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Equilibrium Phase Behavior of Poly(ethylene glycol) 4000 and Biodegradable Salts at Various Temperatures [(20, 30, and 40) °C]

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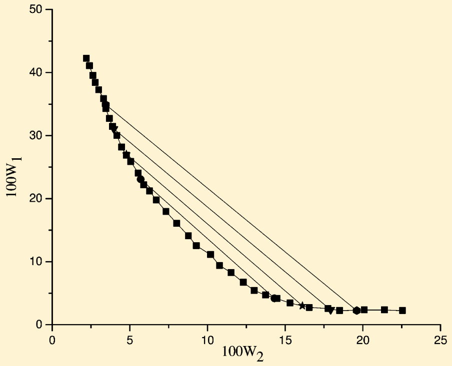
ABSTRACT: Liquid–liquid equilibrium data for the PEG 4000 (poly(ethylene glycol)) + salt [sodium citrate ($Na_3C_6H_5O_7$), potassium citrate ($K_3C_6H_5O_7$), or ammonium citrate ($(NH_4)_3C_6H_5O_7$)] + water systems have been determined experimentally at different temperatures. The effects of temperature on the binodal curves and tie-lines were studied, and it was observed that an increase of the temperature generally led to an increase of the two-phase area. The salting-out ability of the cations follows the ordering sodium citrate > potassium citrate > ammonium citrate. Finally, the Setschenow-type, Othmer–Tobias, and Bancroft equations have been successfully used to correlate the tie-line compositions.



INTRODUCTION

Aqueous two-phase extraction is a technique for the separation, concentration, and purification of proteins, cell organelles, and other biological products. An aqueous two-phase system (ATPS) is expected to be a promising means of separating biological materials because of the mild conditions provided by the phases and their different physical and chemical properties. ATPS's are formed by adding either two structurally different hydrophilic polymers or one polymer and one inorganic salt to water. Albertsson used polymer–polymer ATPS's for separation and purification of biomolecules.[1] However, the ATPS has many advantages: quick separation, easy operation, clear phase boundary, and no emulsification and most importantly, the system avoids organic solvents which are volatile, flammable, or explosive. So ATPS's now have been a widely used extraction and separation technique both in experimental and in industrial applications.[2−5] Aqueous polymer–salt systems have several advantages over the polymer–polymer systems due to the larger differences in density, greater selectivity, lower viscosity, lower costs, and so forth. In recent years, ATPS's, especially poly(ethylene glycol) (PEG) + inorganic salt + water systems, have found widespread applications, mostly because of their use in the separation of macromolecules from the mixture of biological materials.[6,7] Although the aqueous two-phase separation technique is successful, data on the properties and equilibrium of phase systems are necessary for the design of extraction processes and also for the development of models to predict phase partitioning. Several studies have been made on the liquid–liquid equilibrium behavior using PEG with different molecular weights and inorganic salts. The most common inorganic salts consist of selective cations (ammonium, potassium, or sodium) and anions (phosphate, sulfate, or carbonate). These salts, however, lead to high salt concentrations in effluent streams and, therefore, to environmental concern. In this respect, as an alternative approach citrates have been investigated as a substitute for inorganic salts. Citrate is biodegradable and nontoxic and could be discharged into a biological wastewater treatment plant.[8] Equilibrium data for different ATPS formed by PEG of different average molar masses and different citrate based inorganic salts are reported in literature.[9−11] Very limited experimental work on the PEG + citrate + water system has been done, and few data have been reported. However, this kind of data for systems containing PEG 4000 + inorganic salts of cations (NH_3^+, Na^+, K^+) at different temperatures is scarce.

This work reports the systematic study of the phase behaviors of LLE for PEG 4000 and biodegradable salts (sodium citrate, potassium citrate, ammonium citrate) aqueous biphasic systems. In this work, ATPS's composed of PEG 4000 + citrate salt + water were determined, and the phase composition was measured. The influence of the temperature on the phase diagram was also investigated. The tie-line compositions were fitted to the Othmer–Tobias, Bancroft,[12] and Setschenow-type equations.[13] The obtained results are necessary for the design of an extraction process, understanding the general factors determining the partition of solutes and particles in such ATPS's.

EXPERIMENTAL SECTION

Materials. Poly(ethylene glycol), PEG (average molecular weight 4000), was purchased from Merck. Trisodium citrate, tripotassium citrate monohydrate, and triammonium citrate were supplied by the Loba and (with a minimum purity of 99 %)

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used for the study. The polymer and salts were used without further purification. Double-distilled water was used for the preparation of solutions.

