Liquid−Liquid Equilibria of Aqueous Two-Phase Poly(ethylene glycol)−Potassium Citrate System

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Liquid−liquid equilibrium data of poly(ethylene glycol) 6000 + potassium citrate + water have been measured at (25, 30, and 35) °C. The binodal curves were described using a three-parameter equation, and the tie lines were satisfactorily described using the Othmer−Tobias and Bancroft equations.

Introduction

Aqueous two-phase systems (ATPS) present a powerful technique for the separation of biological materials. There are two kinds of ATPS: polymer + polymer ATPS and polymer + salt ATPS. The latter type has more advantages. These systems, introduced in 1956 by the pioneering work of Albertsson, have been applied in separation of biological materials, such as proteins, enzymes, and nucleic acids.[1−3] Poly(ethylene glycol) (PEG), which is a hydrophilic polymer, is often used in the aqueous two-phase partitioning studies. In earlier studies, salting out of PEG has been accomplished by the use of either phosphate or sulfate salts. These salts, however, led to high phosphate or sulfate concentration in the effluent streams[4] and, therefore, to environmental concern. One way to reduce the salt discharged in the wastewater is to recycle the chemicals. In this regard, Hustedt[4] and Greve and Kula[5] have published useful papers. Recently, as an alternative approach, Vernau and Kula[6] have investigated citrates as a substitute for inorganic salts and found that sodium and potassium citrates form aqueous two-phase systems with PEG which are suitable for protein extraction. Citrate is biodegradable and nontoxic and could be discharged into biological wastewater treatment plants. However, for PEG + citrate + water ATPS a limited amount of experimental work has been carried out. In the case of aqueous PEG_{1550} + sodium citrate, Vernau and Kula[6] have measured binodal curves and obtained a few tie line data at room temperature, but they only have represented the results in figures without any experimental data. As far as we know, there is no report on the phase diagram of the PEG + potassium citrate + water system in the literature. It has also been reported that PEG_{1000} + potassium citrate + water forms a two-phase system and can be satisfactorily used for separation of some biomolecules.[6]

This work is devoted to obtaining phase equilibrium data for the PEG_{6000} + potassium citrate + water system at (25, 30, and 35) °C. These results can be used to develop thermodynamic models of aqueous two-phase systems.[7−8]

Experimental Section

Materials. PEG 6000 was obtained from Merck. The number average molar mass of the polymer was previously determined[9] to be 5886 g·mol^{-1}. Potassium citrate with a minimum purity of 99% was obtained from Fluka. The polymer and salt were used without further purification, and double distilled deionized water was used.

Apparatus and Procedure. The experimental apparatus employed is essentially similar to the one used previously.[10−12] A glass vessel, volume 50 cm^3, was used to carry out the phase equilibrium determinations. The glass vessel was provided with an external jacket in which water at constant temperature was circulated using a thermostat. The temperature was controlled to within ±0.05 °C. The binodal curves were determined by a titration method. A salt solution of known concentration was titrated with the polymer solution or vice versa, until the solution turned turbid. The composition of the mixture was determined by mass using an analytical balance with a precision of $\pm 1 \times 10^{-7}$ kg. For the determination of the tie lines, feed samples (about 20 cm^3) were prepared by mixing appropriate amounts of polymer, salt, and water in the vessel. The thermostat was set at a desired temperature, and the sample was stirred for 1 h. Then the mixture was allowed to settle for 24 h. After separation of the two phases, the concentrations of potassium citrate in the top and bottom phases were determined by flame photometry. The uncertainty in the measurement of the mass fraction of the salt was estimated to be ±0.0002. The concentration of polymer in both phases was determined by refractive index measurements performed at 25 °C using a refractometer (QUARTZ RS-232, Ceti, Belgium) with a precision of ±0.0001. 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_1, and salt, w_2, is given by

