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Liquid−Liquid Equilibrium of Poly(ethylene glycol) 6000 + Triammonium Citrate + Water Systems at Different Temperatures

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Liquid−liquid equilibrium for an aqueous two-phase system containing poly(ethylene glycol) 6000 + triammonium citrate + water was studied at five different temperatures, (25, 30, 35, 40, and 45) °C. The binodal curve was fitted to an empirical equation relating the concentrations of PEG 6000 and triammonium citrate, and the coefficients were estimated for the respective temperatures. The effective excluded volume values were obtained from the binodal data. Tie line compositions were estimated and correlated using Othmer−Tobias and Bancroft equations, and the parameters are reported.

Introduction

Aqueous two-phase systems (ATPS) consisting of two incompatible polymers or a polymer and an inorganic salt to water have been developed in recent years, due to the advantage of its predominant water content (70 to 90 %) in both the phases, leading to the formation of a mild environment for the biomolecule separation. Due to the differences in the concentration of polymer and the salt in the top and bottom phases, several solute molecules, such as biological macromolecules will be unevenly distributed in each phase, depending upon their solubility in each phase, which enhances the separation of specific biomolecules from their crude.[1] ATPS have found application in the extraction of metal ions[2] and also for the recovery of biological products.[3] ATPS with PEG and salt have proved to be efficient in partitioning processes both for biomaterials and for metal ions. Further, the ATPS often give good yields with high purity and higher capacity and their ease of scaling up the process. Albertsson[1] listed many aqueous two-phase systems including the PEG−ammonium salt−water system, but no detailed partition data were reported apart from a rough phase diagram. ATPS consisting of polymer−salt−water have the advantages of low viscosity and low cost compared with polymer−polymer−water systems. Recently, citrate salts were used as a substitute for phosphate and sulfate salts to form an aqueous two-phase system with PEG because of the biodegradable and nontoxic nature of the citrates. The PEG + citrate salt + water system could form environmentally safe aqueous two-phase systems.[3−5] However, only a very limited amount of research work has been reported using ammonium citrate salts. Hence, in the present work, it is proposed to develop data on binodal and tie lines at different temperatures, (25, 30, 35, 40, and 45) °C, by conducting the experiments using the PEG 6000 + triammonium citrate + water system.

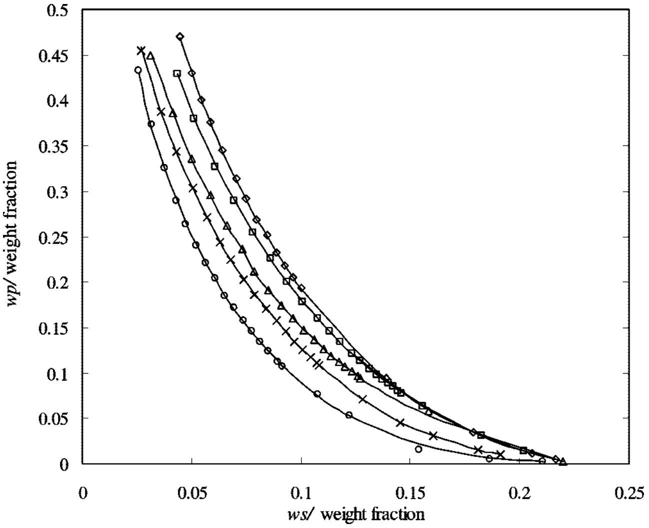

Figure 1. Effect of temperature on the binodal curve for PEG 6000 + triammonium citrate + water. Experimental binodal points for ◇, 25 °C; □, 30 °C; Δ, 35 °C; ×, 40 °C; and ○, 45 °C; −, calculated from eq 3.

Experimental Section

Materials. Analytical grade poly(ethylene glycol) 6000 (Merck-Schuchardt (Hohenbrunn bei Mü nchen, Germany), cat. no: 8.07490.1000) with a molar mass average of 5400 and triammonium citrate ($(NH_4)_3C_6H_5O_7$) (Cat. No. 21315, 'SQ' grade, Qualigens, India) with a minimum purity of 99 % were used. Double distilled, deionized water was used for the present experiments. The polymer and salts were used without further purification.