Apparatus and Procedure. The binodal curves were determined by the cloud point method.[14] Salt (sodium citrate, potassium citrate, and ammonium citrate) of 30 % (w/w) and PEG 4000 of 40 % (w/w) was prepared. A known amount of the salt solution was taken and titrated against PEG. To ensure the end point, PEG was added in drops until the appearance of turbidity. The first appearance of turbidity indicates that the system is about to enter the two-phase area. 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 with a precision of 0.0001 mg. The temperature of the working vessel was maintained by circulating water through an external jacket using a thermostat (Pricillab Equipments, India). The temperature was maintained with an uncertainty of ± 0.1 °C. 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 were properly diluted to determine PEG 4000 and salt equilibrium concentrations. The concentrations of salts in the top and bottom phases were determined by flame photometry (Systronics128 flame photometer). The uncertainty in the measurement of mass fraction of the salts was estimated to be ± 0.001. The concentration of PEG in both phases was determined by refractive index measurement at different temperatures [(20, 30, and 40) °C] using an Abbe-type refractometer with a precision of ± 0.0001. The uncertainty of the mass fraction of PEG was better than ± 0.002. The tie line lengths (TLL) for the different compositions were calculated according to

$$\mathrm{TLL} = \sqrt{(W_1^{\mathrm{T}} - W_1^{\mathrm{B}})^2 + (W_2^{\mathrm{T}} - W_2^{\mathrm{B}})^2} \qquad (1)$$

where W_1^{T}, W_2^{T}, W_1^{B}, and W_2^{B} are the top (T) and bottom (B) equilibrium mass fractions of PEG (1) and citrate (2). The tie line lengths are expressed in mass fractions.

Table 1. Binodal Data for the PEG 4000 (1) + Sodium Citrate (2) + Water (3) System at Temperatures of (20, 30, and 40) °C

T = 20 °C		T = 30 °C		T = 40 °C	
$100W_1$	$100W_2$	$100W_1$	$100W_2$	$100W_1$	$100W_2$
44.67	2.37	42.24	2.21	41.26	1.94
43.09	2.63	41.10	2.41	39.68	2.25
41.96	2.78	39.54	2.63	37.85	2.57
40.68	3.05	38.42	2.78	36.56	2.74
39.4	3.21	37.27	3.00	35.14	2.83
38.12	3.36	35.85	3.31	33.99	3.00
36.99	3.58	34.29	3.47	32.72	3.16
35.85	3.74	32.71	3.69	31.01	3.48
34.57	4.00	31.45	3.90	29.88	3.74
33.15	4.32	30.03	4.17	28.61	3.95
31.86	4.63	28.17	4.48	27.17	4.17
30.87	4.96	26.89	4.79	26.04	4.48
29.59	5.27	25.91	5.06	24.91	4.85
28.31	5.54	24.05	5.54	23.06	5.17
27.17	5.75	22.19	5.90	21.78	5.54
26.18	5.95	21.21	6.28	19.93	5.85
25.19	6.22	19.78	6.71	18.23	6.33
23.57	6.61	17.94	7.33	17.09	6.71
22.77	6.81	16.09	8.02	15.67	7.07
21.92	7.07	14.10	8.77	14.52	7.50
20.93	7.50	12.53	9.29	13.39	7.92
19.78	8.02	11.11	10.19	11.68	8.34
18.51	8.39	9.41	10.77	10.69	8.82
17.51	8.82	8.27	11.52	9.41	9.40
16.65	9.08	6.71	12.31	8.27	9.98
15.38	9.61	5.43	13.00	6.84	10.62
13.24	10.46	4.71	13.74	5.43	11.36
12.39	10.77	4.14	14.48	4.29	11.94
11.4	11.15	3.43	15.32	3.29	12.58
10.26	11.67	2.72	16.55	2.58	13.31
9.98	11.84	2.58	17.76	2.44	14.21
8.42	12.94	2.25	18.5	1.59	15.19
7.27	13.90	2.36	20.09	1.47	15.84
6.13	14.69	2.36	21.39	1.46	16.54
5.56	15.44	2.25	22.54	0.88	17.95
4.57	16.18			0.591	19.39
4.14	16.91				
3.58	17.66				
3.58	18.51				
3.43	19.75				

