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Liquid−Liquid Equilibria of Poly(ethylene glycol) 2000 + Sodium Citrate + Water at (25, 30, 35, 40, and 45) °C

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The effect of temperature on the liquid−liquid equilibrium for the aqueous two-phase system containing poly(ethylene glycol) 2000 + sodium citrate + water was studied at (25, 30, 35, 40, and 45) °C. An equation for the binodal curves was obtained in terms of the concentrations of PEG 2000 and sodium citrate, and the temperature effect was also incorporated into the equation parameters. The tie lines were correlated using the Othmer−Tobias and Bancraft equations as well as osmotic virial equation. The mean deviations between the experimental and calculated equilibrium compositions in the top and bottom phases were <1 %.

Introduction

Aqueous two-phase systems are formed when two mutually incompatible water-soluble polymers or one polymer and one inorganic salt are dissolved in water above a threshold concentration.[1] These systems contain mainly water with the first polymer predominating in one phase and the second polymer or salt predominating in the other phase. Because of their high water mass fraction (>80 %), both equilibrium phases provide a gentle and protective environment for the purification and separation of biomolecules such as proteins, enzymes, plant cells, animal cells, viruses, and nucleic acids. This extraction technique presents a powerful tool for the separation of biological materials in particular to retain the structure and activity of the biomolecules. In most of the earlier studies, PEG had been combined with either phosphate or sulfate salts to form aqueous two-phase systems. These salts, however, led to high concentrations of sulfate and phosphate salts in the effluent streams, causing environmental problems. Recently, Vernau and Kula[2] and Zafarani-Moattar et al.[3] have used citrate as a substitute for phosphate and sulfate salts to form 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, the available experimental data for PEG + citrates + water systems are limited, and data for the binodal and the phase diagram of the PEG 2000 + sodium citrate + water system are not available in the literature. Zafarani-Moattar et al.[3] reported liquid−liquid equilibriam data for the aqueous PEG 6000−sodium citrate system at (25, 35, and 45) °C. Vernau and Kula[2] reported equilibrium compositions for the PEG 1550 + sodium citrate + water system; the experiments were conducted at room temperature, hence the temperature effects were not reported.

In the present work, the binodal data for PEG 2000 + sodium citrate + water at five different temperatures (25, 30, 35, 40, and 45) °C and liquid−liquid equilibrium data at (25, 35, and 45) °C were measured and correlated with model equations.

Experimental Section

Materials. Analytical-grade poly(ethylene glycol) (Merck) with a molar mass average of 2000 (cat. no. 8.21037.1000) and tribasic sodium citrate dihydrate with a minimum purity of 99 % (cat. no. 61770905001046) were used. The polymer was in powdered form, and the polydispersity index was 1.05. 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 100 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 °C. The binodal curve was determined by the cloud point method.[1] From the stock, a PEG solution of known concentration was taken into the vessel. A sodium citrate solution of known mass fraction was added drop by drop to the vessel and mixed. The point where the mixture became turbid or cloudy was noted, and the amount of salt solution added was determined. Then the solution became clear on addition of water, and the mass of the mixture was noted. Again, sodium citrate solution was added dropwise until the mixture turned turbid, and the above procedure was repeated over a whole range of concentrations starting with PEG and sodium citrate. The composition of the aqueous mixture was determined by mass using an analytical balance (Ohaus-Essae-Teraoka, model AR2140) with a precision of 0.1 mg. The aqueous two-phase systems were prepared in a 50 cm^3 centrifuge tube using an analytical balance and were stored in a water bath to maintain the appropriate temperature with an uncertainty of ±0.05 °C. The systems were stirred and mixed well and allowed to settle to ensure the proper phase separation, as indicated by the absence of turbidity in each phase, and then separated to measure the equilibrium compositions at the corresponding set temperature. For the determination of tie line compositions, the concentrations of sodium citrate in the top and bottom phases were determined by flame photometry.[3] The concentrations of PEG in both phases were determined by refractive index measurements. The uncertainties of the estimated mass fraction of sodium

