfobetaine **7** into anionic polyelectrolyte **8**

To a mixture of the PSB **7** (from entry 8, Table 1) (1.00 g, 3.53 mmol) in water (2.5 cm^3) was added NaOH (148 mg, 3.7 mmol) in 3 portions with stirring over a period of 15 min at 20 °C. To the resulting solution was added another 60 mg (1.5 mmol) of NaOH, and the resulting APE **8** was immediately precipitated into acetone (25 cm^3). The polymer was redissolved in water (~1 cm^3), and reprecipitated in acetone (25 cm^3).

Table 1
Effect of concentration of monomer and initiator on polymerization of the monomer **6**/SO_2 copolymerization (polymerization reactions were carried out in DMSO using 10 mmol each of the monomer and SO_2)

Entry number	DMSO (g)	Initiator[a] (mg)	Temperature (°C)	Time (h)	Yield (%)	Intrinsic viscosity[b] (dl/g^{-1})
1	2.2	6.0	55	36	18.4	0.606
2	2.2	15	55	36	49.1	0.791
3	2.2	24	55	36	72.8	0.886
4	2.2	32	55	36	78.4	0.795
5	3.5	32	55	36	87.6	0.936
6	5.7	32	55	36	85.9	0.993
7	2.2	32	60	24	81.5	1.02
8	3.5	32	60	28	87.8	1.09

[a] Azobisisobutyronitrile.

[b] Viscosity of 1–0.0625% polymer solution in the presence of 1 equiv. of NaOH in 0.1N NaCl at 30 °C was measured with a Ubbelohde Viscometer ($K = 0.005718$).

The polymer was soaked in methanol for 12 h, and the white polymer was then dried at 60 °C under vacuum to a constant weight (1.01 g, 88.5%). The onset of thermal decomposition (closed capillary): the color changed to brown at 228 °C, evolution of gas started at 235 °C, and finally the color turned to dark brown at 270 °C; the ^1H and ^{13}C NMR spectra are shown in Figs. 1 and 2, respectively; ν_{max} (KBr) 3456, 2932, 2816, 1418, 1300, 1200, 1124, 1048, 796 and 738 cm^{-1}; (found: C, 33.11; H, 5.82; N, 4.25; S, 19.4. $C_9H_{16}NNaO_5S_2.H_2O$ requires C, 33.43; H, 5.61; N, 4.33; S, 19.83%).

2.5. Solubility measurements and cloud point titration in aqueous salt solutions

Solubility of the PSB **7** (from entry 8, Table 1) and APE **8** (obtained via basification of the PSB **7** from the entry 8, Table 1) 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) required to promote water solubility at 23 °C was measured by titration of a 1% w/w polymer solution at sufficiently high salt concentration with deionized water. The accuracy