RESULTS AND DISCUSSION

The binodal experimental data of the phase system for PEG 4000 + salt (sodium citrate, potassium citrate, ammonium citrate) + water systems obtained at three different temperatures [(20, 30, and 40) °C] are shown in Tables 1, 2, and 3, respectively. The effect of temperature on the binodal curve is shown in Figure 1. The figure shows that the increase in temperature from (20 to 40) °C causes an increase of the area of two-phase region. It is evident to see that the two-phase region expanded with increase in temperature and also the salt solubility increases with increase in temperature. Therefore the binodal curve shifts close to the origin. According to the model proposed by Kjellander and Florin,[15] the entropically unfavorable structuring of water produced by PEG at low temperatures is overcome due to the large decrease in enthalpy (due to the energetically favorable and highly directional interactions, such as hydrogen bonding, between unlike molecules). Even at higher temperatures, the effect of temperature does not break down the structure of water in the PEG hydration shell rapidly, and the unfavorable entropy contribution makes the system phase to separate itself. This trend also agrees with experimental results from the literature for other ATPS's.[16,17] The binodal curve and the tie lines for PEG 4000 + sodium citrate + water systems at a temperature of 30 °C were shown in Figure 2. The border line between one and two phase area was a curved line, and the tie-line describes the compositions of the two phases in equilibrium, that is, the top phase (PEG-rich phase) and bottom phase (salt-rich phase). Figure 3 presents the experimental binodal curves of PEG 4000 + salt + water systems at a temperature of 30 °C. The salts considered share a common anion but contain different-sized monovalent cations. Ammonium, potassium, and sodium ions have radii of (0.137, 0.133, and 0.098) nm, respectively.[18] It can be seen that the size of the two-phase region increases as the size of the cation decreases. It seems that, in these systems, the size of cations may be responsible for this trend.

Table 2. Binodal Data for the PEG 4000 (1) + Potassium Citrate (2) + Water (3) System at Temperatures of (20, 30, and 40) °C

T = 20 °C		T = 30 °C		T = 40 °C	
$100W_1$	$100W_2$	$100W_1$	$100W_2$	$100W_1$	$100W_2$
40.37	4.61	40.93	4.02	39.40	3.35
38.56	5.04	39.51	4.22	37.55	3.54
37.39	5.23	38.59	4.43	36.28	3.98
35.57	5.61	36.91	4.63	34.86	4.17
34.55	5.92	35.24	4.97	33.43	4.42
33.38	6.23	33.95	5.22	31.26	4.79
31.70	6.55	32.78	5.43	30.30	5.29
30.29	7.11	31.75	5.70	29.32	5.55
28.48	7.68	30.33	5.90	27.74	5.79
26.53	8.11	29.04	6.31	26.46	6.04
24.72	8.67	27.99	6.51	25.33	6.36
22.92	9.29	26.97	6.78	24.19	6.67
21.62	9.80	25.93	7.25	22.77	6.92
20.20	10.36	24.51	7.59	21.21	7.29
18.91	10.92	23.22	8.06	19.65	7.61
17.75	11.42	22.06	8.53	18.64	8.04
16.72	11.87	21.02	8.87	17.36	8.48
15.68	12.49	19.87	9.27	16.09	8.86
14.91	12.80	18.57	9.74	15.09	9.11
13.87	13.49	17.54	10.15	13.81	9.49
12.85	14.17	16.24	10.62	12.39	9.92
11.42	14.79	14.83	11.16	10.97	10.48
10.64	15.36	13.66	11.69	9.68	11.05
9.60	15.81	12.62	12.3	8.56	11.79
8.58	16.42	11.07	12.91	7.55	12.36
7.67	16.93	9.51	13.58	6.42	12.93
6.64	17.49	8.49	14.32	5.56	13.68
5.73	18.24	7.32	15.07	4.57	14.30
4.70	18.99	6.29	16.08	3.16	15.18
3.66	19.68	5.12	17.02	2.30	16.11
3.02	20.62	3.83	18.03	1.59	17.11
2.37	21.55	2.93	19.17	1.44	18.24
2.12	22.49	1.89	20.11	1.44	19.25
1.85	23.87	1.52	21.39		

Table 3. Binodal Data for the PEG 4000 (1) + Ammonium Citrate (2) + Water (3) System at Temperatures of (20, 30, and 40) °C