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Table 1. Binodal Data for PEG 2000 (p) + Sodium Citrate (s) + Water at Different Temperatures

$100w_p$	$100w_s$	$100w_p$	$100w_s$	$100w_p$	$100w_s$	$100w_p$	$100w_s$
$t = 25$ °C				$t = 40$ °C			
44.70	2.85	14.65	11.56	38.92	2.66	12.25	11.55
41.55	3.36	13.63	12.13	35.03	3.38	11.05	12.33
38.24	3.97	11.65	13.56	31.92	4.05	10.13	12.97
36.81	4.25	11.05	13.98	28.92	4.79	9.79	13.22
33.11	4.85	10.90	14.16	26.52	5.46	9.34	13.56
32.35	5.26	10.12	14.67	24.10	6.21	8.80	13.98
29.87	5.89	9.72	14.98	21.87	6.98	8.27	14.42
26.45	6.78	9.21	15.39	20.39	7.54	7.77	14.85
27.00	6.98	8.63	15.89	18.66	8.25	7.34	15.23
25.25	7.25	8.13	16.34	15.22	9.87	6.63	15.90
23.14	7.93	7.56	16.89	14.70	10.14	6.41	16.12
21.50	8.42	6.91	17.55	13.36	10.89		
20.39	9.01	6.48	18.02	$t = 45$ °C			
18.44	9.84	4.73	20.55	37.01	2.45	14.87	9.46
17.34	10.25	4.01	21.89	35.69	2.65	13.56	9.84
16.27	10.87	3.95	22.02	33.58	3.12	13.46	10.23
$t = 30$ °C				29.66	3.98	12.09	10.86
43.80	2.70	13.29	12.03	27.75	4.58	11.72	11.28
39.74	3.36	10.93	13.58	26.01	4.96	10.56	11.91
32.44	4.85	10.42	13.95	25.21	5.23	9.58	12.31
30.76	5.26	9.88	14.36	23.68	5.68	10.45	11.85
28.38	5.89	9.47	14.69	22.78	5.98	10.49	12.03
27.02	6.28	9.19	14.92	22.12	6.25	9.73	12.56
24.85	6.95	8.65	15.38	20.88	6.85	8.97	12.98
23.95	7.25	8.10	15.87	19.91	7.02	8.67	13.36
22.02	7.93	7.60	16.35	18.91	7.56	7.96	13.85
20.33	8.58	7.23	16.72	17.45	7.93	7.68	14.34
19.20	9.05	6.54	17.45	16.77	8.56	6.91	14.94
17.66	9.73	5.30	18.97	15.79	8.94		
16.53	10.27	4.87	19.56				
15.41	10.84	4.47	20.17				
14.27	11.46	3.05	22.85				
$t = 35$ °C							
41.51	2.68	14.83	10.89				
36.85	3.58	12.69	11.65				
33.37	4.15	11.83	12.43				
30.67	4.89	11.06	12.87				
28.10	5.56	10.64	13.02				
25.46	6.21	10.28	13.56				
23.46	6.98	9.52	13.98				
21.86	7.54	8.73	14.52				
19.41	8.45	8.83	14.85				
16.82	9.87	7.88	15.23				
15.77	10.14						

citrate and PEG were ±0.001 and ±0.002, respectively. The relation between the refractive index, n, and the mass fraction of polymer, w_p, and salt, w_s, is given by

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

which was originally suggested by Cheluget et al.[4] for the poly(ethylene glycol) + NaCl + water system and later was successfully used by Zafarani-Moattar et al. [3] for the poly(ethylene glycol) 6000 + sodium citrate + water system. Hence, the above equation was applied to the present PEG 2000 + sodium citrate + water system. The parameters of eq 1 were evaluated by the same method of calibration plots used by Cheluget et al.[4] The estimated values of coefficient $a_{0,}$ a_1, and a_2 for the present system were 1.3332, 0.1411, and 0.1478, respectively.

