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Liquid−Liquid Equilibrium of Poly(ethylene glycol) 2000 + Potassium Citrate + Water at (25, 35, and 45) °C

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Liquid−liquid equilibrium for an aqueous two-phase system containing poly(ethylene glycol) 2000 + potassium citrate + water was studied at three different temperatures of (25, 35, and 45) °C. The binodal curve was fitted to an empirical equation relating the concentrations of PEG 2000 and potassium citrate, and the coefficients were estimated for the respective temperatures. Tie line compositions were correlated using Othmer−Tobias and Bancroft equations, and the parameters are also reported.

Introduction

Extraction using volatile organic solvents is a common method for the isolation and purification of components in process industries. When two liquids are brought into contact with each other and later separated, there is an opportunity for the solute or the targeted molecules to distribute unevenly between the two liquids. For example, nonpolar compounds added to water and an organic solvent that is not miscible with water will shun the water and dissolve mostly in the organic solvent. The surface tension between water and organic solvents is so high that they are not suitable for the separation of biological materials. Recently, a new technology gaining importance for the separation and purification of protein and biomolecules is aqueous two-phase systems (ATPSs). ATPSs have also been used for the extraction of metallic ions, extractive crystallization of inorganic salts, recovery of nanoparticles, extraction of sulfide minerals, and separation of cells and cell particles and have also been used in many product recovery systems in biotechnological operations. An ATPS is accomplished by dissolving two water-soluble polymers (PEG−dextran) or one polymer and a salt (PEG−phosphates, sulfates, citrates) that are normally incompatible.[1] The water solution then separates into two individual phases, both predominantly consisting of water, where the top phase is rich in polymer and the bottom phase is rich in salt or other polymer depending on the systems used. The advantages of ATPSs lie in volume reduction, high capacity, rapid separation, and mildness in particular. A reliable and maximized recovery of the targeted biomolecules could be obtained by manipulating the aqueous two-phase conditions such as volume ratio, tie line length, temperature, salt concentration, pH, etc. Among polymer−polymer and polymer−salt systems, the latter is considered to be more advantageous due to low levels of PEG in the salt phase, which reduces losses. Polymer−salt systems also facilitate further purification and concentration of the biomolecules and also the potential cost advantage over the second polymer due to low viscosity, low cost of chemicals, and shorter separation time.[2,3]

Molecular mass and hydrophobicity of polymer, salt selection, salt and PEG system compositions, pH, temperature, and distance from the critical point are the major variables that affect the performance of the ATPSs for the separation of targeted biomolecules.[4] The basis of separation using an ATPS is the selective distribution of substances between the two phases.[1] Phosphates and sulfates of sodium, potassium, and ammonium salts are commonly used as inorganic salts to form the ATPS with PEG. PEG is inexpensive, nontoxic, inflammable, and easy to handle. Recently, Vernau and Kula[4] found citrate as an alternative to form an ATPS with PEG since citrate salts are biodegradable, nontoxic, inflammable, and easily be discharged into wastewater treatment plants.[5,6] Very limited experimental work on the PEG + citrate + water system has been done, and not much data have been reported. Among the available literature, Vernau and Kula[4] have reported data on the PEG 1550 + sodium citrate + water system at room temperature, whereas Cheluget et al.[7] reported data on binodal curves and tie lines for PEG 6000 + potassium citrate + water systems at (25, 35, and 45) °C. Very recently, Murugesan and Perumalsamy[8] reported experiments using PEG 2000 + sodium citrate + water systems from which data on the binodal curve (25, 30,35, 40, and 45 °C) and tie line compositions at (25, 35, and 45) °C were obtained.

In the present study, PEG 2000 + potassium citrate + water is chosen as the phase forming system. Binodal curves and tie line compositions were determined for three different temperatures (25, 35, and 45 °C). Also, a sample comparison of the effect of polymer molecular weight on the binodal curve was made using the data of PEG 6000 + potassium citrate + water system reported in the literature.[6]

Experimental Section

Materials. Poly(ethylene glycol) 2000 (Catalog No. 8.21037.1000) purchased from Merck was used for the present work. The average molar mass of the polymer was found to be 1900−2200. Purified tri-potassium citrate monohydrate (Merck Catalog No. 61756505001046) with a minimum purity of 99 % was used for the study. The polymer and salt were used without further purification. Double-distilled water was used for the preparation of solutions.