$$n_D = a_0 + a_1 w_1 + a_2 w_2 \tag{1}$$

This equation has been used for phase analysis of the poly(propylene glycol) + NaCl + H_2O system by Cheluget et al.[13] We found that the same method of calibration plots and evaluation of parameters given by Cheluget et al.[13] can also be used for analysis of PEG + potassium citrate + water systems. The values of the coefficients a_0, a_1, and a_2 for the system studied are respectively 1.3325, 0.1411, and 0.1564. However, it was found that this equation is only valid for mass fractions of $w_1 \le 0.1$ and $w_2 \le 0.05$, for which linear calibration plots of the refractive index of the solution against polymer and salt concentration are obtained. Therefore, before refractive index measurements, it was necessary to dilute the samples to the above mass

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Table 1. Binodal Data as Mass Fraction for PEG_{6000} (1) + Potassium Citrate (2) + H_2O (3) at Different Temperatures

$100w_1$	$100w_2$	$100w_1$	$100w_2$
	25 °C		
42.30	3.99	11.51	11.67
35.15	4.99	10.10	12.30
33.39	5.23	8.37	13.08
31.46	5.65	7.60	13.46
29.14	6.05	6.88	13.81
26.98	6.57	6.42	14.02
24.20	7.27	5.15	14.63
22.21	7.83	3.85	15.38
20.14	8.46	3.01	15.91
18.54	8.93	2.35	16.34
16.93	9.51	1.79	16.85
15.16	10.14	0.86	17.59
13.43	10.84		
	35 °C		
40.39	3.80	10.49	10.92
37.43	4.13	8.84	11.60
33.24	4.70	7.53	12.16
28.74	5.50	6.53	12.58
25.10	6.21	5.94	12.82
21.92	6.95	5.51	12.97
19.16	7.69	4.54	13.43
15.75	8.89	3.40	13.96
13.87	9.58	2.03	14.82
12.27	10.18		
	45 °C		
35.30	3.79	12.99	8.74
30.25	4.67	11.48	9.30
29.32	4.82	10.16	9.80
28.26	4.98	9.10	10.21
27.02	5.24	8.33	10.51
25.38	5.53	7.13	10.85
23.27	5.94	5.15	11.63
21.09	6.43	3.93	12.17
18.86	7.01	3.03	12.69
16.76	7.59	2.32	13.19
14.87	8.18	1.91	13.43

Table 2. Tie Line Data as Mass Fraction for PEG_{6000} (1) + Potassium Citrate (2) + H_2O (3) at Different Temperatures

polymer rich phase		salt rich phase	
$100w_1$	$100w_2$	$100w_1$	$100w_2$
	25 °C		
41.97	3.80	0.07	27.60
39.24	4.14	0.59	25.00
35.72	4.74	0.54	22.52
27.99	6.30	0.94	18.97
24.34	6.90	2.30	16.98
	35 °C		
41.20	3.70	1.41	25.11
37.57	4.00	2.03	21.56
32.79	4.76	2.07	18.79
29.17	5.25	1.32	17.42
	45 °C		
45.53	2.40	2.13	24.96
42.00	3.00	1.42	21.34
31.01	4.66	0.29	16.83
27.86	5.20	1.22	15.10

fraction range. The precision of the mass fraction of polymer achieved using eq 1 was better than 0.25%.

Results and Discussion

For the aqueous PEG_{6000} + potassium citrate system the binodal and tie line data at (25, 30, and 35) °C are shown in Tables 1 and 2, respectively. The binodal curves at different temperatures are given in Figure 1. Figure 1 shows that the two-phase area is expanded with increasing temperature. This trend is also in agreement with experimental results from the literature[9,14−16] for other aqueous PEG + salt systems.