Apparatus and Procedures. To construct the binodal curves for the present system, all the experiments were carried out using a glass vessel with a working volume of 200 cm^3. The temperature of the working vessel was maintained by circulating water through an external jacket using a thermostat (Schott- Gerate CT 52, Germany). The temperature was

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Table 1. Binodal Data as Mass Fraction for the PEG 6000 + Triammonium Citrate + Water System at Different Temperatures

T/°C = 25		*T*/°C = 30		*T*/°C = 35		*T*/°C = 40		*T*/°C = 45	
100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p
4.48	47.02	4.35	42.94	3.11	44.98	2.68	45.54	2.57	43.30
5.00	43.07	5.12	38.00	4.15	38.65	3.62	38.75	3.17	37.32
5.46	40.09	6.09	32.70	5.11	33.64	4.32	34.34	3.76	32.57
5.86	37.59	6.93	28.89	5.89	29.60	5.04	30.39	4.28	29.00
6.43	34.52	7.79	25.46	6.61	26.28	5.70	27.15	4.76	26.30
7.03	31.34	8.60	22.55	7.32	23.63	6.31	24.45	5.24	23.99
7.47	29.20	9.36	20.05	7.87	21.24	6.81	22.47	5.67	22.05
7.98	26.91	10.08	17.88	8.50	19.10	7.39	20.25	6.08	20.37
8.44	25.19	10.76	16.00	9.09	17.42	7.88	18.58	6.54	18.53
8.88	23.22	11.30	14.61	9.62	16.00	8.39	17.09	6.97	17.18
9.28	21.87	11.81	13.38	10.11	14.77	8.88	15.79	7.37	15.70
9.64	20.49	12.34	12.19	10.59	13.65	9.32	14.65	7.74	14.56
10.02	19.33	12.73	11.35	11.02	12.70	9.71	13.49	8.13	13.48
13.91	9.40	13.14	10.52	11.39	11.93	10.10	12.55	8.51	12.39
17.88	3.44	13.46	9.87	11.72	11.27	10.43	11.77	8.92	11.28
20.59	1.11	13.74	9.35	12.03	10.67	10.73	11.10	9.15	10.69
21.68	0.50	14.00	8.88	12.31	10.16	10.82	10.91	10.76	7.61
		14.22	8.48	12.59	9.67	12.80	7.16	12.25	5.31
		14.46	8.07	12.73	9.43	14.57	4.56	15.40	1.51
		14.61	7.81	15.82	5.80	16.03	3.07	18.63	0.46
		12.36	12.14	22.00	0.26	18.11	1.53	21.04	0.22
		15.57	6.31			19.12	1.00		
		18.25	3.10						
		20.20	1.40						

Table 2. Tie Line Data as Mass Fraction for the PEG 6000 + Triammonium Citrate + Water System at Different Temperatures

