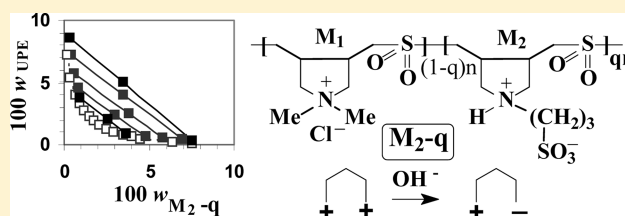


# Coexistence Curves of Aqueous Two-Phase Systems of Some pH-Responsive Homo- and Copolymers of 3-(Diallylammonio)propane-1-sulfonate and Urethanized Poly(ethenol) or Poly(oxyethylene)

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**ABSTRACT:** Poly(ethenol) (PE) was partly urethanized with urea to obtain urethanized PE (UPE) having a urethanated mole fraction of 0.13. A series of pH-responsive polysulfobetaines: homopolymer poly[3-(diallylammonio)propane-1-sulfonate], copolymer poly[3-(diallylammonio)propane-1-sulfonate-*alt*-sulfur dioxide], and terpolymer poly[3-(diallylammonio)propane-1-sulfonate-*alt*-sulfur dioxide-*ran*-diallyldimethylammonium chloride-*alt*-sulfur dioxide], was prepared *via* cyclopolymerization process involving pH-responsive zwitterionic ( $\pm$ ) 3-(diallylammonio)propane-1-sulfonate, cationic diallyldimethylammonium chloride and sulfur dioxide. The composition and phase diagram of aqueous two-phase systems (ATPSs) consisting of UPE or poly(oxyethylene) (POE) and the synthesized polysulfobetaines as polymer components were studied in some details using  $^1\text{H}$  NMR spectroscopy. The presence of trivalent nitrogen and negatively charged oxygen in each repeating units of the polymers permitted the change of the charge types in the polymer chain to zwitterionic/anionic ( $\pm -$ ), zwitterionic/cationic ( $\pm +$ ), ampholytic ( $+ -$ ), or anionic ( $-$ ). The effects of charge types and their densities, and ionic strength on the phase diagrams were investigated. The phase separation happened at a relatively low total polymer mass fraction of below 0.10. The solubility dependence of the polyzwitterions on variable pH make them suitable candidates for potential industrial applications in bioseparations since at lower pH, the polymer in the zwitterionic form can be precipitated out and recycled.



## 1. INTRODUCTION

The high water content along with low interfacial tension in aqueous two-phase systems (ATPSs) comprising water-soluble polymers as main components, provides an environment-friendly compatible avenue for efficient purification as well as separation of a variety of industrially important labile biomolecules including proteins.<sup>1–6</sup> The inexpensive and efficient liquid–liquid extraction technique provided by the ATPSs is also becoming increasingly important in non-biotechnological areas such as remediation of industrial waste.<sup>7–9</sup> The ATPSs can be successfully applied for the removal of color from textile plant wastes,<sup>7</sup> toxic metal ions,<sup>8</sup> and organic pollutants from the environment.<sup>9</sup> The most commonly used ATPS, based on poly(oxyethylene) (POE) and dextran, have some limitations due to the dextran being quite expensive and biodegradable.<sup>10–13</sup> Therefore, there has been an increased emphasis in recent years on the development of environment-friendly ionic liquid-based novel ATPSs.<sup>14,15</sup> The POE-based ATPSs, having hydrophobically modified acrylamide/styrene copolymer<sup>16</sup> or poly(diallylammonioethanoate-*alt*-sulfur dioxide)<sup>17</sup> as the other polymer component, have been successfully employed in the study of protein partitioning. The use of ionic polymers has also been reported in the construction of coexistence curves for a variety of ATPSs using hydrophobically modified pH-responsive poly(diallylammonioethanoate-*alt*-sulfur dioxide) and urethanized poly(ethenol) (UPE) 2 (Scheme 1).<sup>18</sup> UPE 2, an inexpensive

and simple modification of poly(ethenol) (PE) 1, is reported to be readily soluble in water and more stable than PE.<sup>19</sup>

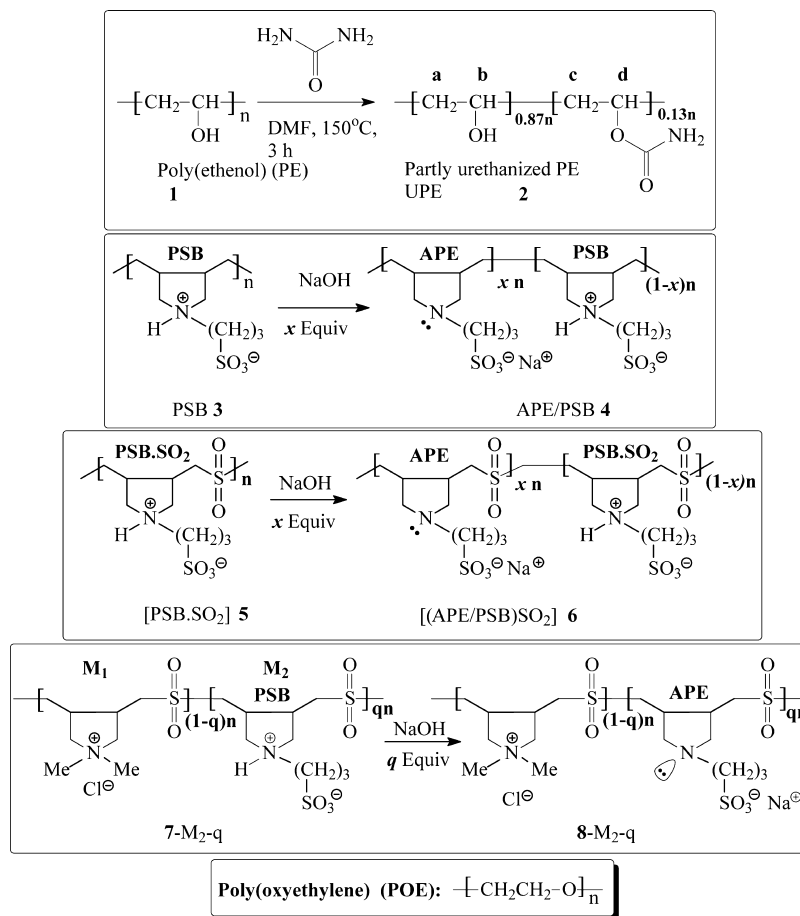
Even though the mechanism of protein partitioning between the phases is a unpredictable complex phenomenon, surface properties of the proteins and hydrophobicity are the main factors that dictate the partitioning.<sup>20</sup> Moreover the distribution of molecules between the two phases depends on hydrogen bond, charge interaction, van der Waals' forces, steric effects, and the molecular weight and the chemical properties of the molecules. As a substitute for filtration and chromatography protocols, the ATPS has been used for retroviral vectors purification as exemplified by fractionation as a function of cell surface properties. Thus microcytic and macrocytic red blood cells from anemic patients have been separated by the countercurrent distribution technique using dextran/poly(ethylene glycol) ATPSs.<sup>21</sup> In the formation of the ATPS, separation into two phases is speeded up by adding iron oxide particles to the mixed system and placing it in a magnetic field.<sup>22</sup> The phase rich in magnetic additives is kept stationary in a column system while the other phase is piped through the column to separate enzymes.<sup>23</sup> Partitioning of the biomolecules in the ATPS is often enhanced in the presence of cosolutes like salts and surfactants which can exhibit specific electrostatic

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Scheme 1. Synthesis of polymers



interactions and hydrophobic attractive forces with the target biomolecule.<sup>24</sup>

Preference of an enzyme into a phase may be further increased by inserting ligands onto the polymer which can show preferential binding of the enzyme to the polymer, thus increasing the effectiveness of the extraction.<sup>25</sup> A recent report describes the use of ATPS for cell assays, for studying cell–cell interactions and tissue engineered systems.<sup>26</sup> A novel approach that combines ATPSs and two-dimensional electrophoresis (2-DE) is introduced as a tool for the isolation and purification of bioparticles from plant origin.<sup>27</sup>