T = 20 °C		T = 30 °C		T = 40 °C	
$100W_1$	$100W_2$	$100W_1$	$100W_2$	$100W_1$	$100W_2$
42.95	5.06	43.59	4.60	39.71	4.35
41.66	5.33	41.66	4.94	37.72	4.63
40.37	5.64	39.97	5.32	36.33	4.91
38.95	5.96	38.68	5.61	35.05	5.24
37.53	6.38	37.26	5.85	33.77	5.56
35.97	6.76	35.72	6.19	32.49	5.81
34.29	7.23	34.29	6.58	30.98	6.09
32.74	7.71	32.74	6.86	29.82	6.37
31.32	8.18	31.45	7.15	28.42	6.69
30.03	8.56	30.16	7.49	27.14	7.01
28.46	8.98	28.87	7.82	25.74	7.30
27.06	9.45	27.45	8.16	24.58	7.62
25.63	9.83	26.16	8.50	23.31	8.29
24.35	10.30	24.87	8.83	22.14	8.55
22.91	10.77	23.57	9.41	20.98	8.77
21.62	11.20	22.54	9.79	20.33	9.29
20.33	11.68	21.49	10.09	19.03	9.83
18.91	12.42	20.07	10.66	17.62	10.36
17.62	13.00	18.53	11.19	16.46	10.83
15.43	14.32	17.37	11.68	14.91	11.41
14.39	14.96	16.07	12.20	13.75	12.04
13.22	15.54	14.79	12.78	12.20	12.79
12.06	16.12	13.87	13.21	11.29	13.37
11.04	16.71	12.85	13.84	10.25	13.95
9.87	17.45	11.56	14.46	9.09	14.48
8.71	18.39	10.39	15.29	8.06	15.22
7.81	19.19	9.35	15.91	7.41	15.92
6.77	19.77	7.93	16.72	5.87	16.75
5.60	20.36	7.29	17.35	5.22	17.44
4.83	21.20	5.99	18.07	4.44	18.24
3.66	22.15	5.47	18.74	3.66	19.08
3.02	23.06	4.70	19.61	2.89	19.88
3.02	24.01	3.66	20.43	2.49	20.88
		3.02	21.24	1.73	21.68
		2.37	22.21		
		1.98	23.03		

It can be seen that three cations promote the formation of the ATPS in the following order: sodium > potassium > ammonium.

The tie-line compositions for the PEG 4000 + salt ($Na_3C_6H_5O_7$, $K_3C_6H_5O_7$, $(NH_4)_3C_6H_5O_7$) + water systems determined at 30 °C are shown in Table 4. The salting-out concept, the Setschenow-type behavior of the phase diagrams for a number of aqueous PEG + salt two-phase systems, has been substantiated by some authors.[19] Recently, eq 2 has been successfully applied for correlating the tie-line data of polymer−salt systems[20] and ionic liquid−salt systems.[21] However, we decided to use a relatively simple two-parameter equation (Setschenow-type) to examine the reliability of the tie-line compositions for the PEG 4000 + salt ($Na_3C_6H_5O_7$, $K_3C_6H_5$ O_7, $(NH_4)_3C_6H_5O_7$) + water systems determined at 30 °C, which can be derived from the binodal theory.[13] The equation used has the following form:

$$\ln\left(\frac{C_1^t}{C_1^b}\right) = k_P(C_1^b - C_1^t) + k_S(C_2^b - C_2^t) \tag{2}$$

where C_1, C_2, k_P, and k_S represent the concentration of the polymer, the concentration of the salt, a parameter relating the activity coefficient of polymer to its concentration, and the salting-out coefficient, respectively. Superscripts "t" and "b" stand for the polymer-rich phase and salt-rich phase, respectively. If the first term on the right-hand side of eq 2 is small compared with the second term, then a Setschenow-type equation is obtained. This would imply that $k_P \ll k_S$ since the absolute values of $(c_1{}^b - c_1{}^t)$ exceed the $(c_2{}^b - c_2{}^t)$ values. The tie-line data values were fitted by a least-squares linear regression method to eq 2, and the obtained slopes which give the salting out coefficients, k_S, together with corresponding intercepts and correlation coefficients are listed in Table 5, for each working salt. To examine the relation between the Setschenow-type behavior and the phase diagram very closely, the Setschenow-type plots of the tie-line data for the studied system have also been shown in Figure 4. The increase of k_S is reflected in the phase diagram by altering the position of the binodal curve to the left corresponding to a decrease in the area representing the single-phase mixture as shown in Figure 2, indicating an increasing phase-forming ability. The values of k_S are in the order $Na_3C_6H_5O_7 > K_3C_6H_5O_7 > (NH_4)_3C_6H_5O_7$, which is in agreement with the salting-out ability of the cations. For the studied systems, it