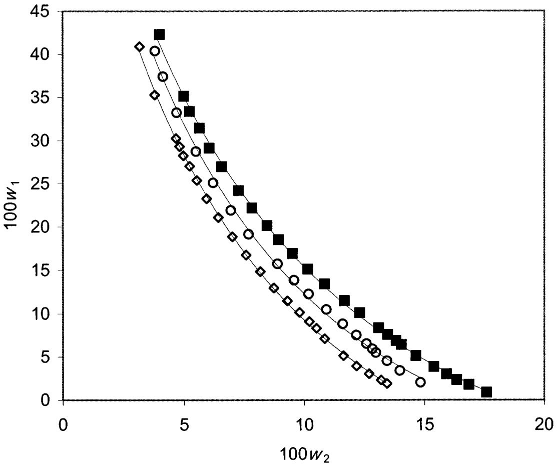

Figure 1. Binodal curves for PEG_{6000} (1) + potassium citrate (2) + H_2O (3) at different temperatures: ■, 25 °C; ○, 35 °C; ◇, 45 °C; —, calculated from eq 2.

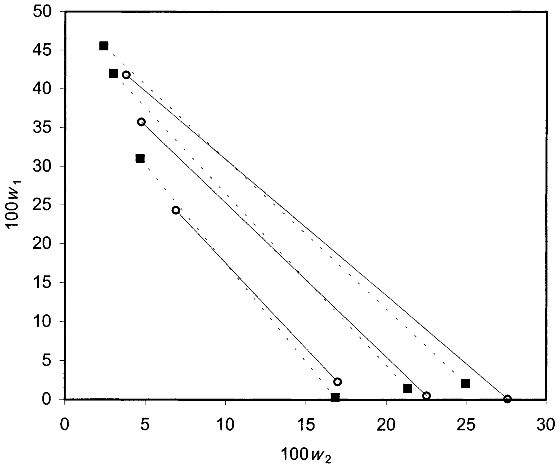

Figure 2. Effect of temperature on the equilibrium phase compositions for PEG_{6000} (1) + potassium citrate (2) + H_2O (3): ○, 25 °C; ■, 45 °C; —, tie lines at 25 °C; - - -, tie lines at 45 °C. These tie lines were obtained by connecting the experimental equilibrium phase composition data.

Additionally, the slopes of the tie lines for the investigated aqueous PEG + salt system at two different temperatures are compared. The slope of the equilibrium tie line increases with increasing temperature, as can be seen in Figure 2. Similar results were obtained by Voros et al.,[14] Zafarani-Moattar and Sadeghi,[9] and Mishima et al.[15] for other aqueous PEG + salt systems.

Recently, for the correlation of binodal data of some aqueous PEG + salt systems, two nonlinear expressions have been used by Graber et al.[17] and Taboada et al.[18] In fitting of the binodal data for the aqueous PEG_{6000} + potassium citrate + water system to different expressions, including those used previously,[17,18] however, better results were obtained with the following empirical equation.

$$w_1 = a + bw_2^{0.5} + cw_2 \tag{2}$$

The coefficients of eq 2 along with the corresponding standard deviations for the investigated system are given

Table 3. Values of Parameters of Eq 2 for PEG_{6000} + Potassium Citrate + H_2O at Different Temperatures

t/°C	a	b	c	sd[a]
25	1.090	−4.139	3.729	0.35
35	1.100	−4.462	4.342	0.45
45	0.997	−4.026	3.694	0.19

[a] $\mathrm{sd} = ((100w_1^{\mathrm{cal}} - 100w_1^{\mathrm{exp}})^2/N)^{0.5}$, where N is the number of binodal data.

Table 4. Values of the Parameters of Eqs 3 and 4 for PEG_{6000} + Potassium Citrate + H_2O at Different Temperatures

t/°C	k	n	R	k_1	r	R	sd_1[a]	sd_2[a]
25	0.338	1.395	0.994	2.282	0.716	0.993	0.69	0.47
35	0.372	1.188	0.985	2.369	0.856	0.989	0.68	0.49
45	0.259	1.335	0.990	2.774	0.791	0.989	0.89	0.56

[a] $\mathrm{sd}_j = \{{}^1/_2L\sum_{i=1}^{L}[(100w^{\mathrm{t}}_{i,j,\mathrm{cal}} - 100w^{\mathrm{t}}_{i,j,\mathrm{exp}})^2 + (100w^{\mathrm{b}}_{i,j,\mathrm{cal}} - 100w^{\mathrm{b}}_{i,j,\mathrm{exp}})^2]\}^{0.5}$, where L is the number of tie lines and j = 1 and 2. sd_1 and sd_2 represent the mass percent standard deviations for PEG and salt, respectively.