The use of nonionic neutral polymers has so far dominated the literature of ATPS; the application of ionic polymers as components of ATPS is somewhat limited. Recently we have reported the use of an ionic polymer having pH-responsive aminomethylphosphonate motifs in the construction of ATPSs.<sup>28</sup> The current article describes our efforts to develop new ATPSs using ionic polymers based on pH-responsive polysulfobetaines (PSBs) [i.e., polyzwitterions (PZs)] poly[3-(diallylammonio)propane-1-sulfonate] 3, poly[3-(diallylammonio)propane-1-sulfonate-*alt*-sulfur dioxide] 5, or poly[3-(diallylammonio)propane-1-sulfonate-*alt*-sulfur dioxide-*ran*-diallyldimethylammonium chloride-*alt*-sulfur dioxide] 7 and UPE 2 or POE (Scheme 1). The objective of this work is to construct pH-responsive recycling ATPSs whose components can be recycled after precipitating at certain pH values by their conversion into water-insoluble polyzwitterions. We examined the effects of pH, ionic strength, and charge types, and their densities in the polymer chain on the

coexistence curves of a variety of new ATPSs. In this study, water-insoluble zwitterionic ( $\pm$ ) PSB 3, ( $\pm$ ) PSB-SO<sub>2</sub> 5 (having charges of both algebraic signs in the same repeating unit) and (+  $\pm$ ) cationic polyelectrolyte (CPE)/PSB-SO<sub>2</sub> 7-M<sub>2</sub>-q were treated with an appropriate equivalent of NaOH to give water-soluble ( $-\pm$ ) anionic polyelectrolyte (APE)/PSB poly[3-(diallylammonio)propane-1-sulfonate-*ran*-sodium 3-(diallylamino)propane-1-sulfonate-*alt*-sulfur dioxide] 4, ( $-\pm$ ) [APE/PSB]·SO<sub>2</sub> poly[3-(diallylammonio)propane-1-sulfonate-*alt*-sulfur dioxide-*ran*-sodium 3-(diallylamino)propane-1-sulfonate-*alt*-sulfur dioxide] 6, and ampholytic (+  $-\pm$ ) polysodium [3-(diallylammonio)propane-1-sulfonate-*alt*-sulfur dioxide-*ran*-diallyldimethylammonium chloride-*alt*-sulfur dioxide] 8-M<sub>2</sub>-q (having cationic and anionic units in the same polymer chain with or without charge symmetry) (Scheme 1). Note that M<sub>2</sub>-q represents a polymer having a mole fraction of 'q' for the repeating unit of comonomer M<sub>2</sub> (Scheme 1). Ionic polymers 4, 6, and 8 seem to mimic biomolecules like proteins; as such the current pH-responsive ATPSs are expected to impart considerable influence in bioseparation involving monomeric amino acids or proteins.

## 2. EXPERIMENTAL SECTION

**2.1. Materials.** Purities and suppliers of materials used in this work are reported in Table 1. Poly(oxyethylene) (POE) of number average molar mass ( $\bar{M}_n$ ) of 35.0 kg·mol<sup>-1</sup> was purchased from MERCK-Schuchardt. PE 1 with a number average molar mass of 72.0 kg·mol<sup>-1</sup>, a degree of hydrolyzation of (0.975 to 0.995) mol fraction and a degree of polymerization

Table 1. Purities of Materials Used in This Work

chemical	supplier	purity <sup>a</sup>
sodium chloride	JT Baker	> 0.995
potassium chloride	Fluka	> 0.995
sodium hydroxide	Fluka	> 0.98
water		deionized water
POE <sup>b</sup>		
urea	Fluka	≥ 0.995
poly(ethanol) (PE) 1 <sup>c</sup>	Fluka	
urethanized poly(ethanol) (UPE) 2		
PSB 3 <sup>d</sup>	ref 22 <sup>c</sup>	c
PSB-SO <sub>2</sub> 5 <sup>e</sup>	ref 23 <sup>d</sup>	d
CPE/PSB-SO <sub>2</sub> 7-M <sub>2</sub> -0.5 <sup>f</sup>	ref 24 <sup>e</sup>	e
CPE/PSB-SO <sub>2</sub> 7-M <sub>2</sub> -0.75 <sup>f</sup>	ref 24 <sup>e</sup>	e

<sup>a</sup>Purities refer to the mass fraction. <sup>b</sup>Poly(oxyethylene) of molar mass 35.0 kg mol<sup>-1</sup>. <sup>c</sup>Poly(ethanol) of molar mass 72.0 kg mol<sup>-1</sup>. <sup>d</sup>Synthesized as described in ref 22 and supported by acceptable elemental analysis. <sup>e</sup>Synthesized as described in ref 23 and supported by acceptable elemental analysis. <sup>f</sup>Synthesized as described in ref 23 and supported by acceptable elemental analysis.

of 1600 was purchased from Fluka Chemie AG (Buchs, Switzerland). Partly urethanized UPE 2 was prepared by heating an equimolar mixture of PE 1 and urea in dimethylformamide (DMF) at 423 K for 3 h as described in the literature (Scheme 1).<sup>29</sup> The degree of urethanization as determined by <sup>1</sup>H NMR (*vide infra*), was found to be 0.13 mol fraction of the total repeating units. The urethanization process is known to be accompanied by some chain cleavage, and as a result the molar mass of 69.0 kg mol<sup>-1</sup> was found to be less than that of the starting PE 1.<sup>19</sup>

The synthesis of pH responsive 3,<sup>30</sup> 5,<sup>31</sup> and 7<sup>32</sup> have been reported elsewhere in the literature (Scheme 1). Some physical properties for the freshly prepared polymers are included in Table 2.

**2.2. Stock Solutions.** All polymer stock solutions having polymer mass fraction (*w*) of 0.20 were prepared in distilled deionized water. Stock solutions of UPE 2 and POE were prepared in salt-free water. A stock solution of (60:40 APE/PSB) 4 having an APE/PSB ratio of 60:40 was prepared by treating PSB 3 with 0.6 equiv of NaOH. In a similar fashion, a stock solution of (60:40 APE/PSB-SO<sub>2</sub>) 6 was prepared from PSB-SO<sub>2</sub> 5. The stock solutions of 8-M<sub>2</sub>-0.5 and 8-M<sub>2</sub>-0.75 were prepared by treating 7-M<sub>2</sub>-0.5 and 7-M<sub>2</sub>-0.75 (MMRU: 0.269 kg·mol<sup>-1</sup>) with 0.5 equiv and 0.75 equiv of NaOH, respectively. The salt concentration in the total systems (Tables

3 to 8) (*vide infra*) was fixed by the addition of an appropriate amount of solid KCl.

All mixtures were prepared by weighing with a Precisa balance accurate to within ± 10<sup>-4</sup> g. The temperature in phase equilibration was determined with an accuracy of ± 0.2 K. Polymer compositions by NMR method were made with an uncertainty of less than ± 1.5 %.

**2.3. Phase Compositions and Phase Diagram of UPE 2 or POE- [Ionic Polymer 3 or 5 or 7 + NaOH]-H<sub>2</sub>O (KCl) System.** **2.3.1. Phase Compositions by NMR.** A set of total systems (~7 cm<sup>3</sup>) of known compositions (*A*<sub>total</sub>) was prepared in calibrated cylinders using the stock solutions of UPE 2 (or POE) and NaOH-treated PSB 3 or PSB-SO<sub>2</sub> 5 or 8-M<sub>2</sub>-0.5 or 8-M<sub>2</sub>-0.75 (Tables 3 to 8).

The mixtures of the total systems were shaken thoroughly, followed by centrifugation for a period of 600 s to ensure complete phase separation. After equilibration at 296 K for 24 h, the volume and the density of the top (~1020 kg·m<sup>-3</sup>) and bottom layers (~1045 kg·m<sup>-3</sup>) were measured. The <sup>1</sup>H NMR spectra of the top and bottom layers after exchanging H<sub>2</sub>O with D<sub>2</sub>O, were measured on a JEOL LA 500 MHz NMR spectrometer at 353 K using sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal standard. To acquire simpler spectra, the top and bottom layers were neutralized with NaOD solution of *m*<sub>NaOD</sub> of 1 mol·kg<sup>-1</sup> for the complete conversion of the polymers into their anionic forms of 4 (*x* = 1) and 5 (*x* = 1) (Scheme 1). The <sup>1</sup>H NMR spectra of several top and bottom layers are displayed in Figures 1 and 2.

Composition of top and bottom phases of UPE 2-[PSB 3 + NaOH] systems were determined by <sup>1</sup>H NMR and dilution method (Table 3). The spectrum of pure UPE 2 revealed the presence of three signals centered on ~δ1.6 (for CH<sub>2</sub>), 3.9 (for nonurethanated CHO) and 4.9 ppm (for urethanated CHO-CON) in an area ratio of 20:8.7:1.3, respectively, thus revealing the degree of urethanization as 0.13. The 16-proton complex signals of NaOH-neutralized PSB 3 appeared in the range δ0.9 to 3.3 ppm (Figures 1b and 2b). The area (*A*) for the nonoverlapping signal at δ3.9 ppm belongs to 0.87 H marked "b" of UPE 2 (Figures 1b and 2b). The area for 1H of UPE 2 thus becomes *A*/0.87. The total area (*B*) in the range δ0.9 to 3.3 ppm now belongs to 16H of PSB 3 and 2H (marked a,c) of UPE 2. The area for the 16 H of PSB 3 alone accounts to [*B* - (2·*A*/0.87)]. The mole ratio of UPE 2/PSB 3 thus becomes