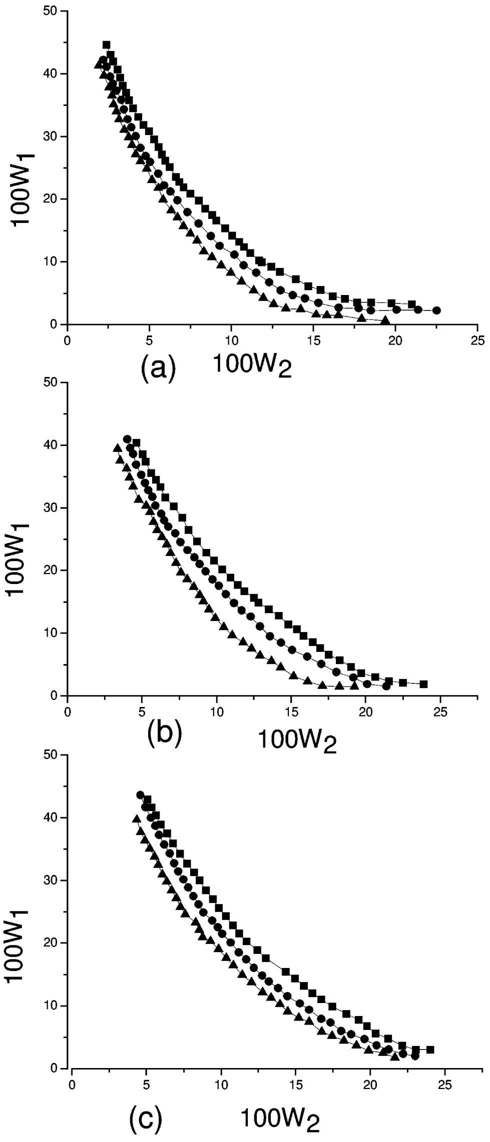

Figure 1. Effect of temperature on the binodal curves for: (a) PEG 4000 (1) + sodium citrate (2) + water (3) systems, (b) PEG 4000 (1) + potassium citrate (2) + water (3) systems, (c) PEG 4000 (1) + ammonium citrate (2) + water (3) systems. ■, 20 °C; ●, 30 °C; ▲, 40 °C.

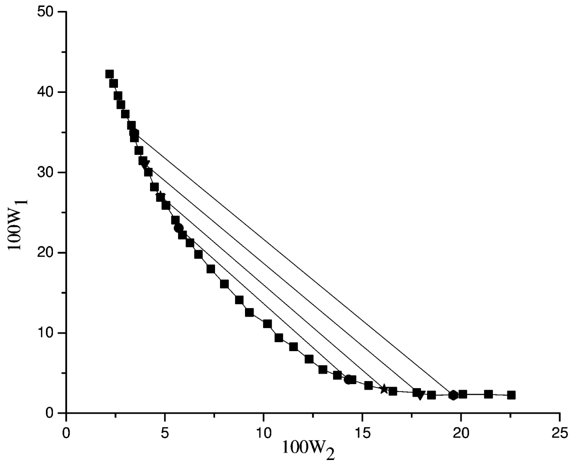

Figure 2. Binodal curve and tie lines for PEG 4000 (1) + sodium citrate (2) + water (3) systems at 30 °C.

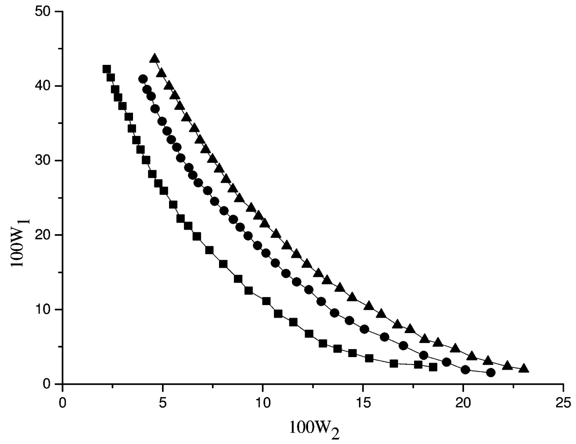

Figure 3. Effect of the type of salt on binodal curves for the PEG 4000 (1) + salt (2) + water (3) systems at 30 °C. ■, $Na_3C_6H_5O_7$; ●, $K_3C_6H_5O_7$; ▲, $(NH_4)_3C_6H_5O_7$.