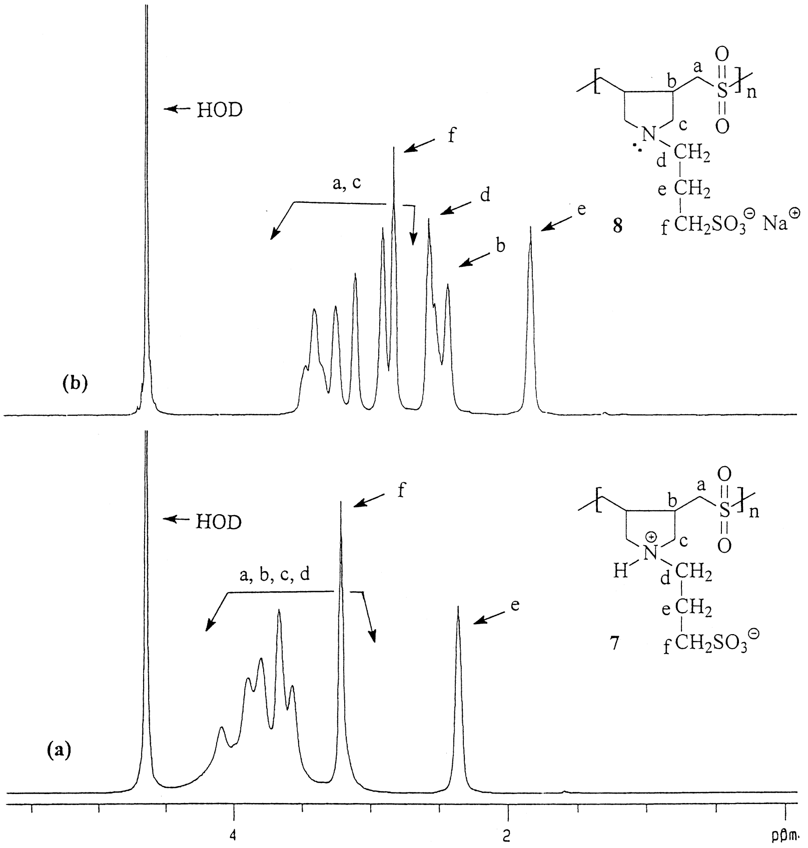


Fig. 1. ^1H NMR spectrum of (a) PSB **7** (in the presence of NaCl) and (b) APE **8** in D_2O.

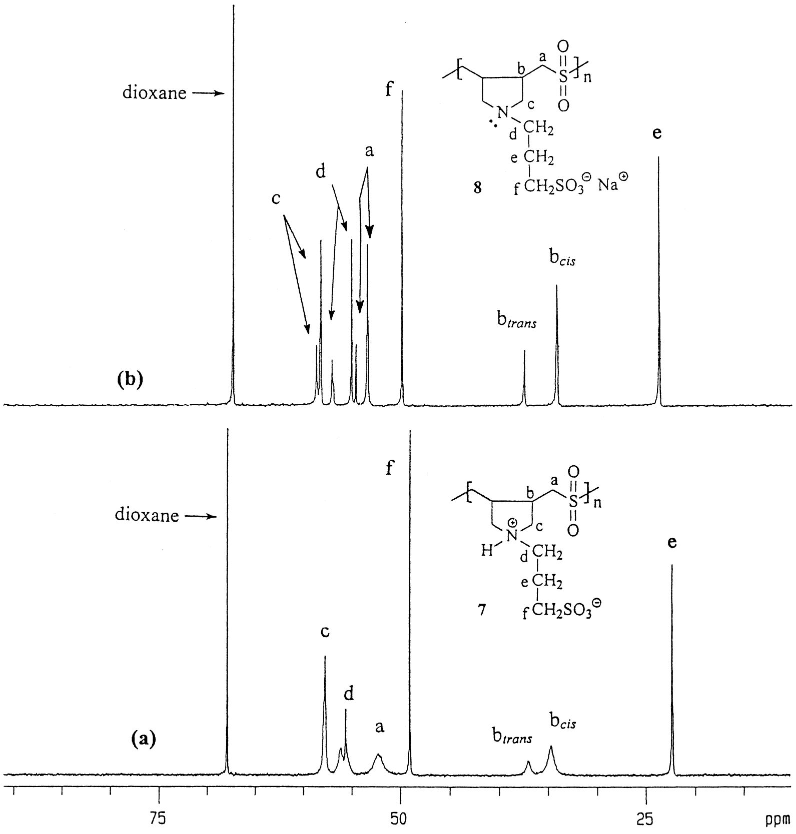


Fig. 2. ^1H decoupled ^{13}C NMR spectrum of (a) PSB **7** (in the presence of NaCl) and (b) APE **8** in D_2O.

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

2.6. Potentiometric titrations

The potentiometric titrations were carried out at 25 °C as described elsewhere [24]. The results of the potentiometric titrations are summarized in Table 4. For each titration, 200 cm^3 of salt-free or 0.1N NaCl solution containing a weighed amount of the polymer was used [(usually around 0.40–0.64 mmol of PSB **7** (3 runs) or APE **8** (one run)]. Four titrations in each solution were performed. In the case of PSB **7**, the polymer was first converted to the APE **8** by dissolving it in 1 equiv. of 0.1046N NaOH, and then diluting it to the required volume. The solution was titrated at 25 °C with 0.1015 M HCl under N_2, and after each addition of the titrant (0.25 cm^3) the pH of the solution was recorded using a Corning pH meter 220. The number of data points from titration curves was in the range 13–25. In our earlier work [27], sodium 3-(*N*,*N*-diallylamino)propanesulfonate, the anionic form of the monomer **6**, was titrated with HCl in salt-free and 0.1N NaCl and the results are included in Table 1 for the purpose of comparison.