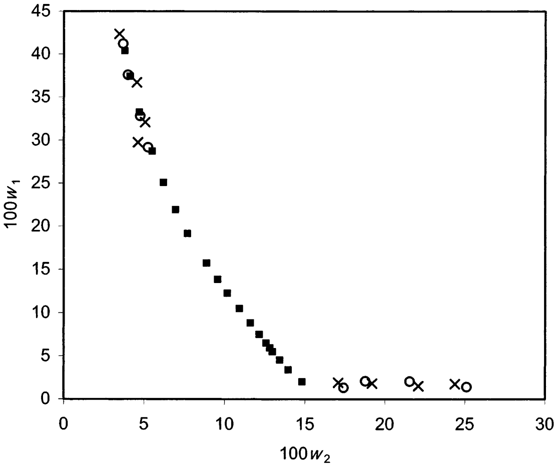

Figure 3. Tie lines for PEG_{6000} (1) + potassium citrate (2) + H_2O (3) at 35 °C: ○, experimental; ×, calculated from eqs 3 and 4; ■, binodal.

in Table 3. On the basis of the obtained standard deviations, it was concluded that eq 2 can be satisfactorily used to correlate the binodal curves of the investigated system at different temperatures. The calculated binodal curves at different temperatures are also shown in Figure 1.

The correlation equations[19] given by Othmer-Tabias (eq 3) and Bancroft (eq 4) have been used to correlate the tie line compositions.

$$\left(\frac{1-w_1^{\mathrm{t}}}{w_1^{\mathrm{t}}}\right) = k\left(\frac{1-w_2^{\mathrm{b}}}{w_2^{\mathrm{b}}}\right)^n \tag{3}$$

$$\left(\frac{w_3^{\mathrm{b}}}{w_2^{\mathrm{b}}}\right) = k_1\left(\frac{w_3^{\mathrm{t}}}{w_1^{\mathrm{t}}}\right)^r \tag{4}$$

where k, n, k_1, and r represent fit parameters. The superscripts t and b stand for the polymer rich phase and the water rich phase, respectively. The values of the parameters are given in Table 4. Recently, eqs 3 and 4 have been used for the assessment and correlation of tie line compositions of PEG + $NaNO_3$ + H_2O and PEG + Na_2CO_3 + H_2O systems.[17,18]

A linear dependency of the plots $\log[(1 - w_1^{\mathrm{t}})/w_1^{\mathrm{t}}]$ against $\log[(1 - w_2^{\mathrm{b}})/w_2^{\mathrm{b}}]$ and $\log(w_3^{\mathrm{b}}/w_2^{\mathrm{b}})$ against $\log(w_3^{\mathrm{t}}/w_1^{\mathrm{t}})$ indicated an acceptable consistency of the results. The corresponding correlation coefficient values, R, are also given in Table 4. Furthermore, on the basis of the standard deviations, sd_1 and sd_2, given in Table 4, we conclude that eqs 3 and 4 can be satisfactorily used to correlate the tie line data of the investigated systems. To show the reliability of the model, comparison between the experimental and correlated tie lines is shown in Figure 3, as an example, for the temperature 35 °C.

Conclusions

For poly(ethylene glycol) + potassium citrate + water, binodal and tie line data have been determined experimentally at (25, 35, and 45) °C. It was found that the two-phase area is expanded with increasing temperature. The tie line data for this system can be satisfactorily described by Othmer−Tobias and Bancroft equations.

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