Experiment and Correlation of Liquid−Liquid Equilibria of an Aqueous Salt Polymer System Containing PEG6000 + Sodium Citrate

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Liquid−Liquid equilibrium data were obtained for an aqueous two-phase system containing poly(ethylene glycol)-6000 (PEG6000) + sodium citrate + water at three different temperatures, 293.15, 303.15, and 313.15 K. An empirical equation relating the concentrations of PEG6000 and sodium citrate was used to fit the binodal curve, and the coefficients were estimated for the corresponding temperatures. The effect of temperature on binodal and tie line data was studied. The Othmer−Tobias and Bancraft equations were used to correlate the tie line compositions, and the parameters are reported.

Introduction

An aqueous two-phase extraction system has become an important emerging technique for separation, concentration, and purification of proteins, cell organelles, and other biological products. Aqueous two-phase systems were first applied to recover biomolecules by Albertsson.[1] He showed that micro-organisms, cell walls, chloroplasts, chloroplast vesicles, and other biological molecules could be selectively partitioned between the phases of an aqueous two-phase systems (ATPS) composed of either two polymers or a polymer and an inorganic salt. Aqueous polymer−salt systems have several advantages over the polymer−polymer systems due to the larger differences in density, greater selectivity, lower viscosity, lower cost, etc.

Most of the research works on aqueous polymer−salt systems were made using poly(ethylene glycol) with salt consisting of selective cations, namely, ammonium, potassium, or sodium, and anions, namely, phosphate, sulfate, or carbonates. These salts, however, led to a high concentration of sulfate and phosphate salts in the effluent streams, causing environmental problems. Recently, Vernau and Kula,[2] Zafarani-Moattar et al.,[3] and Murugesan and Perumalsamy[4] have used citrate as a substitute for phosphate and sulfate salts to form an aqueous two-phase system with PEG. Because citrates are biodegradable and nontoxic, PEG + citrate salts could form environmentally safe aqueous two-phase systems, which are more suitable for the extraction of biological materials. However, only a very limited amount of research work has been reported using citrate salts. Vernau and Kula[2] have determined binodal and tie line data for the aqueous PEG1550 + sodium citrate system at room temperature. Murugesan and Perumalsamy[4] have reported liquid−liquid equilibrium data of the PEG2000 + sodium citrate + water system at (25, 30, 35, 40, and 45) °C. In the present work, the binodal data and liquid−liquid equilibrium data for PEG6000 + sodium citrate + water at 293.15, 303.15, and 313.15 K were measured and correlated using the Othmer−Tobias and Bancraft model equations.

Experimental

Materials. Analytical grade (Merck) poly(ethylene glycol) with a molar mass average of 6000 and tribasic sodium citrate

Table 1. Binodal Data for the PEG6000 (1) + Sodium Citrate (2) + Water (3) System at 293.15, 303.15, and 313.15 K

293.15 K		303.15 K		313.15 K	
100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2
48.23	1.06	48.57	0.86	47.99	0.81
46.37	1.33	44.93	1.39	45.72	1.15
43.05	1.80	41.93	1.76	43.79	1.41
40.29	2.13	39.30	2.08	40.39	1.86
37.87	2.42	36.98	2.37	37.57	2.21
35.53	2.82	34.83	2.69	36.35	2.34
33.63	3.04	32.92	2.98	35.21	2.46
31.70	3.42	31.28	3.18	34.14	2.58
29.98	3.77	29.66	3.47	32.92	2.72
28.50	4.02	28.21	3.74	31.77	2.93
25.74	4.65	26.88	3.98	31.08	3.00
23.50	5.13	25.59	4.30	29.04	3.33
21.59	5.57	24.49	4.49	28.31	3.40
19.22	6.14	23.49	4.67	27.30	3.59
17.20	6.75	22.49	4.92	26.56	3.71
15.05	7.46	21.61	5.11	25.85	3.83
13.25	8.20	20.76	5.32	24.41	4.13
11.57	8.85	20.01	5.48	23.85	4.20
10.21	9.47	19.31	5.63	23.17	4.37
9.12	9.98	18.63	5.80	21.66	4.70
8.24	10.41	17.97	5.99	20.96	4.84
7.57	10.61	17.36	6.17	20.62	4.93
6.45	11.10	16.75	6.40	19.52	5.16
5.63	11.44	15.71	6.70	19.06	5.28
4.98	11.75	14.78	6.99	17.84	5.56
4.06	12.16	13.99	7.20	17.00	5.82
3.39	12.62	13.23	7.45	16.27	6.03
2.73	13.00	12.51	7.77	15.54	6.28
1.67	14.13	11.88	8.01	14.98	6.41
1.05	15.34	11.08	8.27	14.38	6.60
0.58	16.62	10.35	8.57	13.33	6.92
0.27	18.43	9.72	8.79	12.29	7.19
0.26	20.99	9.15	9.02	11.50	7.45
0.23	22.24	8.64	9.25	10.99	7.61
0.22	26.07	7.92	9.51	10.35	7.82
0.20	31.50	7.30	9.78	9.79	8.00
0.16	37.81	6.69	10.00	9.26	8.19
0.16	39.78	6.08	10.25	8.82	8.32
		5.50	10.51	8.58	8.43
		4.93	10.71	8.03	8.57
		4.47	10.88	7.20	8.88
		3.92	11.08	6.64	9.09
		3.48	11.27	6.16	9.28
		3.13	11.44	5.47	9.54
		2.83	11.63	4.54	9.87
		2.52	11.82	3.69	10.22
		2.26	12.04	3.26	10.41
		2.05	12.15	2.87	10.56
		1.88	12.27	2.13	10.89
		1.73	12.39	1.93	11.07
		1.61	12.50	1.69	11.33
		1.44	12.67	1.45	11.65
		1.09	12.92	0.96	12.24
		0.67	13.84	0.63	13.08
		0.40	15.19	0.45	13.89
		0.30	17.79	0.23	14.83
		0.25	20.57	0.17	17.85
		0.22	22.31	0.16	20.61
		0.20	28.19	0.13	22.36
		0.14	29.83	0.11	28.27
				0.11	29.83