Table 2
Solubility of polysulfobetaine **7** and corresponding anionic polyelectrolyte **8**

	ε	PSB **7**	APE **8**
Water	78.4	−	+
Methanol	32.3	−	−
Formic acid	58.5	±	±
Formamide	111.0	+	+
Ethylene glycol	37.3	−	+
Triethylene glycol	23.7	−	+
Acetic acid	6.15	−	−

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. ‘ + ’ indicates soluble; ‘ − ’ indicates insoluble; ‘ ± ’ indicates partially soluble.

The protonation constant of the amine nitrogen, log *K*, is calculated at each pH value by the well-known Henderson-Hasselbalch Eq. (1) where degree of

Table 3
Critical salt concentration (CSC) for aqueous solutions of PSB **7** at 23 °C

Salt	CSC (M)
LiCl	2.04
NaCl	1.49
KCl	1.56
KBr	0.691
KI	0.201
NH_4Cl	1.56
$CaCl_2$	1.15
HCl	1.46

Table 4
Potentiometric results of protonation of polymer **8** and its corresponding monomer at 25 °C

Polymer/monomer	Solvent	$\log K_1^{0\mathrm{a,b,c}}$	n^{b}
APE **8**	Salt-free water	8.51 (5)	1.50 (4)
APE **8**	0.1N NaCl	7.54 (3)	1.20 (3)
Monomer[c]	Salt-free water	8.51 (6)	1.01 (3)
Monomer[c]	0.1N NaCl	8.33 (3)	1.01 (3)

Sodium 3-(*N*,*N*-diallylamino)propanesulfonate [27], the anionic form of the monomer **6**.

[a] For the reaction: $Z^- + H^+ \rightleftharpoons ZH^{\pm}$.

[b] Values in the parentheses are standard deviations in the last digit.

[c] $\log K_1 = \log K_1^0 + (n-1)\log[(1-\alpha)/\alpha]$.

protonation (α) is the ratio $[ZH^{\pm}]_{eq}/[Z]_0$. The $[Z]_0$ is the initial analytical concentration of the monomeric units in the APE **8** (or PSB **7**), and $[ZH^{\pm}]_{eq}$ is the concentration of the protonated species at the equilibrium given by $[ZH^{\pm}]_{eq} = C_H^+ - [H^+] + [OH^-]$, where C_H^+ is the concentration of the added HCl; $[H^+]$ and $[OH^-]$ at equilibrium was calculated from the pH value [29]. The typical electrolytes having apparent basicity constants could be described by Eq. (2) where $\log K^0 =$ pH at $\alpha = 0.5$ and $n = 1$ in the case of sharp basicity constants. The linear regression fit of pH vs. $\log[(1-\alpha)/\alpha)]$ gave $\log K^0$ and n as the intercept and slope, respectively. The correlation coefficients (R^2) were found to be in the range 0.9840–0.9963. Simultaneous protonation of the two basic sites is least likely since the basicity constant for the SO_3^- group is less than that of the amine group by at least 8 orders of magnitude. Inserting the value of pH from Eq. (2) into Eq. (1) leads to the modified Henderson–Hasselbalch equation (Eq. (3)) [30,31] 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).

$$\underset{\mathbf{8}}{Z^-} + H^+ \overset{K_i}{\rightleftharpoons} \underset{\mathbf{7}}{ZH^{\pm}}$$

$$\log K_1 = \mathrm{pH} - \log[(1-\alpha)/\alpha] \tag{1}$$

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

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

2.7. Viscometric titration

The viscometric and pH measurements were performed using earlier procedure [26] at 25 °C in the presence of various amounts of HCl, but the concentration of the polymer (0.01793 M, i.e. 0.5 g/dl, PSB **7** treated with 1 equiv. of NaOH) was kept constant throughout (Fig. 5). The percentage of different ionic species in the solutions was calculated using Eq. (2).