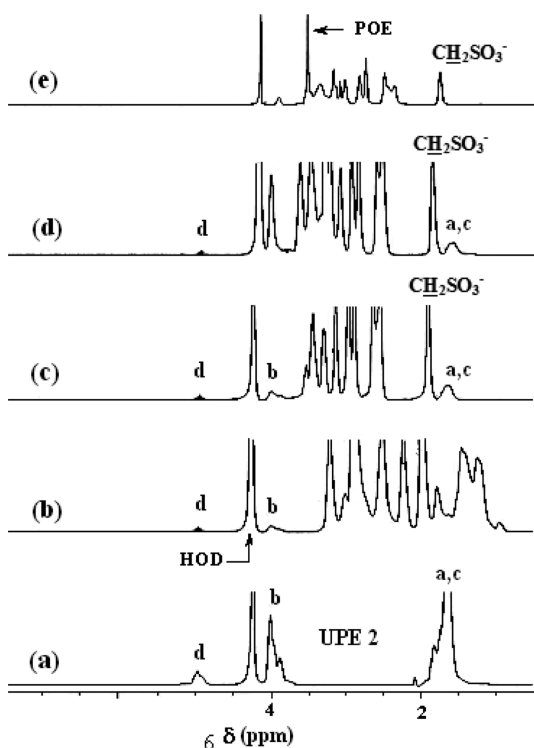
Table 2. Viscosity and Solubility Behaviours of UPE 2 and Ionic Polymers 3, 5, and 7

polymer	solubility <sup>a</sup>		intrinsic viscosity <sup>c</sup> [η]/m <sup>3</sup> kg <sup>-1</sup> ( <i>m</i> <sub>NaCl</sub> /mol·kg <sup>-1</sup> )
	salt-free water	CSC <sup>b</sup> <i>m</i> <sub>NaCl</sub> /mol·kg <sup>-1</sup>	
2	+	NA	0.0767 (0)
3 <sup>d</sup>	—	0.67	0.0363 (0.1)
5 <sup>d</sup>	—	1.49	0.0860 (0.1)
7-M <sub>2</sub> -50 <sup>d</sup>	—	1.77	0.0213 (1.0) <sup>e</sup>
7-M <sub>2</sub> -75 <sup>d</sup>	—	1.73	0.0242 (1.0) <sup>f</sup>

<sup>a</sup>Symbols: +, indicates soluble; —, indicates insoluble. <sup>b</sup>Critical salt concentration (CSC) for promoting water-solubility of polymer (0.01 mass fraction) at 296 K. <sup>c</sup>Viscosity of (1 to 0.125) % polymer solution (after neutralization of the zwitterionic units in 3, 5, and 7 using equivalent amounts of NaOH) in *m* kg·mol<sup>-1</sup> NaCl at 303 K as measured with an Ubbelohde viscometer (*K* = 0.005718). Concentration of NaCl is given in the parentheses. <sup>d</sup>Neutralization of the zwitterionic units in the polymers with NaOH led to water-soluble polymers while 7-M<sub>2</sub>-0.5 gave a cloudy mixture which has a CSC of *m*<sub>NaCl</sub> of 0.57 mol·kg<sup>-1</sup>. <sup>e</sup>[η] in *m*<sub>NaCl</sub> of 0.6 mol·kg<sup>-1</sup> and 0.8 mol·kg<sup>-1</sup> are 0.00803 and 0.0114 m<sup>3</sup> kg<sup>-1</sup>, respectively. <sup>f</sup>[η] in *m*<sub>NaCl</sub> of 0.1 mol·kg<sup>-1</sup> and 0.5 mol·kg<sup>-1</sup> are 0.0360 and 0.0253 m<sup>3</sup> kg<sup>-1</sup>, respectively.

Table 3. Phase Composition of the PSB 3 (Treated with 0.6 equiv NaOH)-UPE 2-H<sub>2</sub>O System at 296 K (Shown in Figure 3)

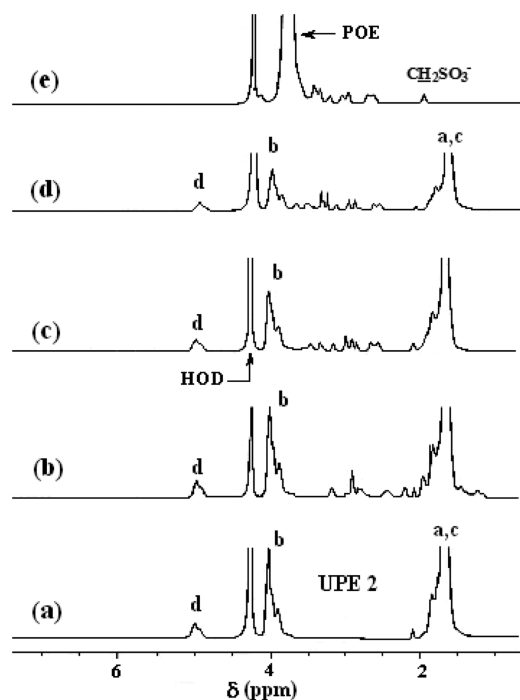
NMR method								
system	total system			top phase		bottom phase		volume ratio <sup>a</sup>
	UPE 2 100-w	PSB 3 100-w	KCl/mol·kg <sup>-1</sup>	UPE 2 100-w	PSB 3 100-w	UPE 2 100-w	PSB 3 100-w	
1	7.57	8.13	0.1	0.960	11.4	22.3	0.570	2.62
2	8.64	5.52	0.1	1.42	9.15	18.7	0.993	1.42
3	6.50	4.15	0.1	1.90	6.14	13.9	1.14	1.61
4	4.48	2.86	0.1	2.54	3.69	8.95	1.31	2.22
5	4.49	2.28	0.1	3.33	2.68	7.08	1.55	1.54
dilution method								
system	total system (two-phase)			total system (one-phase)		total system (one-phase)		
	UPE 2 100-w	PSB 3 100-w	KCl/mol·kg <sup>-1</sup>	UPE 2 100-w	PSB 3 100-w	KCl/mol·kg <sup>-1</sup>	UPE 2 100-w	PSB 3 100-w
a <sub>1</sub>	3.98	4.61	0.1	1.94	2.25	0.3	1.69	1.95
b <sub>1</sub>	5.27	3.61	0.1	2.71	1.85	0.3	2.23	1.53
c <sub>1</sub>	7.59	3.43	0.1	3.58	1.62	0.3	3.09	1.40

<sup>a</sup>Volume ratio of top and bottom phase.

**Figure 1.** <sup>1</sup>H NMR spectra in D<sub>2</sub>O in the presence of NaOH at 353 K of (a) UPE 2; (b) top phase, system 2 (Figure 3, Table 3), UPE 2-PSB 3; (c) top phase, system 3 (Figure 5, Table 4), UPE 2-PSB-SO<sub>2</sub> 5; (d) bottom phase, system 5 (Figure 6a, Table 5), UPE 2-[7-M<sub>2</sub>-0.5]; (e) bottom phase, system 5 (Figure 6b, Table 6), POE-[7-M<sub>2</sub>-0.5].

$$\begin{aligned}
 \frac{\text{mol UPE}}{\text{mol PSB}} &= \frac{\text{area of 1H of UPE}}{\text{area for 1H of PSB}} \\
 &= \frac{A/0.87}{[B - (2 \times A/0.87)]/16} \quad (1)
 \end{aligned}$$

The phase compositions of UPE 2-[PBS·SO<sub>2</sub> 5 + NaOH] systems, as determined by <sup>1</sup>H NMR analysis, are given in Table 4. The sixteen-proton complex signals of NaOH neutralized PSB·SO<sub>2</sub> 5 appeared in the range δ1.7 ppm to 3.5 ppm. The area (A) for the nonoverlapping signal at δ3.9 ppm was considered belonging to 0.87 H as marked “b” of UPE 2 (Figures 1c and 2c). The CH<sub>2</sub>SO<sub>3</sub><sup>-</sup> protons appeared as an



**Figure 2.** <sup>1</sup>H NMR spectra in D<sub>2</sub>O in the presence of NaOH at 353 K of (a) UPVA; (b) bottom phase, system 5 (Figure 3, Table 3), UPE 2-PSB 3; (c) bottom phase, system 4 (Figure 5, Table 4), UPE 2-PSB-SO<sub>2</sub> 5; (d) top phase, system 4 (Figure 6a, Table 5), UPE 2-7-M<sub>2</sub>-0.5; (e) top phase, system 3 (Figure 6b, Table 6), POE-7-M<sub>2</sub>-0.5.

overlapping signal at δ1.8 ppm while the area (B) in the range δ2.4 ppm to 3.5 ppm was assigned to its remaining 14H. Therefore, the mole ratio of UPVA 2/PSB·SO<sub>2</sub> 5 was determined as

$$\frac{\text{mol UPE}}{\text{mol PSB} \cdot \text{SO}_2} = \frac{\text{area of 1H of UPE}}{\text{area for 1H of PSB} \cdot \text{SO}_2} = \frac{A/0.87}{B/14} \quad (2)$$

The chemical composition of the top and bottom phases of POE or UPE 2-[7-M<sub>2</sub>-q + NaOH] systems as calculated by <sup>1</sup>H NMR, are given in Tables 5 to 8. For the UPE 2-7-M<sub>2</sub>-0.5 systems, the area (A) for the proton marked “a,c” belongs to 2H of UPE (Scheme 1, Figure 1d). Both M<sub>1</sub> and M<sub>2</sub> in 7-M<sub>2</sub>-0.5 contain 16 H whose complex signals appeared in the range δ1.9 to 4.0 ppm. The area (B) for the two-proton in CH<sub>2</sub>SO<sub>3</sub><sup>-</sup>