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Table 1. Binodal Data as Mass Fraction for PEG 2000 + Potassium Citrate + Water System at Different Temperatures

25 °C		35 °C		45 °C	
100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s
4.38	42.61	4.20	40.91	3.53	42.91
5.61	37.21	4.96	37.58	3.80	40.95
7.59	31.63	5.51	35.35	3.91	38.36
9.31	27.16	6.79	30.10	4.52	35.04
10.97	23.37	7.15	28.58	4.93	33.22
11.87	20.96	8.68	24.53	5.92	29.00
12.23	20.51	8.24	25.60	7.08	24.70
13.46	18.01	9.14	23.20	8.07	21.19
14.56	15.91	9.34	22.89	8.51	19.94
15.43	14.41	10.22	20.41	9.61	16.77
15.51	14.19	11.51	17.85	10.16	15.30
16.31	12.76	11.31	18.19	10.96	13.43
16.97	11.60	12.97	15.05	13.27	9.49
19.18	8.39	12.15	16.44	17.22	5.17
21.91	5.43	13.10	14.77	19.26	4.04
24.98	2.55	13.68	13.57	20.57	2.52
27.87	0.81	14.67	11.87	23.92	1.95
30.40	0.44	16.39	8.86	25.86	0.99
		19.02	5.62	30.04	0.93
		20.10	4.46		
		21.76	3.00		
		22.46	2.55		
		24.03	1.80		
		25.59	1.03		
		28.47	0.68		
		31.46	0.44		

Table 2. Tie Line Data as Mass Fraction for PEG 2000 + Potassium Citrate + Water System at Different Temperatures

polymer-rich phase		salt-rich phase	
100 w_p	100 w_s	100 w_p	100 w_s
	25 °C		
30.34	8.18	5.93	22.69
33.38	7.03	2.91	25.61
36.31	6.00	0.49	29.26
	35 °C		
27.6	7.33	1.25	26.08
30.02	6.75	1.004	27.12
33.85	5.94	1.002	28.69
	45 °C		
27.05	6.22	1.57	22.22
33.39	4.76	1.06	26.17
36.73	4.28	0.05	28.73

Table 3. Values of Parameter for Equation 2 for PEG 2000 + Potassium Citrate + Water System at Different Temperatures

temp/°C	a	b	c	R^2
25	0.9008	−2.6204	1.7649	0.9979
35	0.9733	−3.2901	2.7534	0.9984
45	1.0224	−3.9430	3.8444	0.9984

Table 4. Values of Parameters in Equations 3 and 4 for the PEG 2000 + Potassium Citrate + Water System at Different Temperatures

temp/°C	k	n	R^2	k_1	r	R^2
25	0.8757	0.7818	0.9959	1.4461	1.1104	0.9918
35	0.2527	2.2464	0.9961	1.8639	0.4757	0.9989
45	0.4672	1.3993	0.9998	1.6844	0.8149	0.9998

Apparatus and Procedure. A jacketed glass vessel of 200 cm^3 was used for carrying out the phase equilibrium study. Water was circulated at constant temperature through the external jacket of the glass vessel. The temperature was controlled within ± 0.05 °C. The binodal curves were determined by the cloud point method[3] (titration method). Potassium citrate of 30 % (w/w) and PEG 2000 of 50 % (w/w) was prepared. A known amount of the salt solution was taken and titrated against PEG 2000. To ensure the end point, PEG 2000 was added in drops till the appearance of turbidity, which indicates the two-phase formation. Water was then added until the disappearance of turbidity. The procedure was repeated to get the other binodal points. 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. For the determination of tie lines, samples were prepared (50 g) by mixing polymer, salt, and water in appropriate proportions in a centrifuge tube. The samples were thoroughly mixed and kept in a thermostat at constant temperature for 24 h. The individual phases were separated, and the concentrations of polymer and salt were determined by refractive index measurement and flame photometry (Systronics128 flame photometer), respectively. For dilute aqueous solutions containing a polymer and a salt, the relation between the refractive index (n_D) and the mass fractions of PEG (w_p) and salt (w_s) is given by

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

where a_0, a_1, and a_2 are the fitting parameters. For phase analysis, 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 Zafarani-Moattar and Hamidi[6] for the PEG 6000 + potassium citrate + water system. Hence the same method was adopted for the present system. The calibration plot was drawn between refractive index versus PEG 2000 (10 to 50 % w/w) for the different concentrations of potassium citrate (1 to 10 % (w/w)). The parameters a_0, a_1, and a_2 were estimated, and the values are found to be 1.3334, 0.1459, and 0.1538, respectively, for the PEG 2000 + potassium citrate + water system. The estimated errors are within ± 1.0 %.