2.8. Phase compositions and phase diagram

2.8.1. The NMR method

Several systems of PEG-35,000 and PSB **7** (treated with 0.60 equiv. of NaOH) in 0.1N KCl of known compositions as described by the points *A–D* in Fig. 6 were made. The phase separation happened within minutes after thorough shaking, and the mixture was kept at 23 °C for 24 h. The weight percent of the polymers in each phase was determined using ^{1}H NMR technique as described elsewhere [4,27]. The top and bottom layers were found to be rich in PEG and PSB, respectively. Basification (K_2CO_3) followed by ^{1}H NMR measurements allowed us to determine the approximate mole ratios of the polymers by careful integration of the two-proton signal for the APE **8** at δ 1.77 ppm and the four-proton PEG signal at δ 3.58 ppm. The tie lines *A–D* were constructed using the wt% of the polymers in the top, bottom and total systems.

2.8.2. The turbidity method

The binodal curve was constructed by using procedure as described elsewhere [24]. About 2.0 g of a concentrated solution (25% w/w) of the PEG-35,000 in 0.1N KCl was titrated at 23 °C with a concentrated solution (20% w/w in 0.1N KCl) of the PSB **7** (treated with 0.60 equiv. NaOH). Points obtained by the turbidity method are joined together in Fig. 6.

3. Results and discussion

3.1. Synthesis of the copolymers

Cyclopolymerization of the monomer **6**-SO_2 in DMSO using AIBN as the initiator went smoothly to give the copolymer PSB **7** in excellent yields (Scheme 2). The PSB **7**, on treatment with sodium hydroxide, afforded the APE **8**. The results of the polymerization under various conditions, and the intrinsic viscosities of the resultant polymers are given in Table 1. As is evident from the Table, keeping the monomer concentration and temperature constant, both the viscosity and yield increases with increasing amount of the initiator (entries 1–3). On further increase in the amount of the initiator, while the yield increases, the viscosity of the resultant polymer decreases (entry 4). There are ample evidence in the literature [4,23–25,32] that in cyclopolymerization and cyclocopolymerization reactions of diallyl quaternary ammonium salts, the viscosity increases with increasing catalyst ratio up to a certain point, then declines. High monomer and high initiator concentration are required in order to obtain high yields and viscosity values. This is attributed to degradative chain transfer reactions characteristic for allylic monomers [33]. Increasing the amount of the solvent (DMSO) is found to give polymers with higher yield and viscosity values (entries 4–6). Among all the polymerization reactions, the highest viscosity value is obtained under entry 8, and this sample and its corresponding APE **8** were used for the study of the solution properties (vide infra).

3.2. Infrared and NMR spectra

The IR spectrum of **7** and **8** indicates the presence of the sulfonate group by its characteristic bands at ~1200 and ~1040 cm^{-1}. The two strong bands at ~1300 and ~1120 cm^{-1} were assigned to the asymmetric and symmetric vibrations of SO_2 unit. ^1H and ^{13}C NMR spectra of the polymers **7** and **8** are shown in Figs. 1 and 2, respectively. The absence of any residual alkene proton or carbon signal in the spectra suggested the degradative chain transfer process [34] for the termination reaction. The proton signals of PSB **7** are, as expected, shifted downfield in compare to APE **8** due to the presence of positively charged nitrogen in the former. The assignments of the ^{13}C peaks are based on earlier works [15,33,35,36]; the spectral data (specially the number of signals) point to the formation of the pyrrolidine rings rather than the unsymmetrical six-member ring structure. Integration of the relevant peaks in the ^{13}C spectrum yields the *cis*/*trans* ratio of the ring substituents to be 72/28, which is similar to that observed for the polymers derived from quaternary ammonium salts [15,16].