Results and Discussion

The phase equilibrium for an aqueous two-phase system can be represented as a binodal curve relating the concentration of the salt and poly(ethylene glycol) in the corresponding phases. The binodal data for the PEG 2000 + sodium citrate + water system is given in Table 1. The binodal curves at different temperature are represented in Figure 1. It is observed that the two-phase area is expanded with increasing temperature, which is in good agreement with the reported experimental results for the other aqueous two-phase systems.[3] In the previous studies, Graber et al.[5] and Taboada et al.[6] have used two nonlinear expressions to fit the binodal data of aqueous two-phase systems. Zafarani-Moattar et al.[7] have also developed an empirical equation to fit the binodal data for the PEG 6000 + potassium citrate + water system. In the present study, the binodal is found to vary with temperature. An attempt was made to fit the binodal data using different expressions, including those used previously; however, better results were obtained with the following empirical equation:

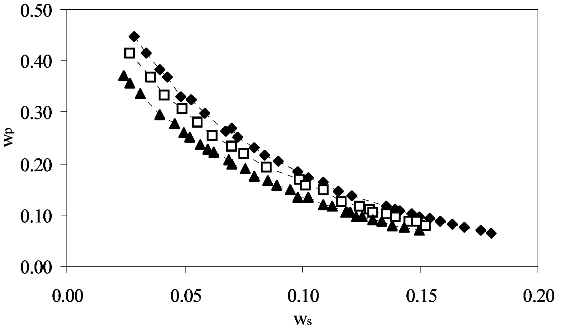

Figure 1. Binodal curves for PEG 2000 (p) + sodium citrate (s) + water at different temperatures: ◆, 25 °C; □, 35 °C; ▲, 45 °C; —, calculated using eqs 2 and 3.

$$w_p = A + B w_s^{0.5} + C w_s \tag{2}$$

where w_p and w_s are the mass fractions of PEG 2000 and sodium citrate, respectively. From the analysis of the present data, constants A, B, and C are found to be functions of temperature and are given by

$$A = a_0 + \frac{a_1 t}{°C} \tag{3a}$$

$$B = b_0 + \frac{b_1 t}{°C} \tag{3b}$$

$$C = c_0 + \frac{c_1 t}{°C} \tag{3c}$$

where t is the temperature of the system.The coefficients of eq 3 are given in Table 2. Tie line data for the PEG 2000 + sodium citrate + water system is given in Table 3. The tie line composition for the total composition of $w_p = 0.15$, $w_s = 0.125$ at different temperatures is shown in Figure 2. From the Figure, it was observed that the slope of the tie line increases with increasing temperature as reported by Voros et al.,[8] Zafarani-Moattar, and Sadeghi[9] for other aqueous PEG + salt systems. Hence, the poly(ethylene glycol) + sodium citrate + water system forms two phases at high temperatures more easily, that is, a smaller mass fraction of polymer or salt is sufficient for phase separation.

The correlating equations proposed by Othmer−Tobias[10] (eq 4) and Bancroft[11] (eq 5) have been used to correlate the tie line compositions:

$$\frac{1 - w_p^t}{w_p^t} = K\left(\frac{1 - w_s^b}{w_s^b}\right)^n \tag{4}$$

$$\left(\frac{w_w^b}{w_s^b}\right) = K_1\left(\frac{w_w^t}{w_p^t}\right)^r \tag{5}$$

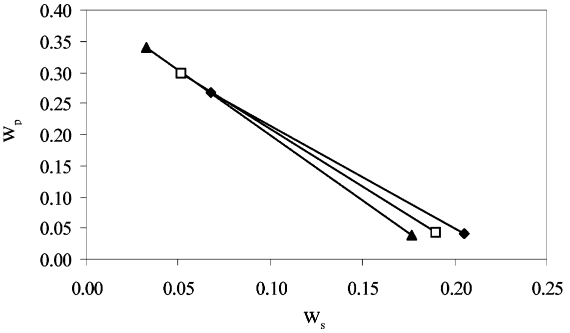

Figure 2. Effect of temperature on the equilibrium phase compositions for PEG 2000 (p) + sodium citrate (s) + water. These tie lines were obtained by connecting the experimental equilibrium phase composition for the total composition. ($w_p = 0.15$, $w_s = 0.125$): ◆, 25 °C; □, 35 °C; ▲, 45 °C.