**Table 4.** Phase Composition of the PSB·SO<sub>2</sub> 5 (Treated with 0.60 equiv NaOH)-UPE 2-H<sub>2</sub>O System at 296 K (Shown in Figure 5)

NMR method								
system	total system			top phase		bottom phase		Volume ratio <sup>a</sup>
	UPE 2 100-w	PSB·SO <sub>2</sub> 5 100-w	KCl/mol·kg <sup>-1</sup>	UPE 2 100-w	PSB·SO <sub>2</sub> 5 100-w	UPE 2 100-w	PSB·SO <sub>2</sub> 5 100-w	
1	9.89	6.60	0.1	0.506	13.1	18.7	0.408	1.00
2	7.20	5.54	0.1	0.938	9.58	14.5	0.888	1.07
3	5.60	4.05	0.1	1.08	6.01	11.7	1.25	1.35
4	4.05	3.02	0.1	1.80	4.04	8.03	1.54	1.88
5	3.18	2.02	0.1	2.53	2.50	4.13	1.41	1.35
dilution method								
system	total system (two phase)			total system (one-phase)		total system (one-phase)		Volume ratio <sup>a</sup>
	UPE 2 100-w	PSB·SO <sub>2</sub> 5 100-w	KCl/mol·kg <sup>-1</sup>	UPE 2 100-w	PSB·SO <sub>2</sub> 5 100-w	KCl mol·kg <sup>-1</sup>	UPE 2 100-w	
a <sub>1</sub>	3.30	6.00	0.1	1.50	2.73	0.3	1.25	2.28
b <sub>1</sub>	5.05	3.52	0.1	2.66	1.85	0.3	2.16	1.51
c <sub>1</sub>	6.34	2.34	0.1	3.52	1.30	0.3	2.91	1.08

<sup>a</sup>Volume ratio of top and bottom phase.**Table 5.** Phase Composition of the 7-M<sub>2</sub>-0.5 (Treated with 0.5 equiv NaOH)-UPE 2-H<sub>2</sub>O (*m*<sub>KCl</sub> of 0.6 mol·kg<sup>-1</sup>) System at 296 K (Shown in Figure 6a)

system	total system		top phase		bottom phase		volume ratio <sup>a</sup>
	7-M <sub>2</sub> -0.5 100-w	UPE 2 100-w	7-M <sub>2</sub> -0.5 100-w	UPE 2 100-w	7-M <sub>2</sub> -0.5 100-w	UPE 100-w	
1	3.50	5.03	0.305	8.61	7.56	0.306	1.28
2	3.47	4.03	0.384	7.30	6.92	0.421	1.12
3	3.78	2.52	0.610	5.77	5.77	0.453	0.667
4	3.01	2.38	0.830	4.65	4.76	0.702	0.868
5	2.55	2.08	0.941	3.76	3.67	0.952	0.841

<sup>a</sup>Volume ratio of top and bottom phase.**Table 6.** Phase Composition of the 7-M<sub>2</sub>-0.5 (Treated with 0.5 equiv NaOH)-POE-H<sub>2</sub>O (*m*<sub>KCl</sub> of 0.6 mol·kg<sup>-1</sup>) System at 296 K (Shown in Figure 6b)

system	total system		top phase		bottom phase		volume ratio <sup>a</sup>
	7-M <sub>2</sub> -0.5 100-w	POE 100-w	7-M <sub>2</sub> -0.5 100-w	POE 100-w	7-M <sub>2</sub> -0.5 100-w	POE 100-w	
1	3.72	4.43	0.459	5.46	17.8	0.0616	4.5
2	6.33	2.72	0.721	4.31	14.7	0.144	1.53
3	7.21	1.97	0.705	3.75	13.7	0.181	1.01
4	4.32	2.26	0.971	3.21	11.9	0.265	2.36

<sup>a</sup>Volume ratio of top and bottom phase.

of M<sub>2</sub> in 7, appearing as a nonoverlapping signal at ~δ2.0 ppm, accounts for <sup>1</sup>H of the repeating unit since it consists of 0.5 mol fraction each of M<sub>1</sub> and M<sub>2</sub> (Scheme 1). The mole ratio of UPE 2/7-M<sub>2</sub>-0.5 thus can be determined using

$$\frac{\text{mol UPE}}{\text{mol 7-M}_2\text{-0.5}} = \frac{\text{area of 1H UPE}}{\text{area for 1H of 7-M}_2\text{-0.5}} = \frac{A/2}{B} \quad (3)$$

For the UPE 2-7-M<sub>2</sub>-0.5 system shown in Figure 2d, the nonoverlapping proton marked “d” represent an area (A) for 0.13H of UPE 2. The area for 1H of UPE thus becomes A/0.13. The total area (B) in the range δ1.4 to 4.0 ppm now belongs to 16H of 7-M<sub>2</sub>-0.5 and 2.87 H (marked a, c, and b) of UPE 2. The area for the 16H of 7-M<sub>2</sub>-0.5 alone accounts for [B - (2.87 × A/0.13)]. Therefore, the mole ratio of UPE 2/7-M<sub>2</sub>-0.5 can be determined by using eq 4.

$$\frac{\text{mol UPE}}{\text{mol (7-M}_2\text{-0.5)}} = \frac{\text{area of 1H of UPE}}{\text{area for 1H of 7-M}_2\text{-50}} = \frac{A/0.13}{[B - (2.87 \times A/0.13)]/16} \quad (4)$$

Figures 1e and 2e represent a POE-7-M<sub>2</sub>-0.5 system. For POE, the 4H signal appears as a singlet at δ3.7 ppm while the area (A) under the nonoverlapping two-proton CH<sub>2</sub>SO<sub>3</sub><sup>-</sup> signal (a part of repeating unit M<sub>2</sub>), displaying at ~δ2.0 ppm in the <sup>1</sup>H NMR spectrum, represents the area of 1H for the total repeating unit. The total area (B) in the range δ2.5 ppm to 4.0 ppm belongs to 16H of M<sub>1</sub> and the remaining 14H of M<sub>2</sub> in 7-M<sub>2</sub>-50 and 4H of POE. Therefore, the area for the 4H of POE alone accounts for [B - (16 × 0.5 + 14 × 0.5) × A]. The mole ratio of POE/7-M<sub>2</sub>-0.5 is determined using eq 5.

$$\frac{\text{mol POE}}{\text{mol 7-M}_2\text{-50}} = \frac{\text{area of 1H POE}}{\text{area for 1H of 7-M}_2\text{-50}} = \frac{[B - 15 \times A]/4}{A} \quad (5)$$

The calculation of molar ratios of POE/7-M<sub>2</sub>-0.75 follows similar steps as in the case of systems containing 7-M<sub>2</sub>-0.5 except the two-proton CH<sub>2</sub>SO<sub>3</sub><sup>−</sup> signal represents 2 × 0.75 that is, 1.5 H proton for the entire repeating unit (data not shown).

**2.3.2. The Tie Lines by NMR Method.** The tie lines were constructed using the systems mentioned in the Tables 3 to 8. Mass fraction (*w*) percent of each polymer, for instance in the case of the UPE–PSB systems, is determined by using eqs 6 and 7 as described in our earlier work.<sup>33</sup>

$$[(PSB)_b] = \frac{UPE_0/0.0496 - (PSB_0/0.219)([UPE]/[PSB])_t}{V_b\{([UPE]/[PSB])_b - ([UPE]/[PSB])_t\}} \quad (6)$$

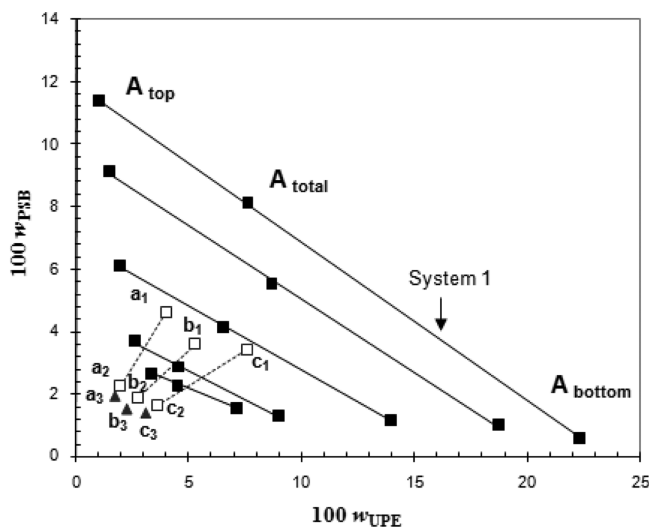
where, subscript t and b represents top and bottom phases, respectively. [PSB] and [UPE] represents concentration of the repeating units in mol·kg<sup>−1</sup>. PSB<sub>0</sub> and UPVA<sub>0</sub> represent total mass in kg of the polymers, and *V* represents the volume in m<sup>3</sup>. Molar masses of the repeating units of the PSB 3 and UPE 2 were taken as 0.219 and 0.0496 kg·mol<sup>−1</sup>, respectively. [UPE]/[PSB] represents molar ratio of the polymers as determined by <sup>1</sup>H NMR. Similarly, the same eqs 6 and 7 were used to determine the mass fractions of the polymers in the UPE 2-PSB-SO<sub>2</sub> 5, UPE 2-7-M<sub>2</sub>-0.5, and UPE 2-7-M<sub>2</sub>-0.75 systems except that the molar masses of the repeating units of the ionic polymers were replaced by 0.283, 0.255, and 0.269 kg·mol<sup>−1</sup>, respectively. For the POE-based system, the molar masses of the repeating units of POE were taken as 0.04405 kg·mol<sup>−1</sup>.