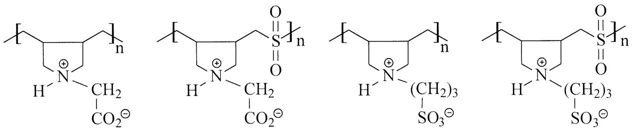
3.3. Solubility and critical salt concentrations (CSC)

The solubility behavior of the polymers is shown in Table 2. While the APE **8** was found to be very soluble in most of the protic solvents except methanol and acetic acid, the polysulfobetaine **7**, like the overwhelming majority of reported polybetaines [11,18,19], was found to be 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 the polymer PSB **7** (100 mg) was soaked in 5 cm^3 of deionized water, 0.1N NaCl and 0.1N HCl at 25 °C for 48 h; at the end 96, 98 and 98.5 mg, respectively, of the polymer were recovered thus, indicating virtual insolubility in these aqueous systems.

However, as anticipated for zwitterionic polymers, PSB **7** was found to be soluble in concentrated aqueous solution of HCl and various salts including divalent cation such as Ca^{2+}, which is known to precipitate out polyelectrolytes. The low molecular weight anions and cations of the added electrolyte enter and partially neutralize a portion of the intra-chain 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 to promote water solubility of PSB **7** at 23 °C are shown in Table 3. For a common anion, Cl^-, the sequence of increasing solubility power (decreasing order of CSC values) was found to be: $Li^+ < Ca^{2+} < K^+ = NH_4^+ \approx Na^+ \approx H^+$, which is not quite the same sequence $Li^+ < H^+ < NH_4^+ < Ca^{2+} < Na^+ \approx K^+$ observed for the homopolymer PSB **11** [27] (Scheme 3). The copolymer **7** requires almost twice the amount of salts required to promote solubility of the homopolymer. However, it is to be noted that the copolymer is of higher molecular weight since the APE **8** has almost twice the intrinsic viscosity of the corresponding homopolymer **5**. The Ca^{2+} is placed before Na^+ because each mole of the former is associated with 2 mol of the chloride ions; the anions are known to play a more dominant role in deciding the solubility behavior [18]. For a common cation, K^+, the CSC values are very sensitive to the nature of the anions. The sequence of increasing solubilizing power is found to be:

$$Cl^- < Br^- \ll I^-$$

The concentration of KCl required to promote water solubility is found to be 7.8 times more than that of KI. The iodide anion is the most polarizable (soft), hence it is particularly effective in neutralizing ionic cross-links and increasing the solubility of the PSB. There is a considerable difference in the concentration of common electrolytes required to promote solubility of PSB **7**; amounts of the salts required were found to be much higher than required in the cases involving polymers of quaternary ammonium sulfobetaines [18,19]. The nitrogen in PSB **7** is less crowded since H instead of an alkyl group is attached to it, and as such the negatively charged SO_3^- is expected to have closer approach to the positive nitrogen for effective internal neutralization of the charges, thus making the polymeric backbone tightly coiled and less hydrated.



Scheme 3.

3.4. Viscosity measurements

Viscosity data for APE **8** (entry 8, Table 1, obtained by treating the PSB **7** with 1 equiv. of NaOH) is shown in Figs. 3 and 4. In the absence of added salt (NaCl), the plot for APE **8** is typical for polyelectrolyte i.e. concave upward. However, at higher dilution, the reduced viscosity tends to fall off owing to the formation of the zwitterionic species (as in PSB **7**) 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 of any polyelectrolyte, the viscosity values for the APE decrease in the presence of added salt NaCl (Fig. 3).

The reduced viscosities of the PSB **7** in aqueous solution containing various added salts were studied and are shown in Fig. 4. An increase in the intrinsic viscosity of the PSB **7** with increasing NaCl concentration (1.5 and 2N) is a demonstration of the 'anti-polyelectrolyte behavior' of the