	feed composition			top-phase composition			bottom-phase composition		
no.	w_p	w_s	w_w	w_p	w_s	w_w	w_p	w_s	w_w
				T/°C = 25					
1	0.12	0.14	0.74	0.2041	0.0973	0.6985	0.0090	0.2049	0.7862
2	0.15	0.14	0.71	0.2547	0.0848	0.6605	0.0038	0.2236	0.7726
3	0.18	0.14	0.68	0.3001	0.0736	0.6263	0.0032	0.2421	0.7548
4	0.21	0.14	0.65	0.3489	0.0623	0.5888	0.0035	0.2566	0.7399
5	0.24	0.14	0.62	0.3959	0.0523	0.5518	0.0069	0.2768	0.7164
				T/°C = 30					
1	0.12	0.14	0.74	0.2299	0.0861	0.6840	0.0031	0.2053	0.7916
2	0.15	0.14	0.71	0.2804	0.0736	0.6460	0.0042	0.2207	0.7752
3	0.18	0.14	0.68	0.3239	0.0661	0.6100	0.0033	0.2354	0.7613
4	0.21	0.14	0.65	0.3632	0.0573	0.5795	0.0029	0.2542	0.7429
5	0.24	0.14	0.62	0.4042	0.0511	0.5447	0.0057	0.2733	0.7210
				T/°C = 35					
1	0.12	0.14	0.74	0.2607	0.0736	0.6657	0.0049	0.1980	0.7970
2	0.15	0.14	0.71	0.3095	0.0623	0.6282	0.0069	0.2116	0.7815
3	0.18	0.14	0.68	0.3494	0.0573	0.5933	0.0058	0.2299	0.7643
4	0.21	0.14	0.65	0.3913	0.0486	0.5601	0.0055	0.2463	0.7482
5	0.24	0.14	0.62	0.4315	0.0411	0.5275	0.0025	0.2632	0.7343
				T/°C = 40					
1	0.12	0.14	0.74	0.2970	0.0542	0.6488	0.0026	0.1977	0.7998
2	0.15	0.14	0.71	0.3229	0.0598	0.6173	0.0021	0.2119	0.7860
3	0.18	0.14	0.68	0.3729	0.0523	0.5748	0.0070	0.2295	0.7635
4	0.21	0.14	0.65	0.4095	0.0473	0.5432	0.0048	0.2452	0.7500
5	0.24	0.14	0.62	0.4411	0.0436	0.5153	0.0027	0.2625	0.7348

Table 3. Values of Parameters for Equation 3 for the PEG 6000 + Triammonium Citrate + Water System at Different Temperatures

temp/°C	a_1	b_1	c_1	AARD/%	sd
25	1.3873	−5.4835	5.40116	0.2409	0.069
30	1.2013	−4.61829	4.39782	0.2567	0.067
35	1.0640	−5.9274	4.06842	0.7569	0.207
40	1.0597	−4.3873	4.54337	0.7956	0.205
45	0.9670	−4.2563	4.69339	1.5414	0.712

Table 4. Effective Excluded Volumes as Determined by Regression of Statistical Geometry Model (Equation 4) for the PEG 6000 + Triammonium Citrate + Water System at Different Temperatures

temp/°C	EEV/g·mol^{-1}	AARD/%	sd
25	45.8113	2.2966	5.3005
30	45.3431	1.3412	3.6585
35	48.3751	1.5822	4.2694
40	52.1873	1.5178	6.1415
45	58.5834	1.3129	7.7085

maintained with an uncertainty of ± 0.05 K. The titration method (cloud point method) was used to determine the phase equilibrium concentrations for establishing binodal curves. Stock solutions of known concentrations were prepared and kept in a constant-temperature bath. To ensure the uniform concentration of the constituents of ATPS in the jacketed vessel, constant stirring was applied by using a magnetic stirrer. A salt solution of known concentration was titrated against the polymer solution or vise versa, until the clear solution turned turbid. An analytical balance with a precision of ± 0.1 mg (OHAUS Essae-Teraoka, model AR2140) was used to determine the composition of the mixture.

The phase equilibrium studies were carried out in glass separators (100 cm^3). The feed samples (50 g basis) were prepared by mixing appropriate amounts of polymer, salt, and water in the vessel. The sample was stirred for (3 to 4) h at a desired temperature, and then the mixture was allowed to settle for 24 h at constant temperature using a thermostat. The two phases were then separated. The refractive index method[4,5] (Abbe-type refractometer) and conductivity methods[6,7] were used to measure the polymer and salt compositions, respectively. The precision of the mass fraction of triammonium citrate and PEG measurements was better than ± 0.002 and ± 0.001, respectively.