Table 2. Parameters of Equation 3 for PEG 2000 + Sodium Citrate + Water at Different Temperatures

a_0	a_1	b_0	b_1	c_0	c_1
1.20179	−0.01093	−4.42209	0.04329	4.31343	−0.04914

Table 3. Tie Line Data for PEG 2000 (p) + Sodium Citrate (s) + Water at Different Temperatures

top phase		bottom phase	
$100w_p$	$100w_s$	$100w_p$	$100w_s$
	$t = 25$ °C		
26.79	6.77	4.17	20.50
30.78	5.66	3.23	23.25
34.43	4.89	2.58	25.40
38.32	3.89	1.70	28.11
	$t = 35$ °C		
24.86	6.58	5.99	15.85
29.89	5.16	4.30	19.00
34.22	4.41	3.30	21.51
37.44	3.37	2.09	23.63
41.04	2.64	1.15	26.08
	$t = 45$ °C		
30.53	3.88	4.67	15.65
34.05	3.23	3.76	17.68
38.31	2.20	2.36	19.76
40.78	1.88	1.81	21.28
43.43	1.37	1.04	23.05

Table 4. Parameters of Equations 4 and 5 for PEG 2000 (p) + Sodium Citrate (s) + Water at Different Temperatures

t/°C	K	n	K_1	r	σ_p	σ_s
25	0.4824	1.2816	1.8231	0.7689	0.03	0.24
35	0.4136	1.1919	2.1657	0.8101	0.03	0.24
45	0.3097	1.1850	2.7291	0.8127	0.09	0.39

where K, n, K_1, and r are the fit parameters. Superscripts t and b represent the polymer-rich phase (top phase) and the salt-rich phase (bottom phase), respectively. Subscripts p and s stand for PEG and salt, respectively. Equations 4 and 5 are linearized by taking the logarithm on both sides of the equations to determine the fit parameters. The values of fit parameters K, n, K_1, and r along with the mass percent standard deviations σ_p and σ_s are given in Table 4. Figures 3 and 4 show plots of $\log[(1 - w_p^t)/w_p^t]$ 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. Recently, eqs 4 and 5 have been successfully used for the correlation of tie line compositions of the PEG 6000 + potassium citrate + water system.[7]

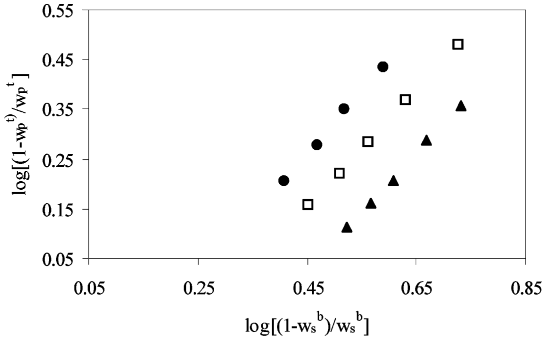

Figure 3. Linear dependency of the Othmer−Tobias equation: ●, 25 °C; □, 35 °C; ▲, 45 °C.

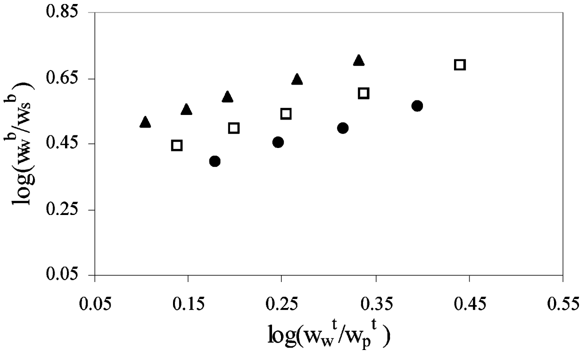

Figure 4. Linear dependency of the Bancroft equation: ●, 25 °C; □, 35 °C; ▲, 45 °C.