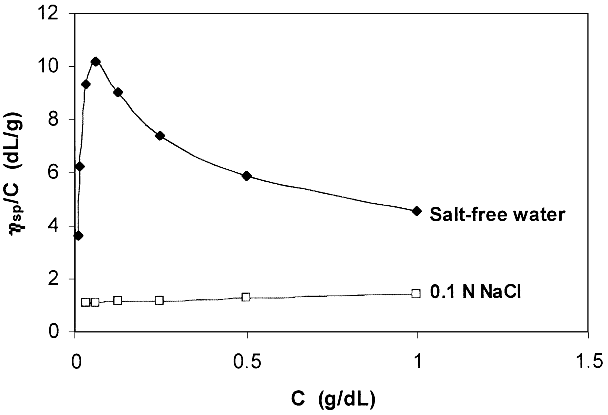


Fig. 3. The viscosity behavior of the APE **8** (obtained via basification of the PSB **7** from entry 8, Table 1) in salt-free water and 0.1N NaCl at 30 °C using an Ubbelohde Viscometer.

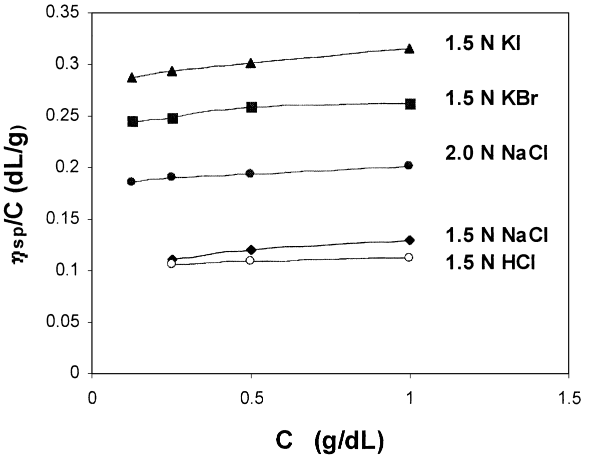


Fig. 4. Effect of added salts on the viscosity behavior of the PSB **7** (from entry 8, Table 1) at 30 °C using an Ubbelohde Viscometer.

PSB **7**. The sequence of increasing intrinsic viscosity in 1.5N solutions is found to be:

$$\mathrm{HCl} \approx \mathrm{NaCl} < \mathrm{KBr} < \mathrm{KI}$$

It is noteworthy that the salt effects on the viscosity and CSC values (vide supra) are very similar.

3.5. Basicity constants

In order to gain further information on the conformational transitions, we have determined the basicity constant, K_1^0, and corresponding n values relative to the protonation of the tertiary amine of APE **8** in salt-free water and 0.1N NaCl solution, and are reported in Table 4. The second step protonation constant ($\log K_2$) involving the protonation of SO_3^- is 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 since the APE **8** starts to precipitate in 0.1N NaCl and salt-free water after the addition of around 0.79 and 1.04 equiv. of HCl, respectively.

While the basicity constants in this polymer **8** are found to be apparent ($n > 1$) [30,31,37,38], the basicity constants for the anionic form of the corresponding monomer **6**, as expected, are found [27] to be real ($n = 1$). The $\log K_1$ decreases with the increase in the degree of protonation (α) implying that the approach of the incoming protons to the amine nitrogen groups becomes more and more difficult owing to a decrease in the electrostatic field force as a result of decreasing overall negative charge density in the macromolecule. It is to be noted that the presence of SO_2 spacer decreases the 'apparentness' of the basicity constant of the copolymer **8** as indicated by the decreased 'n' values of 1.50 and 1.20 in salt-free and 0.1N NaCl, respectively; the corresponding values for the homopolymer **5** were found to be 1.81 and 1.51 [27]. The SO_2 units in the polymer backbone thus shield nitrogen of the adjacent units, thereby minimizing the influence of the protonated unit on the basicity of the adjacent nitrogen.