The concentration of PEG in both phases was determined by refractive index measurements using an Abbe-type refractometer. The relation between the refractive index, n_D, and the mass fraction of polymer, w_p, and salt, w_s, is given by

$$n_D = a_o + a_1 w_p + a_2 w_s \qquad (1)$$

The values of the fitting parameters a_0, a_1, and a_2 for the PEG 6000 + triammonium citrate + water system were obtained as 1.3285, 0.1522, and 0.2150, respectively, and the estimated errors are within ± 1.0 %. The above equation was originally suggested by Cheluget et al.[7] for the poly(propylene glycol) + NaCl + H_2O system, which was later successfully applied by many authors[3−5,8−10] for various

Table 5. Values of Parameters in Equations 5 and 6 for the PEG 6000 + Triammonium Citrate + Water System at Different Temperatures

	Othmer−Tobias equation (eq 5)				Bancroft equation (eq 6)			
temp/°C	K	n	R^2	AARD/%	K_1	r	R^2	AARD/%
25	0.1507	2.3913	0.9974	1.5392	2.2689	0.4334	0.9953	0.8304
30	0.1737	2.1527	0.9852	3.1145	2.3325	0.4776	0.9842	1.4918
35	0.1626	2.0204	0.9923	1.8176	2.5353	0.5068	0.9939	0.8016
40	0.2025	1.7605	0.9946	1.5958	2.5819	0.5717	0.9972	0.6563

combinations of PEG + salt + water systems. Similarly, the relation between the conductivity and the salt concentrations is represented by

$$\kappa = b_o + b_1 w_s \qquad (2)$$

where κ is the conductivity (μS·cm^{-1}); the values of b_o and b_1 for triammonium citrate solution are 2.1786 and 799.4, respectively; and the estimated errors are within ± 1.0 % (AARD = 0.42 %).

Results and Discussion

The formation of the two phases in the polymer–polymer system is enthalpy driven, whereas the polymer−salt system is due to the salting out effect on the polymer. The incompatibility existing between water-soluble polymers/salts leads to the formation of two aqueous phases. At low polymer concentrations, the other polymer/salt is miscible and coexists in a single phase. Above a critical concentration, they will separate into two phases, where each phase is enriched in one of the polymers/salt. The phase system for the PEG 6000 + triammonium citrate + water is described (Figure 1) for five different temperatures, (25, 30, 35, 40, and 45) °C, and the corresponding values are presented in Table 1. The binodial curve describes the border between the single-phase area and the two-phase area. The area above the binodial describes all compositions giving rise to two-phase systems. Table 2 provides the tie line data for the present system at different temperatures, (25, 30, 35, and 40) °C. From Figure 1, it can be observed that an increase in temperature expands the two-phase area of the binodal curve due to the increase in the salt solubility. Further, the experimental binodal data for the PEG 6000 + triammonium citrate + water system at five different temperatures were fitted by modifying the constants and coefficients of the expressions available in the literature. Recently, the binodal data of the PEG 2000 + sodium citrate + water,[4] PEG 6000 + sodium citrate + water,[5] PEG 2000 + potassium citrate + water,[9] and PEG 4000 + diammonium hydrogen phosphate + water[10] were fitted to the following empirical equation

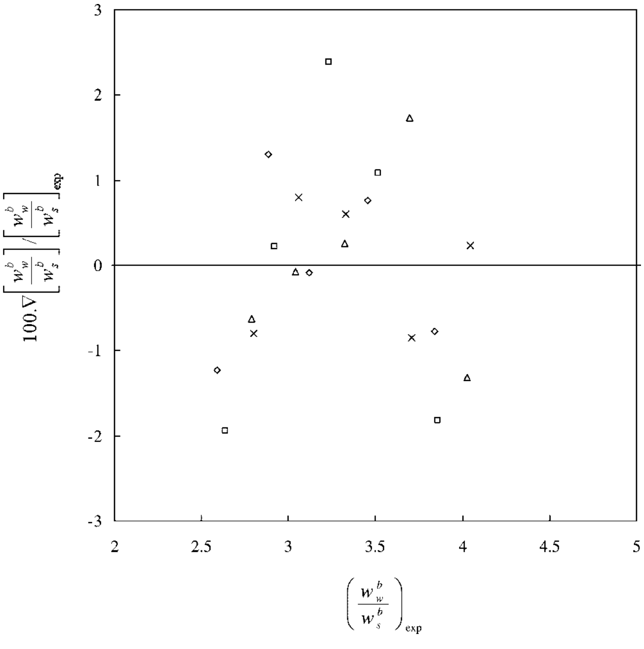

Figure 2. Relative deviation ($\nabla[w_w^b/w_s^b] = [w_w^b/w_s^b]_{exp} - [w_w^b/w_s^b]_{cal}$) of the tie line composition calculated through eq 6 for different temperatures. ◇, 25 °C; □, 30 °C; Δ, 35 °C; ×, 40 °C.