Mishima et al.[12] and Zafarani-Moattar et al.[3] have used the osmotic virial equation as adopted by Edmond and Ogston[13] for the correlation of LLE data of aqueous two-phase systems. The osmotic virial equation relates the chemical potentials of polymer (p) and salt (s) as a function of the molality of polymer and salt and can be written as

$$\mu_p = \mu_p^{\ 0} + RT(\ln m_p + \beta_{pp}m_p + \beta_{ps}m_s) \tag{6}$$

$$\mu_s = \mu_s^{\ 0} + RT(\ln m_s + \beta_{ss}m_s + \beta_{ps}m_p) \tag{7}$$

where β_{pp}, β_{ss}, and β_{ps} are the interaction parameters between a molecule of polymer (p) and salt (s) and μ_p^0 and μ_s^0 are the standard-state chemical potentials of polymer and salt, respectively. Using the Gibbs−Duhem relation, the chemical potential of water (w) is then obtained as

$$\mu_w = \mu_w^{\ 0} - RTV_3\rho\left(m_p + m_s + \frac{1}{2}(\beta_{pp})m_p^{\ 2} + \frac{1}{2}(\beta_{ss})m_s^{\ 2} + \beta_{ps}m_pm_s\right) \tag{8}$$

where μ_w^0 is the standard-state chemical potential of pure water. The Gibbs energy is a minimum when the system is in thermodynamic equilibrium. From classical thermodynamics, it is obvious that the top and bottom phases of the aqueous two-phase system containing polymer (p), salt (s), and water (w) will obey the following constraints at equilibrium:[3]

$$\mu_p^{\ top} = \mu_p^{\ bot} \tag{9a}$$

$$\mu_s^{\ top} = \mu_s^{\ bot} \tag{9b}$$

$$\mu_w^{\ top} = \mu_w^{\ bot} \tag{9c}$$

The interaction parameters are obtained by fitting the experimental LLE data to eq 9 using the objective function suggested by Zafarani-Moattar et al.[3] The evaluated interaction parameters are given in Table 5. To prove the

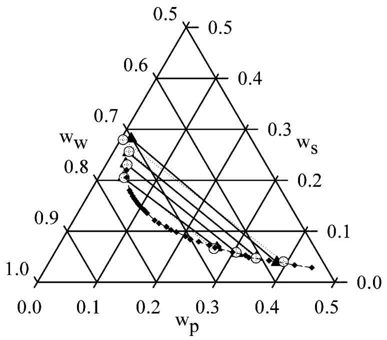

Figure 5. Binodal and tie lines for the PEG 2000 (p) + sodium citrate (s) + water system at 25 °C: ◆, binodal; ▲, experimental; ○, calculated using eq 9.

Table 5. Interaction Parameters of the Osmotic Virial Equation for the System PEG 2000 (p) + Sodium Citrate (s) + Water at Different Temperatures

t/°C	β_{pp}	β_{ps}	β_{ss}
25	−40.7096	−8.61399	−4.07036
35	35.47568	15.86263	3.538937
45	36.99634	19.56183	5.526164

reliability of predictions using eq 9, a comparison between the experimental and correlated tie lines for 25 °C is shown in Figure 5. Figure 5 shows the good agreement of experimental binodal data with the experimental and predicted tie line data with an error of <1 %.

Conclusions

Binodal data at (25, 30, 35, 40, and 45) °C and tie line compositions at (25, 35, and 45) °C were measured. From the data, it was observed that the two-phase region is expanded with increasing temperature. The binodal data is fit with an empirical equation relating the concentration of PEG and sodium citrate at different temperatures. The tie line data for the PEG 2000 + sodium citrate + water system was correlated satisfactorily by Othmer−Tobias and Bancroft equations as well as osmotic virial equations.

Note Added after ASAP Publication

This paper was released ASAP on June 17, 2005. Due to a production error, eq 2 was incorrect. The corrected version was posted on June 28, 2005.

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