Table 4. Tie-Line Data for PEG 4000 (1) + Salt (2) + Water (3) Systems at 30 °C

top phase		bottom phase		
$100W_1$	$100W_2$	$100W_1$	$100W_2$	TLL
		$PEG + Na_3C_6H_5O_7 + H_2O$		
23.06	5.71	4.18	14.32	20.75
27.06	4.79	3.01	16.10	26.58
31.06	3.98	2.37	17.93	31.90
34.93	3.45	2.25	19.61	36.46
		$PEG + K_3C_6H_5O_7 + H_2O$		
28.89	6.76	9.11	14.50	21.24
32.02	6.09	6.79	16.41	27.26
35.05	5.38	4.81	18.39	32.92
38.19	5.21	3.99	19.90	37.22
		$PEG + (NH_4)_3C_6H_5O_7 + H_2O$		
26.93	8.34	5.87	18.29	23.29
30.29	7.44	4.96	19.39	28.01
33.14	6.84	3.79	20.39	32.33
36.09	6.08	3.15	21.29	36.28

Table 5. Values of k_s and Intercept of Setschenow-Type Equation (eq 2) for the PEG 4000 (1) + Salt (2) + Water (3) Systems at 30 °C

salt	k_s kg·mol^{-1}	intercept	R^2
sodium citrate	4.89	0.57	0.975
potassium citrate	4.02	−0.09	0 0.998
ammonium citrate	3.56	−0.26	0 0.994

appears that the k_S value may have a close relationship with the salting-out ability of salt.

The Othmer−Tobias (eq 3) and Bancroft (eq 4) equations have also been used to correlate the tie-line compositions of the PEG 4000 (1) + salt (2) + water (3) systems, which have been successfully used in the correlation of LLE compositions of other ATPS's.[22,23]

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

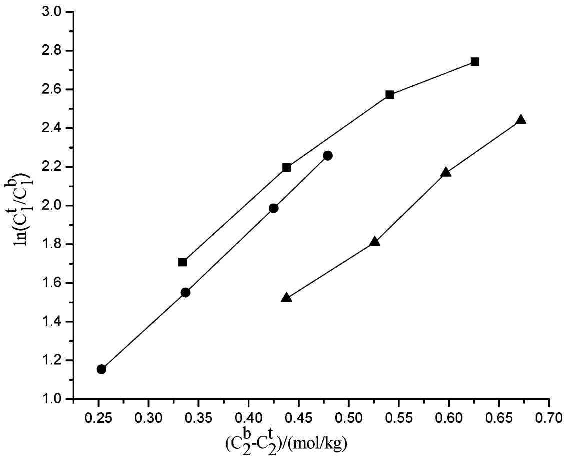

Figure 4. Setschenow-type plots of the PEG 4000 (1) + salt (2) + water (3) systems at 30 °C. ■, $Na_3C_6H_5O_7$; ●, $K_3C_6H_5O_7$; ▲, $(NH_4)_3C_6H_5O_7$.

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

where *k*, *n*, k_1, and *r* represent the fitting parameters. The superscripts t and b stand for polymer-rich (top) and salt-rich (bottom) phases. The values of the parameters *k*, *n*, k_1, and *r* are given in Table 6. Subscript 1, 2, and 3 stands for polymer, salt, and water, respectively. A linear dependency of the plots $\log[(1 - w_2{}^t)/w_2{}^t]$ against $\log[(1 - w_3{}^b)/w_3{}^b]$ and $\log(w_1{}^b/w_3{}^b)$ against $\log(w_1{}^t/w_2{}^t)$ are shown in Figures 5 and 6. It indicates an acceptable consistency of the results.