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Table 2. Experimental Liquid−Liquid Equilibrium Data (w/w %) for PEG6000 (1) + Sodium Citrate (2) ATPS at 293.15, 303.15, and 313.15 K

293.15 K				303.15 K				313.15 K			
top phase		bottom phase		top phase		bottom phase		top phase		bottom phase	
W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2
30.24	3.65	1.86	20.76	26.49	4.08	1.00	18.93	23.45	4.43	0.99	16.35
33.91	3.04	0.93	24.16	28.53	3.62	0.91	19.45	29.15	3.30	0.23	18.14
39.03	2.31	0.81	25.36	32.12	3.01	0.79	20.30	34.51	2.62	0.22	20.47
41.49	2.04	0.61	26.62	33.49	2.91	0.57	21.85	37.41	2.26	0.21	22.20
43.21	1.80	0.20	28.05	35.31	2.65	0.18	23.14	39.47	2.04	0.19	23.18
46.14	1.36	0.12	30.59	37.23	2.42	0.12	23.89	45.32	1.21	0.13	24.84

dihydrate (Merck) with a minimum purity of 99 % were used. The polymer and salts were used without further purification. Double distilled, deionized water was used for the present experiments.

Apparatus and Procedure. The experiments were carried out using a glass vessel with a working volume of 500 cm^3 to determine the phase equilibrium (binodal curve). The glass vessel was provided with an external jacket in which water was circulated at constant temperature using a thermostat (Schott-Gerate CT 52, Germany). The temperature was maintained with an uncertainty of ± 0.05 K.

The binodal curves were determined by a titration method. Stock solutions of known concentrations were prepared and kept in a constant temperature bath, and their densities were measured. A magnetic stirrer was used to ensure constant stirring in the jacketed vessel. A salt solution of known concentration was titrated with the polymer solution or vice versa, until the clear solution turned turbid. The composition of the mixture was determined by mass using an analytical balance (OHAUS-Essae-Teraoka, model AR2140) with a precision of ± 0.1 mg.

Glass separators (100 cm^3) were used to carry out the phase equilibrium studies. The feed samples (60 g basis) were prepared by mixing appropriate amounts of polymer, salt, and water in the vessel. The sample was stirred for 1 h at a desired temperature, and then the mixture was allowed to settle for 24 h at constant temperature using a thermostat. After separation of the two phases, an Abbe-type refractometer and flame photometer were used for the determination of phase compositions. The concentrations of sodium citrate in the top and bottom phases were determined by flame photometry. The top and bottom phases were diluted to concentrations of less than 100 ppm of sodium ions. The concentration of sodium in the sample as given by the flame photometer was then converted in terms of the amount of salt. The precision of the mass fraction of sodium citrate and PEG was better than ± 0.002 and ± 0.001, respectively.