$$w_p = a_1 + b_1 w_s^{0.5} + c_1 w_s \qquad (3)$$

where w_p and w_s are the mass fractions of PEG 6000 and triammonium citrate, respectively. The present experimental binodal data of the poly(ethylene glycol) 6000 + triammonium citrate + water system were also fitted to eq 3 by regression analysis, and the coefficients are listed in Table 3 for different temperatures along with corresponding standard deviation and AARD.

An aqueous two-phase system can be applied as a primary recovery step to recover the targeted protein (whole cells and cell debris), in which generally the proteins moved to the light PEG-rich phase. The other molecules (nucleic acids) are left behind in the heavy salt-rich phase. Protein has no net charge at its isoelectric point, but if the pH is increased or decreased by the addition of appropriate salts, the net charge of the protein changes and will become either negative or positive. This net charge can be used to direct the protein partitioned in the system.[11,12] If the top phase is more hydrophobic relative to the bottom phase, then the partition coefficient increases in combination with hydrophobic anions for proteins carrying positive net charges. The salting-out strength of the salt could to be related to effective excluded volume (V_{123}^*), as proposed by Huddleston et al.[13] In the present work, the effective excluded volume of the of PEG 6000 + triammonium citrate + water system was predicted by using the model developed by Guan et al.[14] which is based on the statistical geometry methods for aqueous polymer−polymer systems. The binodal equation for the aqueous polymer−salt systems can be written as

$$\ln\left(V_{123}^* \frac{w_p}{M_p}\right) + V_{123}^* \frac{w_s}{M_s} = 0 \qquad (4)$$

where V_{123}^* is the effective excluded volume (EEV). M_P and M_s are the molar mass of polymer (5400 g/mol) and salt (243.22 g/mol), respectively. In the present work, the effective excluded volume along with the corresponding standard deviations were determined for all five temperatures using the present experimental binodal data and given in Table 4.

The Othmer−Tobias (eq 5) and Bancroft (eq 6) equations[15] were used to predict the tie line compositions of the polyethylene glycol 6000 + triammonium citrate + water system, as given below.

$$\frac{1 - w_p^t}{w_p^t} = K\left(\frac{1 - w_s^b}{w_s^b}\right)^n \qquad (5)$$

$$\left(\frac{w_w^b}{w_s^b}\right) = K_1\left(\frac{w_w^t}{w_p^t}\right)^r \qquad (6)$$

where K, n, K_1, and r are the fitting parameters. Superscripts t and b represent the polymer-rich phase (top phase) and the salt-rich phase (bottom phase), respectively. Subscripts p, s, and w stand for PEG, salt, and water, respectively. Equations 5 and 6 were earlier successfully applied for other similar polymer−salt systems,[3−5,8−10] hence those equations were used to correlate the tie line composition of the polyethylene glycol 6000 + triammonium citrate + water system. The constants and coefficients of eqs 5 and 6 along with the AARD are reported in Table 5. With these estimated constants, both eq 5 and 6 were used to verify the material balance for the top and bottom phases. The relative deviation between the experimental and correlated data using eq 6 for the temperatures (25, 30, 35, and 40) °C are shown in Figure 2.

Conclusion

Binodal curves and tie line compositions were measured for the PEG 6000 + triammonium citrate + water systems at different temperatures, (25, 30, 35, 40, and 45) °C. It was found that as the temperature increases the binodal curve shifts down due to the increase in solubility, resulting in an increase in the two-phase region. The binodal and tie line data were correlated using the available empirical equations, and the values of the fitting parameters were also reported. The effective excluded volume values were estimated from the bimodal data, and the salting out ability was discussed

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