Table 6. Values of *k*, *n*, k_1, and *r* of Othmer−Tobias Equation (eq 3) and Bancroft Equation (eq 4) for the PEG 4000 (1) + Salt (2) + Water (3) Systems at 30 °C

	Othmer−Tobias equation			Bancroft equation		
salt	*k*	*n*	R^2	k_1	*r*	R^2
sodium citrate	0.369	1.07	0.999	2.78	0.794	0.948
potassium citrate	0.212	1.54	0.995	2.89	0.569	0.995
ammonium citrate	0.093	2.25	0.999	2.96	0.386	0.999

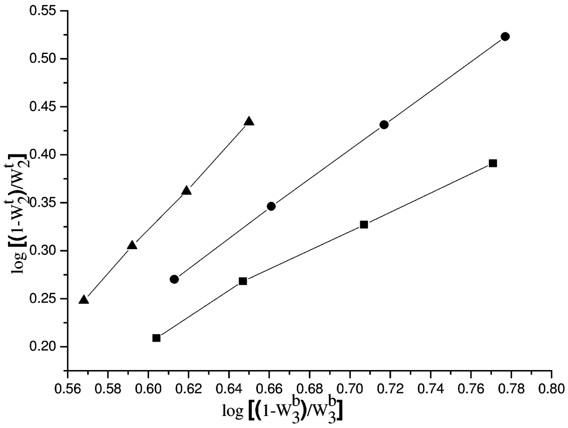

Figure 5. Linear dependency of the Othmer−Tobias equation. ■, $Na_3C_6H_5O_7$; ●, $K_3C_6H_5O_7$; ▲, $(NH_4)_3C_6H_5O_7$.

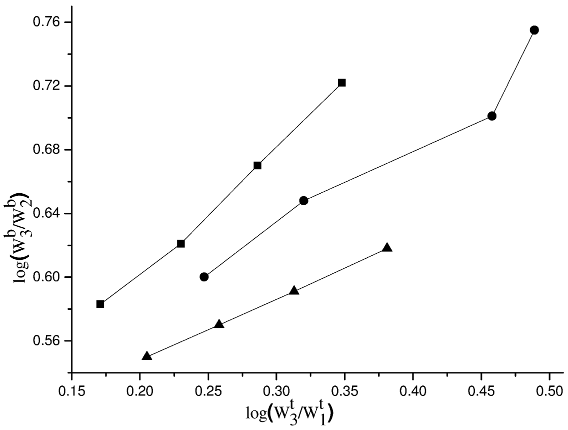

Figure 6. Linear dependency of the Bancroft equation. ■, $Na_3C_6H_5O_7$; ●, $K_3C_6H_5O_7$; ▲, $(NH_4)_3C_6H_5O_7$.

■ CONCLUSIONS

The aqueous two-phase partitioning method of liquid−liquid extraction is useful for separating material of biological origin. PEG + citrate salt could form an environmentally safe ATPS, which is more suitable for the extraction of protein. Liquid−liquid equilibrium data for the PEG 4000 + salt (sodium citrate, potassium citrate, ammonium citrate) + water systems were studied at different temperatures. The effects of temperature and salt on the binodal curves were studied. The salting-out abilities of different salts were evaluated by the salting-out coefficient (k_S), and the results show that the phase separation abilities of the investigated cations are in the order of sodium citrate > potassium citrate > ammonium citrate. Finally, the tie-line compositions for the investigated systems were satisfactorily correlated with the Setschenow-type, Othmer−Tobias, and Bancroft equations.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Albertsson, P. A.; Cajarville, A.; Brooks, D. E.; Tjerneld, F. Partition of proteins in aqueous two-phase systems and the effect of molecular weight of the polymer. *Biochem. Biophys. Acta* **1987**, *926*, 87−93.

(2) Naganagouda, K.; Mulimani, V. H. Aqueous Two-phase Extraction (ATPE): An Attractive and Economically Viable Technology for Downstream Processing of Aspergillus Oryzae R-Galactosidase. *Process Biochem.* **2008**, *43*, 1293−1299.

(3) Mokhtarani, B.; Karimzadeh, R.; Amini, M. H.; Darvish Manesha, S. Partitioning of ciprofloxacin in aqueous two-phase system of poly(ethylene glycol) and sodium sulphate. *Biochem. Eng. J.* **2008**, *38*, 241−247.

(4) Patil, G.; Raghavarao, K. S. M. S. Aqueous two phase extraction for purification of C-phycocyanin. *Biochem. Eng. J.* **2007**, *34*, 156−164.

(5) Ferreira, L. A.; Teixeira, J. A. Salt effect on the aqueous two-phase system PEG 8000-sodium sulfate. *J. Chem. Eng. Data* **2011**, *56*, 133−137.