While the $\log K^0$ values (at $\alpha = 0.5$) of the basic nitrogen in the homopolymer **5** [27], copolymer **8** and monomer in salt-free water were found to be 10.82, 8.51, 8.51, respectively, the corresponding values in 0.1N NaCl were measured to be 9.88, 7.54 and 8.33. In comparing the $\log K^0$ values between the homo- and the copolymer it is evident that the presence electron-withdrawing SO_2 unit lowers the basicity constants by 2.31 and 2.34 units in salt-free water and 0.1N NaCl, respectively. On the other hand, the polyelectrolyte effects (i.e. the higher electrostatic field force generated by the many negative charges on the macromolecule) increase the protonation constant of the homopolymer **5** by 2.31 units (in salt-free water) and 1.55 units (in 0.1N NaCl) in comparison to that of the monomer. As a result of the equal but opposing influence of the presence of polyelectrolyte and SO_2 effects, there is no difference in the K^0 values between the monomer and the copolymer in the salt-free water. However, in 0.1N NaCl, weak polyelectrolyte effects, due to a less favorable entropy of protonation (vide infra), cannot balance the effect of electron-withdrawing SO_2 unit, and as such the copolymer becomes a weaker base than the monomer by 0.79 unit.

Several studies [38,39] have indicated that the entropy effects involving release of water molecules from the hydration shell of the repeating unit that is being protonated dictate the protonation behavior of the polymers. The APE **8** in salt-free water is highly extended as indicated by the higher viscosity values (Fig. 3) and as such more hydrated (more water molecules in each hydration shell) than in 0.1N NaCl; in salt solution the polymer chain adapts a compact conformation due to screening of the negative charges by the sodium ions. With progressive protonation of APE **8**, leading to the formation of zwitterionic species **7**, both the highly extended polymer chains in salt-free water and the already compact polymer chains in NaCl become more compact (hence less hydrated) (Figs. 3–5). However, the

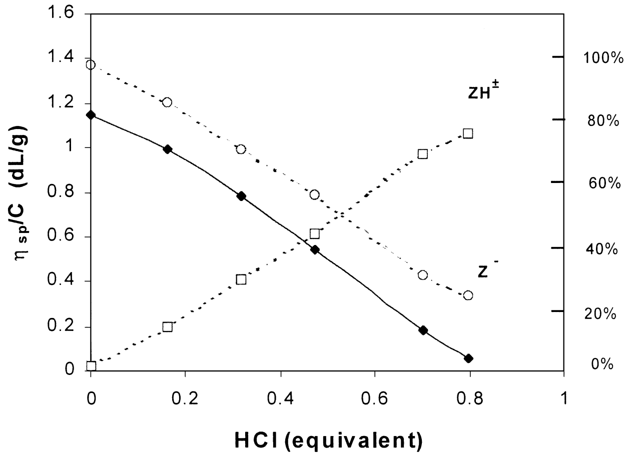


Fig. 5. Reduced viscosity (η_{sp}/C) of a 0.01793 M (i.e. 0.50 g/dl) solution of polymer PSB **7** (+1 equiv. NaOH) in 0.1N NaCl (◆) versus equivalent of added HCl at 25 °C. Distribution curves (dashed lines) of the various ionized species calculated from the basicity constants log K in 0.1N NaCl at 25 °C.

higher degree of change in the hydration shell in salt-free water leads to the higher values of basicity constant and n owing to a more favorable entropy change as a result of a greater liberation of water molecules.

3.6. Viscometric titrations

A viscometric titration of a 0.01793 M (0.5 g/dl) solution of the PSB **7** in the presence of 1 equiv. of NaOH at 25 °C is shown in Fig. 5. The figure also includes the distribution curves of the different ionized species $ZH^{\pm}$ (PSB **7**) and Z^{-} (APE **8**) calculated from the basicity constants (vide supra) and pH values. Reduced viscosity decreases continuously as the concentration of the zwitterionic species, $ZH^{\pm}$ increases and that of the anionic species, Z^{-}, decreases. The observations presented above are supported by the viscometric studies (vide supra).