The mass of polymer PSB in the bottom phase is then calculated using eq 7.<sup>33</sup>

$$PSB_b = [PSB_b]V_b \cdot 0.219 \quad (7)$$

Once one of the polymer concentrations in a phase is known, then the concentrations and mass fractions of the polymers in both phases are calculated from the known density, mass, and volume of the phases. The tie lines were then constructed as shown in Figures 3, 5, and 6.

**2.3.3. Binodals by the Turbidity Method.** The points for the construction of the binodals were obtained by turbidity method



**Figure 3.** Phase diagram [■ and (□, Δ) represent data obtained by respective NMR and dilution method] of UPE 2-[PSB 3 + 0.6 equiv NaOH]-water (■ and □, *m*<sub>KCl</sub> of 0.1 mol·kg<sup>−1</sup>; ▲, *m*<sub>KCl</sub> of 0.3 mol·kg<sup>−1</sup>) at 296 K. [a<sub>1</sub>, b<sub>1</sub>, and c<sub>1</sub> are the compositions of three two-phase total systems which become single phase at a<sub>2</sub>, b<sub>2</sub>, c<sub>2</sub> and a<sub>3</sub>, b<sub>3</sub>, c<sub>3</sub> in respective 0.1 mol·kg<sup>−1</sup> and 0.3 mol·kg<sup>−1</sup> KCl].

at 296 K.<sup>18</sup> A stirred concentrated solution of mass fraction of ~0.20 (1.5 g) of NaOH-treated PSBs 3, 5, or 7 in the presence of the appropriate concentrations of KCl was titrated with a concentrated solution (*w*: 0.20) of UPE 2 in *x m* KCl (where, *x m* = 0, 0.1, 0.3, or 0.5 mol·kg<sup>−1</sup>) until the transparent system turned turbid. A known weight of an *x m* KCl solution was then added dropwise until the system became transparent again. At this point, the final composition of the two polymers corresponds to a point on the binodal curve. After obtaining the first point, further additions of a UPE 2 (leading to turbidity) followed by dilution with water (KCl) were repeated to obtain a sufficient number of points for the construction of the binodal. To obtain points on the other side of the binodal curve, the concentrated solution of the UPE 2 was titrated with NaOH-treated PSBs 3, 5, or 7. Points obtained by turbidity method are shown in Figures 4 and 6 (*vide infra*).

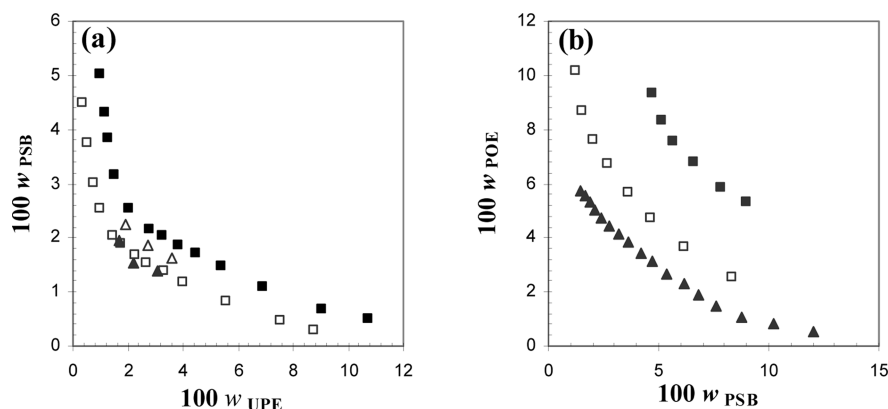
**2.3.4. Binodals by the Dilution Method.** When the turbidity method does not provide a clear-cut visualization of a phase transition, the binodals can be constructed by the dilution method. For the current work, however, the construction of binodals was successfully achieved by the turbidity method. As a complementary to the turbidity method, binodals can also be constructed using the dilution method. Thus a two-phase system with a known total composition was diluted with water (KCl), a few drops at a time, until the two phases became one as indicated after centrifugation. The composition of the diluted system (before the last drop makes the system homogeneous) gives a point on the binodal.

### 3. RESULTS AND DISCUSSION

#### 3.1. Synthesis and Characterization of Ionic Polymers.

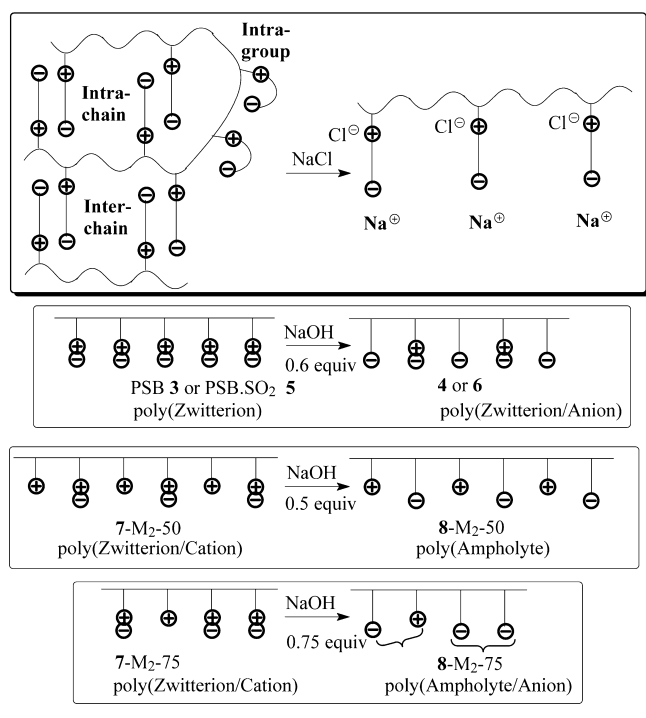
Poly(oxyethylene) (POE)  $-(CH_2CH_2O)_n-$  of number average molecular weight (*M*<sub>n</sub>) of 35.0 kg·mol<sup>−1</sup> and UPE 2 of *M*<sub>n</sub> 69.0 kg·mol<sup>−1</sup> were used as neutral polymers for the construction of ATPSs. The <sup>1</sup>H NMR spectrum revealed the presence of nonurethanized and urethanized fractions of UPE 2 in a ratio of 87:13 as measured by the integration of signals of the protons marked “b” and “d” (Scheme 1) which appeared at δ3.9 ppm and 4.9 ppm, respectively. The synthetic procedure of PSB 3,<sup>30</sup> PSB-SO<sub>2</sub> 5,<sup>31</sup> 7-M<sub>2</sub>-50,<sup>32</sup> and 7-M<sub>2</sub>-75<sup>32</sup> were reported previously in the literature; some solution properties of the freshly prepared polymers are given in Table 1. For terpolymer 7, *M*<sub>1</sub> and *M*<sub>2</sub> represent the cationic (+) and sulfobetaine (±) units, respectively. Terpolymer 7-M<sub>2</sub>-0.75, for instance, represents a polymer consisting of 0.75 mol fraction of sulfobetaine (±) (*M*<sub>2</sub>) and 0.25 mol fraction of cationic (+) (*M*<sub>1</sub>) units. All these zwitterionic polymers (PZs), having an ionically cross-linked network in a collapsed coil conformation, are insoluble in salt-free water. These cross-linked networks cause a reduction in hydrodynamic radius due to the strong electrostatic attractive interactions between the charges of opposite algebraic signs as well as dipolar interactions promoting intragroup and intra- and interchain associations (Scheme 2).<sup>34</sup> Amphoteric PZs antipolyelectrolyte behavior, like enhancements in the viscosity and solubility in the presence of added electrolytes (e.g., NaCl), is attributed to the added ability of the salt to neutralize the ionic cross-links thus permitting expansion of the polymer backbone and globule-to-coil transition (Scheme 2).<sup>35–37</sup>

PSB 3 and PSB-SO<sub>2</sub> 5 require the presence of a minimum critical salt concentration (CSC) of 0.67 mol·kg<sup>−1</sup> and 1.49 mol·kg<sup>−1</sup> NaCl, respectively, to break the zwitterionic interactions and thus promote solubility,<sup>30,31</sup> whereas the



**Figure 4.** Phase diagram at 296 K of (a) UPE 2-PSB 3 (in the presence of 0.85 equiv NaOH, ■ and □ represent data at respective  $m_{\text{KCl}}$  of 0.1 and 0.3 mol·kg<sup>-1</sup>, while in the presence of 0.60 equiv NaOH, ▲ and △ represent data at respective  $m_{\text{KCl}}$  of 0.1 and 0.3 mol·kg<sup>-1</sup>); (b) POE-PSB 3 (in the presence of 0.85 equiv NaOH, ■ and □ represent data at respective  $m_{\text{KCl}}$  of 0.3 and 0.5 mol·kg<sup>-1</sup>, while in the presence of 0.60 equiv NaOH, ▲ represents data at  $m_{\text{KCl}}$  of 0.1 mol·kg<sup>-1</sup>).