Results and Discussion

For the PEG 2000 + potassium citrate + water based ATPS, the binodal and tie line data at (25, 35, and 45) °C are shown in Tables 1 and 2, respectively.

Effect of Temperature. The effect of temperature on the binodal curve is shown in Figure 1. It is found that as temperature increases the solubility also increases and hence the two-phase region expands. Therefore the binodal curve shifts down. The same trend was observed for other PEG + salt systems.[7,8] The binodal data of the PEG 2000 + potassium citrate + water system was fitted using the following equation:

$$w_p = a + b w_s^{0.5} + c w_s \tag{2}$$

where w_P and w_s are the mass fractions of PEG 2000 and potassium citrate, respectively. Using regression analysis, the constants of eq 2 were estimated and are shown in Table 3. The estimated errors are within ± 1.0 %.

Correlation of Tie Line. The tie line compositions for PEG 2000 + potassium citrate + water were correlated using the suggested relations of Othmer−Tobias (eq 3) and Bancroft (eq 4) as given below, which were earlier successfully applied for other polymer−salt systems:[8]

$$(1 - w_p^{\,t})/w_p^{\,t} = k((1 - w_s^{\,b})/w_s^{\,b})^n \tag{3}$$

$$(w_w^{\,b}/w_s^{\,b}) = k_1(w_w^{\,t}/w_p^{\,t})^r \tag{4}$$

where k, n, k_1, and r represent the fitting parameters. The superscripts t and b stand for polymer-rich (top) and salt-rich

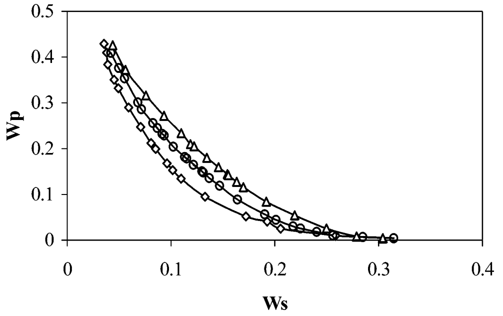

Figure 1. Effect of temperature on binodal curve for PEG 2000 + potassium citrate + water. Experimental binodal points for △, 25 °C; ○, 35 °C; and ◇, 45 °C. Calculated from eq 2.

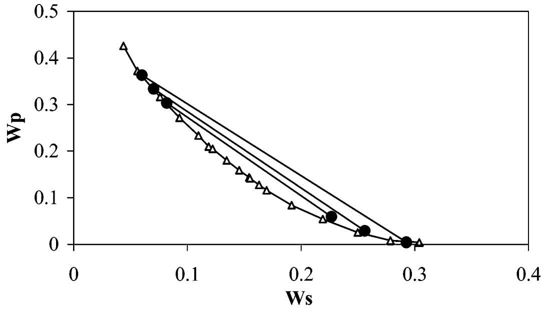

Figure 2. Tie lines for PEG 2000 + potassium citrate + water at 25 °C; ● and △, experimental; —, calculated from eqs 3 and 4.

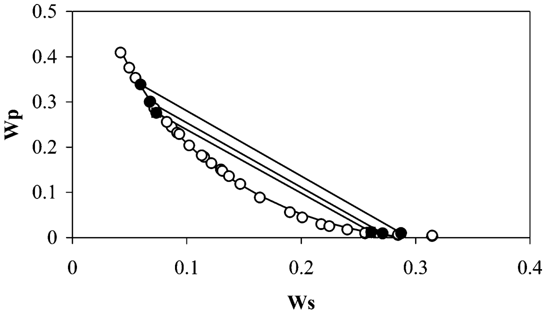

Figure 3. Tie lines for PEG 2000 + potassium citrate + water at 35 °C. ○ and ●, experimental; —, calculated from eqs 3 and 4.