The concentration of PEG in both phases was determined by refractive index measurements performed at 303.15 K using an Abbe-type refractometer. The relation between the refractive index, n, and the mass fraction of polymer, w_p, and salt, w_s, is given by

$$n = a_0 + a_1 w_p + a_2 w_s \tag{1}$$

The values of coefficients a_0, a_1, and a_2 for the PEG6000 + sodium citrate + water system were obtained as 1.3324, 0.1434, and 0.1572, respectively, and the estimated errors are within ± 1.0 %.

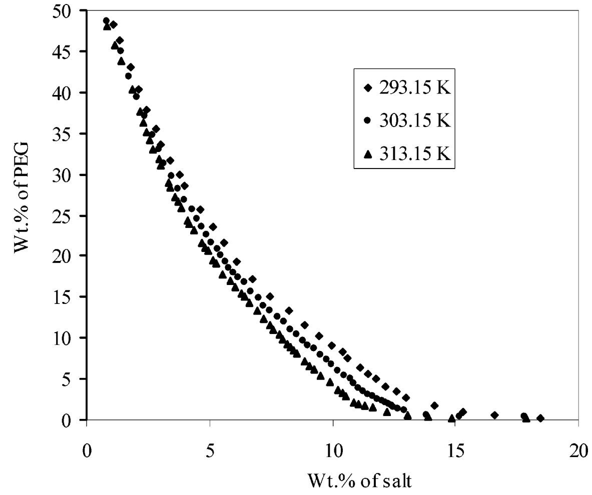

Figure 1. Binodal curves for the PEG6000 + sodium citrate + water system at 293.15, 303.15, and 313.15 K.

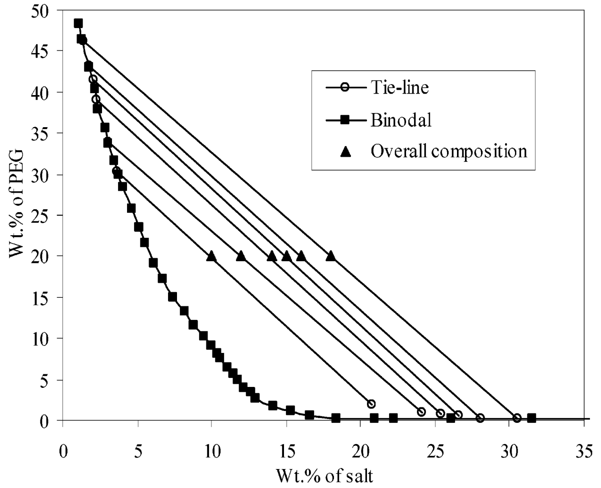

Figure 2. Binodal curves and the tie lines for the PEG6000 + sodium citrate + water system at 293.15 K.

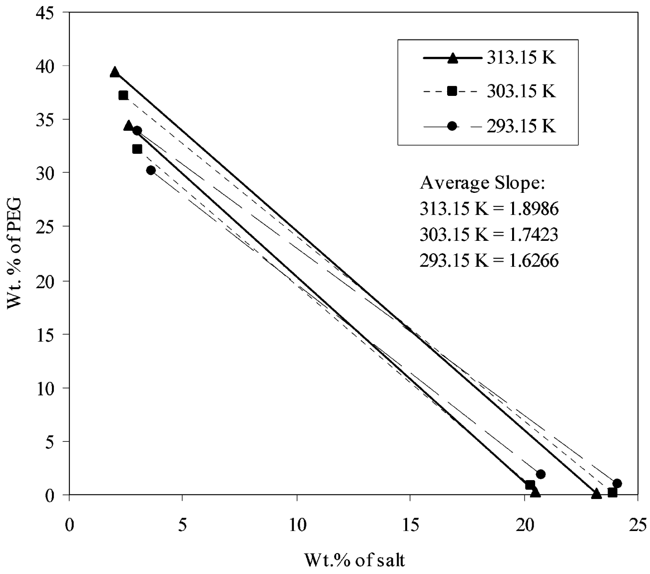

Figure 3. Effect of temperature on the tie lines for the PEG6000 + sodium citrate + water system.

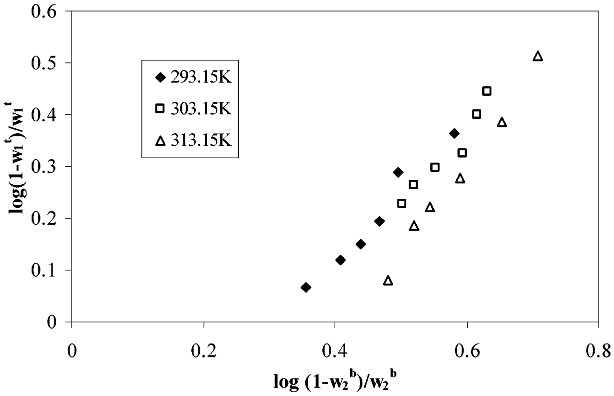

Figure 4. Linear dependency of the Othmer−Tobias equation at 293.15, 303.15, and 313.15 K.