## Scheme 2. Zwitterionic Interaction and Change of Charge Types and Their Densities



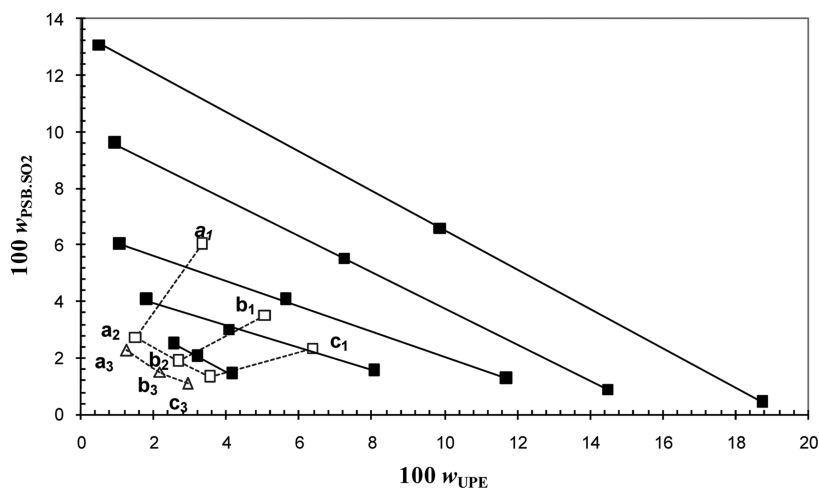
respective CSCs of 7-M<sub>2</sub>-0.5 and 7-M<sub>2</sub>-0.75 were found to be 1.77 mol·kg<sup>-1</sup> and 1.73 mol·kg<sup>-1</sup> NaCl (Table 2).<sup>32</sup> Intrinsic viscosity of the freshly prepared ionic polymers were measured in the presence of 1 equiv of NaOH which converts water-insoluble zwitterionic 3 and 5 to water-soluble anionic polyelectrolytes 4 ( $x = 1$ ) and 6 ( $x = 1$ ) (Table 2). For the construction of the ATPSs, the (±) PSB 3 and (±) PSB-SO<sub>2</sub> 5 were treated with 0.6 equivalent of NaOH to give water-soluble (− ±) APE/PSB 4 and (− ±) APE/PSB-SO<sub>2</sub> 6 with an anionic (−)/zwitterionic (±) ratio of 60:40 (Scheme 2). The dominance of anionic over zwitterionic fraction thus dictates the solubility behavior. Note that water-insoluble polycationic (+)/zwitterionic (±) 7-M<sub>2</sub>-0.5 (i.e.,  $q = 0.5$ ) on treatment with 0.5 equiv of NaOH leads to water-insoluble polyampholyte 8 with a charge symmetry [i.e., having equal number of (+) and (−) charges] (Scheme 2); the CSC to break ampholytic

interactions and promote water-solubility of 8 was found to be 0.57 mol·kg<sup>-1</sup> NaCl.<sup>32</sup> On the other hand, 7-M<sub>2</sub>-0.75 ( $q = 0.75$ ) in the presence of 0.75 equiv of NaOH was converted to polyampholyte/anion 8 in which the dominance of the anionic fraction over the ampholytic group makes it water-soluble.

The knowledge of water-solubility behavior presented above is indeed helpful in the construction of the ATPSs. For the current study, pH-responsive PSB 3 and PSB-SO<sub>2</sub> 5 were treated with 0.6 equiv of NaOH to obtain water-soluble (APE/PSB) 4 ( $x = 0.6$ ) and (APE/PSB-SO<sub>2</sub>) 6 ( $x = 0.6$ ) which were used as components of ATPSs. The polymer thus has the biomimicking influence retained as a result of the remaining zwitterionic motifs, whereas the water solubility is maintained by the presence of sufficient proportion of the anionic fraction (Schemes 1 and 2). The pH-responsive zwitterionic fractions of 7-M<sub>2</sub>-0.5 and 7-M<sub>2</sub>-0.75 were completely neutralized with 0.5 and 0.75 equivalent of NaOH to obtain polyampholyte 8-M<sub>2</sub>-0.5 and polyampholyte/anion 8-M<sub>2</sub>-0.75 which were then used as components for the construction of ATPS.

**3.2. Phase Diagrams using UPVA 2 –[PSB 3 + NaOH]-water (KCl) systems.** Several systems of known compositions of UPE 2 and PSB 3/(0.60 equiv NaOH) were prepared (Table 3). The base treatment changed the PSB 3 to APE/PSB 4 having 0.60 mol fraction anionic electrolyte (APE) and 0.40 mol fraction polysulfobetaine (PSB) motifs. Polymer concentrations of the phases at equilibrium, as determined by <sup>1</sup>H NMR, are connected by tie lines (Figure 3). Turbidity and/or dilution methods are used to obtain the binodal curves. The top and bottom layers of the phase-separated systems were found to be overwhelmingly rich in (APE/PSB) 4 and UPE 2, respectively. Note that PSB 3 is almost insoluble in common solvents but soluble in the presence of NaCl (0.7 mol·kg<sup>-1</sup>); however, the corresponding (APE/PSB 4) is soluble in salt-free water.<sup>30</sup>

In the current (Figure 3) and subsequent phase diagrams, the weight percent (100  $w$ ) of the polymer rich in the top and bottom phases are assigned the  $y$ - and  $x$ -axis, respectively. The tie lines are helpful in the construction of ATPS with suitable volume or mass ratio of the two phases. For a total system of composition  $A_{\text{total}}$ , the volume or mass ratio of the top and bottom phases can be calculated from the ratio of the lengths of  $A_{\text{total}}-A_{\text{bot}}$  and  $A_{\text{total}}-A_{\text{top}}$ , where  $A_{\text{top}}$  and  $A_{\text{bot}}$  denote their equilibrium compositions in the top and bottom phases, respectively.



**Figure 5.** Phase diagram [■ and (□, Δ) represent data obtained by respective NMR and dilution method] of UPE 2-[PSB·SO<sub>2</sub> 5 + 0.6 equiv NaOH]-water (■ and □,  $m_{\text{KCl}}$  of 0.1 mol·kg<sup>-1</sup>; Δ,  $m_{\text{KCl}}$  of 0.3 mol·kg<sup>-1</sup>) at 296 K. [ $a_1$ ,  $b_1$ , and  $c_1$  are the compositions of three two-phase total systems which become single phase at  $a_2$ ,  $b_2$ ,  $c_2$  and  $a_3$ ,  $b_3$ ,  $c_3$  in respective 0.1 mol·kg<sup>-1</sup> and 0.3 mol·kg<sup>-1</sup> KCl].

The binodal was constructed by dilution method since the turbidity method failed to give a clear-cut indication of phase transitions. In the dilution method, three total systems  $a_1$ ,  $b_1$ , and  $c_1$  were allowed to walk from a two-phase to a one-phase systems (Figure 3). For instance, the two-phase system  $a_1$  was diluted with 0.1 mol·kg<sup>-1</sup> KCl to reach the single phase at  $a_2$ . Upon the concentration of KCl being changed from 0.1 mol·kg<sup>-1</sup> to 0.3 mol·kg<sup>-1</sup>, the system became two-phase again. On further dilution with 0.3 mol·kg<sup>-1</sup> KCl, it became single phase at  $a_3$ . The polymers displayed segregative phase separation behavior. The phase separation took place at a relatively low total polymer mass fraction of <0.10, which could be useful from an industrial point of view. It can be seen that the binodal curve is shifted downward as the concentration of KCl increases from 0.1 to 0.3 mol·kg<sup>-1</sup>, indicating that lower concentration of the polymers is required to form ATPS in the latter system. The zwitterionic (0.40 mol fraction) and anionic (0.60 mol fraction) portions in (PSB/APE) 4 (Schemes 1 and 2) are expected to ascertain “antipolyelectrolyte” and “polyelectrolyte” behavior, respectively. The addition of salt (KCl) will demonstrate a dominant polyelectrolyte effect (i.e., decrease of viscosity and hydrodynamic volume in the presence of a salt) since anionic motifs constitute the larger fraction. As a result, at a higher concentration of KCl, the polymer chain will be contracted more, and thus making it less compatible with the UPE, thereby requiring a lesser amount of polymers for phase separation to occur. It is shown that UPE 2 has a higher intrinsic viscosity hence higher hydrodynamic volume than APE 4 ( $x = 1$ , 100 % anionic fraction) (Table 1) in 0.1 mol·kg<sup>-1</sup> NaCl, while (APE/PSB) 4 ( $x = 0.6$ , 60 % anionic fraction) is expected to have even lower viscosity value and hydrodynamic volume owing to the presence of lesser proportion of mutually repulsive anionic units.