3.7. Aqueous two-phase polymer systems

The study of phase diagram is an important step in this work. The phase diagram and the tie lines A–D for a system composed of PEG-35000 and PSB **7** (+0.60 equiv. NaOH) at 0.1N KCl are constructed by turbidity method and ^{1}H NMR technique [4,24], respectively. The binodal curve (Fig. 6) distinguishes between single- and two- phase regions and provides information about the concentration of both polymers required for protein separation work. The phase separation takes place at relatively low total polymer concentrations, typically well below 10%; phase separation in a low concentration could be useful from an industrial point of view. The PEG and PSB displayed preference to remain in the top and bottom phase, respectively. The tie lines are helpful in constructing two phases with suitable volume ratio of the top and bottom phases. For instance, the total system represented by the point A will have the volume or mass ratio of the top and bottom phases determined by the ratio of length A–A_{bot} and A–A_{top}, respectively. Aqueous two-phase polymer systems as constructed above may offer a friendly environment for the separation [28,40–42] of labile proteins. Purified dextran, used in the most common dextran–poly(ethylene glycol) system for protein separation, is quite expensive and biodegradable [43]. The pH dependent solubility behavior of the new polymer described in this work may allow its effective removal and recycling.

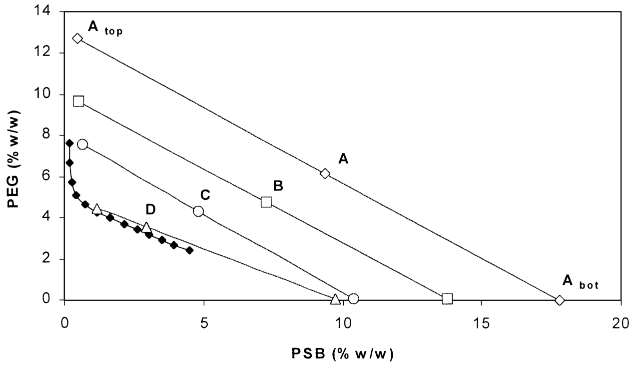


Fig. 6. Phase diagram of the system containing PSB **7** (treated with 0.60 equiv. NaOH) and PEG-35,000 in 0.1N KCl at 23 °C.

3.8. Polymer structure versus solution properties

It is interesting to note that while the homopolymer polycarbobetaine **9** (Scheme 3) is readily soluble in salt-free water, 0.1N NaCl or HCl solution [26], the corresponding copolymer **10** is virtually insoluble in the absence or presence of any amount of added salt (NaCl or KI) or acid (HCl) [24]. However, both the polysulfobetaines (homopolymer **11** or the copolymer **7**) are insoluble in salt-free water, but soluble in either the acid or salt solution (vide supra). The presence of SO_2 seems to be a requirement for the insolubility of the copolycarbobetaines. The solubility difference between the polycarbobetaines and polysulfobetaines could be attributed to the acidity difference between the carboxylic acid and sulfonic acid. While the carbobetaine copolymer **10**/polyethyleneglycol aqueous two-phase polymer system, currently tested for protein separation in our laboratory,[1] showed very promising results, the polymer system could not be used below pH 7 because of the solubility problem. The polysulfobetaines **7** and **11**, however, remain soluble in the entire pH range 0–14.

The presence of SO_2 unit in the polymer backbone of **8** has a significant effect on the log K^0 value; the protonation constant is decreased by about 2.3 orders of magnitude as compared to the homopolymer **5**. We are currently investigating the possible use of the homopolymer (PSB **5**)/copolymer (PSB **8**) in an aqueous two-phase polymer system. As a consequence of the difference in the K values, the nitrogens in the polymers (**5** or **8**) can be selectively protonated (or deprotonated) under different pH leading to the insoluble zwitterionic forms (**7** or **11**), and thus removed from the system and recycled. The more effective internal neutralization of the negative charge on SO_3^- by the combined electron withdrawing ability of the N^+ and SO_2 units, decreases the hydrophilicity i.e. solubility of the copolymer PSB **7**. As a result, twice as much salt is required for the disruption of the internal neutralizations of the charges and dissolution of the copolymer PSB **7** in compare to the corresponding homopolymer PSB **11**.

4. Conclusions

A very convenient synthetic route for obtaining the new polymers **7** and **8** using inexpensive starting materials is described. Conformational behavior of the polymers is strongly influenced by the nature and the net charge on the chain. As indicated by the viscosity and basicity constant data, the hydrophilicity and the hydrodynamic volume of the 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. Further studies on the hydrophobic modification of this polymer as well as its potential in protein separation are currently under investigation in our laboratory.

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