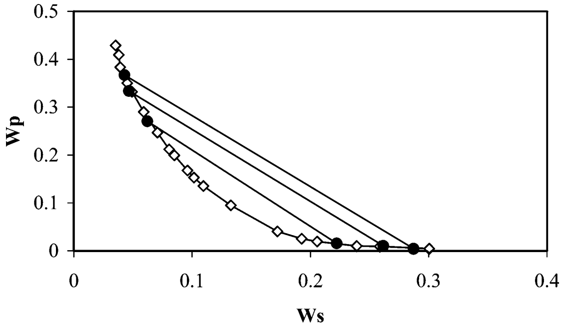

Figure 4. Tie lines for PEG 2000 + potassium citrate + water at 45 °C. ◇ and ●, experimental; —, calculated from eqs 3 and 4.

(bottom) phases. The values of the parameters k, n, k_1, and r are given in Table 4. Subscripts p, s, and w stands for polymer, salt, and water, respectively. The experimental and correlated tie lines for all the three temperatures (25, 35, and 45 °C) are compared and shown in Figures 2 to 4, respectively.

Effect of Polymer Molecular Weight. The effect of polymer molecular weight on the binodal curve was studied. The data for the binodal curve for the PEG 6000 + potassium citrate + water system[6] were compared with that of the present systems for the three different temperatures (25, 35, and 45 °C) and are shown in Figures 5 to 7, respectively. It is found that as the polymer molecular weight increases the binodal curve shifts down, indicating a clear increase in the two-phase region.

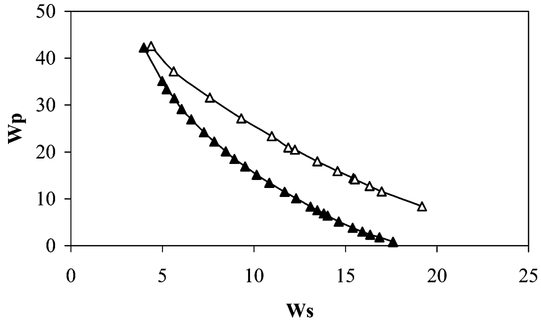

Figure 5. Effect of polymer molecular weight on binodal curve at 25 °C. △, PEG 2000 + potassium citrate + water; ▲, PEG6000 + potassium citrate + water.

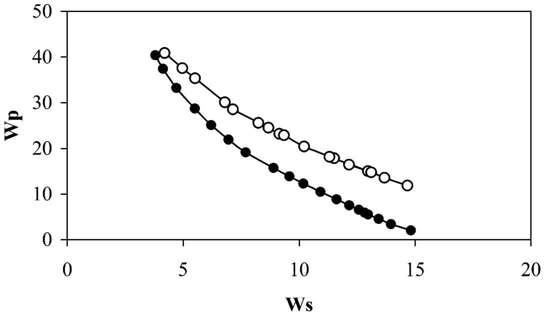

Figure 6. Effect of polymer molecular weight on binodal curve at 35 °C. ○, PEG 2000 + potassium citrate + water; ●, PEG6000 + potassium citrate + water.

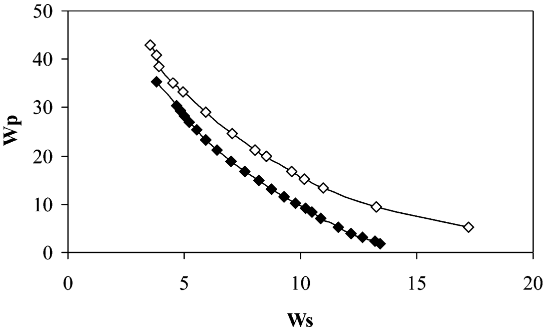

Figure 7. Effect of polymer molecular weight on binodal curve at 45 °C. ◇, PEG 2000 + potassium citrate + water; ◆, PEG6000 + potassium citrate + water.

Conclusion

The binodal and tie line compositions were measured at three different temperatures (viz., 25, 35, and 45 °C) for PEG 2000 + potassium citrate + water based ATPSs. It is found that as temperature increases the binodal curve shifts down due to an increase in solubility, which results in the increase in the two-phase region. The binodal and tie line data were correlated using the available literature equations, and the values of the parameters are reported. In addition, a sample comparison of bimodal data for two different polymer molecular weights was made.

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