The effect of APE/PSB ratio on the phase diagram of (PSB/APE)-UPE-water is shown in Figure 4a. Changing the APE/PSB ratio in 4 from 60:40 and 85:15 by treating PSB 3 with respective 0.60 equiv and 0.85 equiv of NaOH, does not seem to have a considerable effect on the phase diagram either in 0.1 mol·kg<sup>-1</sup> or 0.3 mol·kg<sup>-1</sup> KCl (Figure 4). The sample 4 having a APE(-)/PSB(±) ratio of 85:15 was found to have its binodal moved slightly upward compared to APE/PSB with a ratio of 60:40. The presence of a higher percentage of the

zwitterionic fraction, leading to decreased hydrodynamic volume and greater mismatch with the size of UPE 2, thus requires a lesser amount of polymers for phase separation to occur. It is worth mentioning that the presence of salt (KCl) has the opposite effect on the contribution of zwitterionic and anionic fraction to the hydrodynamic volume of the polymer chain; expansion and contraction of the former and latter fraction, respectively, minimizes the effect of the salt on the polymer having mixed motifs of (±) and (-).

The effect of PSB/APE ratio on the phase diagram of (PSB/APE)-POE-H<sub>2</sub>O is shown in Figure 4b. The samples having a APE/PSB ratio of 85:15 were found to have the binodals moved upward compared to APE/PSB 4 with the corresponding ratio of 60:40. The presence of a higher percentage of the zwitterionic portion thus requires a lesser amount of polymers for phase separation to occur as a result of greater incompatibility with POE. Raising the concentration of KCl from 0.3 mol·kg<sup>-1</sup> to 0.5 mol·kg<sup>-1</sup> shifted the binodal downward as a result of contraction of the polymer chain, thereby leading to lesser compatibility with POE and requiring a lesser amount of 15:85 (PSB/APE) 4 for phase separation to occur.

**3.3. Phase Diagrams using UPE 2-[PSB·SO<sub>2</sub> 5 + NaOH]-water (KCl) Systems.** Several systems of known compositions of UPE 2 and PSB·SO<sub>2</sub> 5 treated with 0.6 equiv of NaOH are given in Table 4 (Scheme 1). The top and bottom layers were found to be overwhelmingly rich in 60:40 APE/PSB·SO<sub>2</sub> 6 and UPE 2, respectively. It was found that the PSB·SO<sub>2</sub> 5 is almost insoluble in every common solvent but soluble in the presence of NaCl (~1.7 mol·kg<sup>-1</sup>); however, the corresponding 60:40 APE/PSB·SO<sub>2</sub> 6 is water-soluble.<sup>31</sup> Using <sup>1</sup>H NMR spectroscopy, the tie lines 1 to 5 were constructed (Figure 5, Table 4). The phase separation occurs at relatively low total polymer concentrations, much below mass fraction of 0.10.

It can be seen that the binodal curve shifts downward as the concentration of KCl increases, indicating that smaller concentrations of the phase polymers are required to form ATPS in 0.3 mol·kg<sup>-1</sup> KCl than in 0.1 mol·kg<sup>-1</sup> KCl. (APE/PSB·SO<sub>2</sub>) 6 (Schemes 1, 2) having 0.40:0.60 ratio of zwitterionic/anionic mole fractions, are expected to ascertain “antipolyelectrolyte” and “polyelectrolyte” behavior of the polymer, respectively. As discussed earlier, the addition of salt

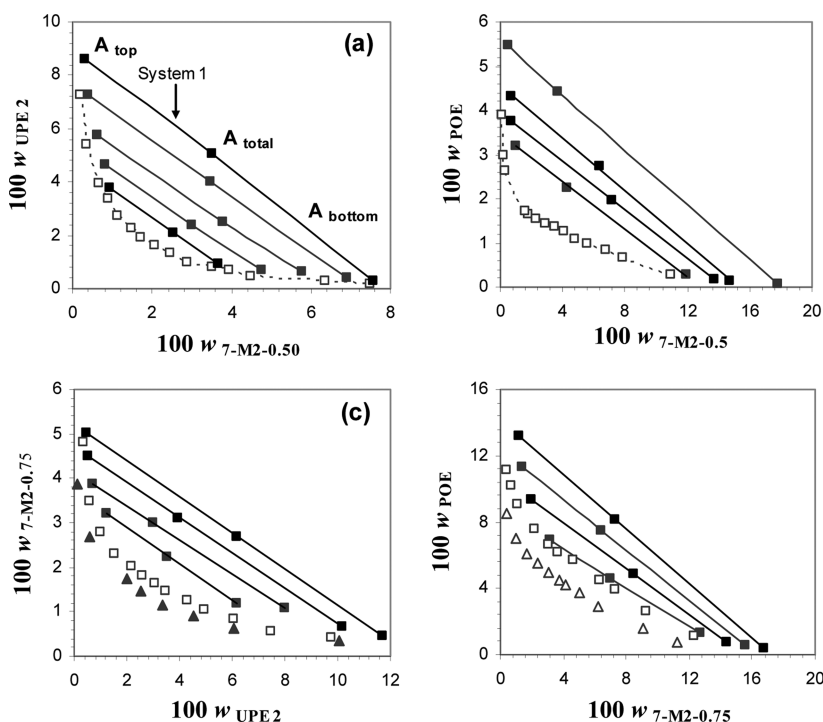


**Table 7.** Phase Composition of the 7-M<sub>2</sub>-0.75 (Treated with 0.75 equiv NaOH) –UPE 2-H<sub>2</sub>O ( $m_{\text{KCl}}$  of 0.1 mol·kg<sup>-1</sup>) System at 296 K (Shown in Figure 6c)

system	total system		top phase		bottom phase		volume ratio <sup>a</sup>
	UPE 2 100-w	7-M <sub>2</sub> -0.75 100-w	UPE 2 100-w	7-M <sub>2</sub> -0.75 100-w	UPE 2 100-w	7-M <sub>2</sub> -0.75 100-w	
1	6.15	2.67	0.45	5.04	11.7	0.461	0.962
2	3.95	3.12	0.510	4.51	10.2	0.65	1.78
3	3.02	2.98	0.712	3.87	8.01	1.08	2.12
4	3.52	2.23	1.22	3.21	6.18	1.20	1.08

<sup>a</sup>Volume ratio of top and bottom phase.**Table 8.** Phase Composition of the 7-M<sub>2</sub>-0.75 (Treated with 0.75 equiv NaOH)-POE-H<sub>2</sub>O ( $m_{\text{KCl}}$  of 0.3 mol·kg<sup>-1</sup>) System at 296 K (Shown in Figure 6d)

system	total system		top phase		bottom phase		volume ratio <sup>a</sup>
	7-M <sub>2</sub> -0.75 100-w	POE 100-w	7-M <sub>2</sub> -0.75 100-w	POE 100-w	7-M <sub>2</sub> -0.75 100-w	POE 100-w	
1	7.30	8.11	1.19	13.2	16.8	0.402	1.47
2	6.40	7.53	1.35	11.3	15.6	0.578	2.00
3	8.45	4.85	2.01	9.31	14.4	0.751	0.974
4	7.00	4.62	3.13	6.88	12.7	1.28	1.75

<sup>a</sup>Volume ratio of top and bottom phase.**Figure 6.** Phase diagram (■ and □ represent data obtained by respective NMR and turbidity method at 296 K of (a) UPE 2-[7-M<sub>2</sub>-0.5 + NaOH]-water ( $m_{\text{KCl}}$  of 0.6 mol·kg<sup>-1</sup>); (b) UPE 2-[7-M<sub>2</sub>-0.5 + NaOH]-water ( $m_{\text{KCl}}$  of 0.6 mol·kg<sup>-1</sup>); (c) UPE 2-[7-M<sub>2</sub>-0.75 + NaOH]-water ( $m_{\text{KCl}}$ , □ and ■ 0.1 mol·kg<sup>-1</sup>; ▲, 0.3 mol·kg<sup>-1</sup>); (d) POE-[7-M<sub>2</sub>-0.75 + NaOH]-water ( $m_{\text{KCl}}$ , □ and ■ 0.3 mol·kg<sup>-1</sup>; ▲, 0.6 mol·kg<sup>-1</sup>).