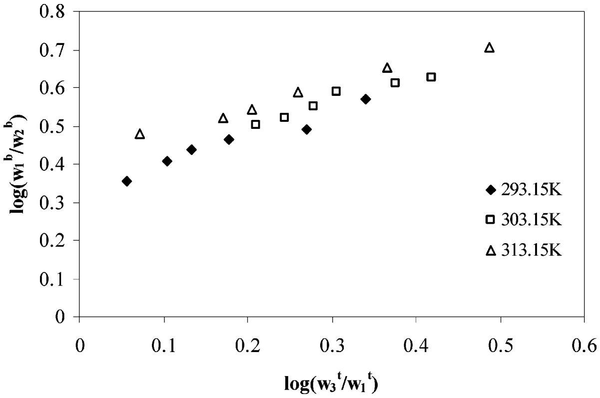

Figure 5. Linear dependency of the Bancroft equation at 293.15, 303.15, and 313.15 K.

Table 3. Parameters of Equations 2 and 3 for PEG6000 + Sodium Citrate + Water at Different Temperatures

temp	K	n	R^2	K_1	r	R^2
293.15 K	0.3593	1.3983	0.9666	2.1466	0.6812	0.9660
303.15 K	0.2895	1.5158	0.9883	2.3875	0.6183	0.9818
313.15 K	0.1754	1.7735	0.9879	2.7134	0.5681	0.9874

Results and Discussion

The binodal data, for the PEG6000 + sodium citrate + water system, representing the minimum concentration required for the formation of two phases at temperatures 293.15, 303.15, and 313.15 K are given in Table 1. The tie line compositions at three different temperatures, 293.15, 303.15, and 313.15 K, are given in Table 2. The effect of temperature on the binodal curves of the investigated aqueous PEG6000 + sodium citrate system is represented in Figure 1, which shows that an increase in temperature expands the two-phase area of the binodal curve, which is attributed to the increase in solubility. The experimental tie line data at 293.15 K are shown in Figure 2, as an example. Also, the effects of temperature on tie line data are shown in Figure 3. From the figure, it was observed that the slope and the length of the tie line increase with an increase in temperature. In the aqueous two-phase system, PEG and salt are predominantly present in the top and bottom phase, respectively, due to the increase in hydrophobicity of PEG molecules with an increase in temperature. Voros et al.[5] and Mishima et al.[6] reported similar results for other aqueous PEG + salt systems.

Correlation of Tie Line

The suggested correlating equations proposed by Othmer−Tobias[7] (eq 2) and Bancroft[8] (eq 3) have been used to correlate the tie line compositions for the PEG6000 + sodium citrate + water system.

$$[(1 - w_p^t)/w_p^t] = K[(1 - w_s^b)/w_s^b]^n \qquad (2)$$

$$(w_w^b/w_s^b) = K_1(w_w^t/w_p^t)^r \qquad (3)$$

where K, n, K_1, and r are the fit parameters. The superscripts t and b represent the polymer-rich phase (top phase) and the salt-rich phase (bottom phase), respectively. The subscripts p and s stand for the PEG and salt, respectively. Figures 4 and 5 show the linear dependency of the plots $\log[(1 - w_p^t)/w_p^t]$ against $\log[(1 - w_s^b)/w_s^b]$ and $\log(w_w^b/w_s^b)$ against $\log(w_w^t/w_p^t)$, respectively. The values of the estimated parameters are given in Table 3. Using the estimated parameters, uncertainties of the tie lines are found to be within an rms error of ± 1 % indicating an acceptable consistency of the results. The corresponding correlation coefficient values and the constants of eqs 2 and 3 are given in Table 3. Recently, eqs 2 and 3 have been successfully used for the correlation of tie line compositions of the PEG6000 + potassium citrate + water system.[9]

Conclusion

The binodal data and tie line data for the system PEG6000 + sodium citrate + water at 293.15, 303.15, and 313.15 K were obtained. As the temperature increases, the two-phase area expands due to an increase in solubility. It was also observed that the slope and length of the tie lines increased with increasing temperature. The tie line data for the PEG6000 + sodium citrate + water system were correlated satisfactorily using the Othmer−Tobias and Bancroft equations.

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