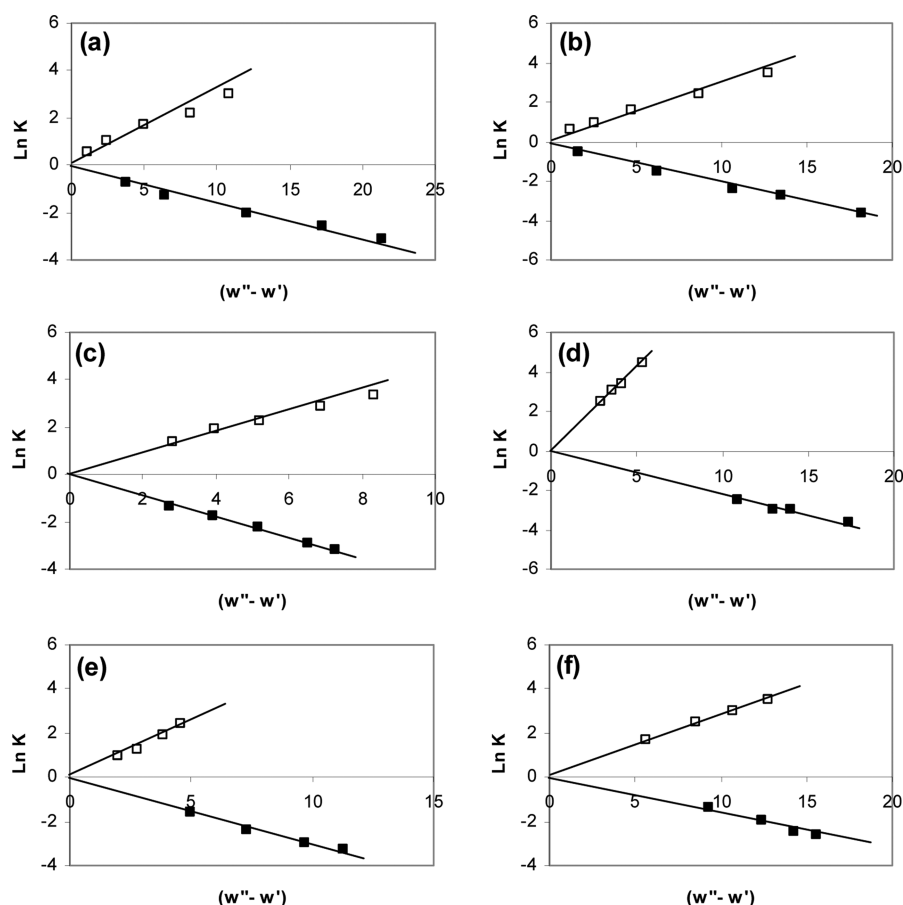
will demonstrate dominant a polyelectrolyte effect since anionic motifs constitute the larger fraction. As a result, at a higher concentration of KCl, the polymer chain will be contracted more and thus result in the decrease of hydrodynamic volume and lesser compatibility with UPE.

**3.4. Phase Compositions and Phase Diagram of UPE 2–CPE/PSB 7 (treated with  $\gamma$  equiv NaOH)-H<sub>2</sub>O System.** Stock solutions of CPE/PSB samples of 7-M<sub>2</sub>-0.5 and 7-M<sub>2</sub>-0.75, having 0.50 and 0.75 mol fraction of pH-responsive zwitterionic monomer M<sub>2</sub> units, were treated with 0.5 and 0.75 equivalent of NaOH to generate 8-M<sub>2</sub>-0.5 and 8-M<sub>2</sub>-0.75, respectively (Scheme 1). Several systems of known composi-

tions of component polymers are described in the Tables 5 to 8.

The phase diagrams are shown in Figure 6a–d. Polymer 7-M<sub>2</sub>-0.5 was found to be overwhelmingly rich in the bottom phase (Figure 6a,b), while 7-M<sub>2</sub>-0.75 remain rich in the top and bottom phase with UPE and POE, respectively (Figure 6c,d). Increasing the concentration of KCl makes 7-M<sub>2</sub>-0.75 less compatible with either UPE (Figure 6c) or POE (Figure 6d).

Aqueous two-phase polymer systems as constructed above may offer a friendly environment for the separation<sup>1,38,39</sup> of labile proteins. The pH dependent solubility behavior of the



**Figure 7.** Correlation of the phase diagrams of [PSB 3 or PSB-SO<sub>2</sub> 5 or 7]-[UPE 2 or PEG]-water (KCl) systems using the method of Diamond and Hsu: Using Tie-line data from (a) (Figure 3, Table 3); (b) (Figure 5, Table 4); (c) (Figure 6a, Table 5); (d) (Figure 6b, Table 6); (e) (Figure 6c, Table 7); (f) (Figure 6d, Table 8).

**Table 9.** Values for Constant Parameter *A* Introduced in eqs 8 and 9 along with the rmsd of the Model from the Experimental Data of the Partition Coefficients

system	polymer (1) <sup>a</sup>		polymer (2) <sup>b</sup>	
	<i>A</i> <sub>1</sub>	rmsd	<i>A</i> <sub>2</sub>	rmsd
PSB 3 + UPE 2 + 0.6 equiv NaOH + <i>m</i> <sub>KCl</sub> of 0.1 mol·kg <sup>-1</sup>	-0.154	0.021	0.288	0.62
PSB-SO <sub>2</sub> 5 + UPE 2 + 0.6 equiv NaOH + <i>m</i> <sub>KCl</sub> of 0.1 mol·kg <sup>-1</sup>	-0.207	0.024	0.282	0.80
7-M <sub>2</sub> -0.5 + UPE 2 + 0.5 equiv NaOH + <i>m</i> <sub>KCl</sub> of 0.6 mol·kg <sup>-1</sup>	-0.444	0.0083	0.422	0.83
7-M <sub>2</sub> -0.5 + POE + 0.5 equiv NaOH + <i>m</i> <sub>KCl</sub> of 0.6 mol·kg <sup>-1</sup>	-0.219	0.0027	0.833	0.66
7-M <sub>2</sub> -0.75 + UPE 2 + 0.5 equiv NaOH + <i>m</i> <sub>KCl</sub> of 0.1 mol·kg <sup>-1</sup>	-0.306	0.016	0.503	0.27
7-M <sub>2</sub> -0.75 + POE + 0.75 equiv NaOH + <i>m</i> <sub>KCl</sub> of 0.3 mol·kg <sup>-1</sup>	-0.165	0.0084	0.280	0.92

<sup>a</sup>Polymer rich in bottom phase. <sup>b</sup>Polymer rich in top phase.

new polymer described in this work may allow its effective removal and recycling.

**3.5. The Correlation of the Phase Diagrams Using the Method of Diamond and Hsu.** The correlation of the phase diagrams, developed by Diamond and Hsu<sup>40</sup> based on Flory-Huggins theory, in order to check the consistency of the tie-lines of the 3 or 5 or 7-UPE 2-water (KCl) systems is given by the following equations:

$$\ln K_1 = A_1(w_1'' - w_1') \quad (8)$$

and

$$\ln K_2 = A_2(w_2'' - w_2') \quad (9)$$

where *w''* and *w'* are the polymer weight percent in the top and bottom phase, respectively, the slopes *A*<sub>1</sub> and *A*<sub>2</sub> are related to the polymer molar mass and their interactions with water, *K*<sub>1</sub> and *K*<sub>2</sub> represent the partition coefficient (*C*<sub>t</sub>/*C*<sub>b</sub>) of the polymer between the top and bottom phase, and the subscripts 1 and 2 represent polymer 1 (3 or 5 or 7) and polymer 2 (UPE 2 or POE). The correlation results are shown in Figure 7. Straight lines having a very good square of correlation coefficients indicate satisfactory representation of the phase behavior by this model. The simplicity of the model lies in its ability to correlate phase system data since only a single phase equilibrium composition is required to determine *A*<sub>1</sub> and *A*<sub>2</sub>, which helps to avoid extensive phase equilibrium determination.

Using eqs 8 and 9, the adjustable parameter  $A_1$  and  $A_2$  of the partition constant correlation was obtained by using a linear regression with an intercept value of zero. The experimental and the calculated partition constants,  $K_{\text{exp}}$  and  $K_{\text{cal}}$  were used to determine the root mean-square deviation (rmsd) by eq 10<sup>41</sup>

$$\text{rmsd} = \frac{\sqrt{\sum_{i=1}^N (K_{\text{exp}} - K_{\text{cal}})_i^2}}{N} \quad (10)$$

where  $N$  is the number of tie lines. The adjustable parameters and rmsd values are presented in Table 9 which shows that that Diamond and Hsu equations based on Flory–Huggins model can accurately correlate with the data collected in this work.

#### 4. CONCLUSIONS

A series of ATPSs was constructed using UPE or POE and several pH-responsive polysulfobetains as polymer components. The effect of charge type and their densities on the polymer chain and salt concentration on the phase diagram have been investigated in some details. Dependence of the solubility behavior of the polyzwitterions on the pH of the media makes them suitable candidates for industrial applications involving bioseparations since at lower pH the polymer in the zwitterionic form can be precipitated out and recycled. The charge types and their densities on the polymer chain may be judiciously chosen to lure certain biomolecules like protein to selectively enter into the phase containing the biomimicking polymer-rich phase. The presence of increasing amount of zwitterionic fractions in the polymer chain makes the polymer less compatible with UPE 2. Further studies on the effect of the presence of charge types and their densities on the polymer chain in protein separation are currently under investigation in our laboratory. The phase separation occurred at relatively low total polymer concentrations (below a mass fraction of 0.10), making the ATPSs useful from an industrial point of view.

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##### Notes

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