(6) Kula, M. R.; Kroner, K. H.; Hustedt, H.; Fiechter, A. *Advances in Biochemical Engineering*; Springer-Verlag: Berlin, 1982.

(7) Zaslavsky, B. Y. *Aqueous two-phase partitioning. Physical chemistry and bioanalytical applications*; Marcel Dekker Inc.: New York, 1995.

(8) Vernau, J.; Kula, M. R. Extraction of Proteins from Biological Raw Material Using Aqueous PEG/citrate Phase Systems. *Biotechnol. Appl. Biochem.* **1990**, *12*, 397−404.

(9) Oliveira, R. M.; Coimbra, J. R.; Minim, L. A.; Mendes da Silva, L. H.; Ferreira Fontes, M. P. Liquid-Liquid Equilibria of Biphasic Systems Composed of Sodium Citrate + Polyethylene(glycol) 1500 or 4000 at Different Temperatures. *J. Chem. Eng. Data* **2008**, *53*, 895−899.

(10) Zafarani-Moattar, M. T.; Sadeghi, R.; Hamidi, A. A. Liquid-Liquid Equilibrium of an Aqueous Two Phase System containing Polyethylene glycol and Sodium citrate: Experimental and Correlation. *Fluid Phase Equilib.* **2004**, *219*, 149−155.

(11) Malathy, J.; Regupathi, I.; Murugesan, T. Liquid-Liquid Equilibrium of Poly(ethylene glycol) 2000 + Potassium Citrate + Water at (25, 35, and 45) °C. *J. Chem. Eng. Data* **2007**, *52*, 56−59.

(12) Othmer, D. F.; Tobias, P. E. Liquid-liquid extraction data-toluene and acetaldehyde systems. *Ind. Eng. Chem* **1942**, *34*, 690−692.

(13) Hey, M. J.; Jackson, D. P.; Yan, H. The Salting-Out Effect and Phase Separation in Aqueous Solutions of Electrolytes and Poly(ethylene glycol). *Polymer* **2005**, *46*, 2567−2572.

(14) Hatti-Kaul, R., Ed. *Aqueous Two-Phase Systems: Methods and Protocols; Methods in biotechnology*; Humana Press: Totowa, NJ, 2000.

(15) Kjellander, R.; Florin, E. Water structure and change in thermal stability of the system poly(ethylene oxide)-water. *J. Chem. Soc., Faraday Trans.* **1981**, *77*, 2053−2077.

(16) Se, A. G.; Aznar, M. Liquid-Liquid Equilibrium of Aqueous Two-Phase System Water + PEG 4000+ Potassium phosphate at Four Temperatures: Experimental Determination and Thermodynamic Modeling. *J. Chem. Eng. Data* **2002**, *47*, 1401−1405.

(17) Khederlou, K.; Pazuki, G. R.; Taghikhani, V.; Vossoughi, M.; Ghotbi, C. Measurement and Modeling Process Partitioning of Cephalexin Antibiotic in Aqueous Two-Phase Systems Containing Poly(ethylene glycol) 4000, 10000 and K_2HPO_4, Na_3Citrate. *J. Chem. Eng. Data* **2009**, *54*, 2239−2244.

(18) Barthel, J. *Topics in Current Chemistry*; Springer: Berlin, 1983.

(19) Xueqiao, X.; Juan, H.; Yun, W.; Yongsheng, Y.; Guowu, Y.; Weisheng, G. Measurement and Correlation of the Phase Diagram Data for PPG400 + (K_3PO_4, K_2CO_3, and K_2HPO_4) + H_2O Aqueous Two-Phase Systems at T = 298.15 K. *J. Chem. Eng. Data* **2010**, *55*, 4741−4745.

(20) Zafarani-Moattar, M. T.; Emamian, S.; Hamzehzadeh, S. Effect of Temperature on the Phase Equilibrium of the Aqueous Two-Phase Poly(propylene glycol) + Tripotassium Citrate System. *J. Chem. Eng. Data* **2008**, *53*, 456−461.

(21) Zafarani-Moattar, M. T.; Hamzehzadeh, S. Phase Diagrams for the Aqueous Two-Phase Ternary System Containing the Ionic Liquid 1-Butyl-3-methylimidazolium Bromide and Tri-potassium Citrate at T = (278.15, 298.15, and 318.15) K. *J. Chem. Eng. Data* **2009**, *54*, 833−841.

(22) Wang, Y.; Yan, Y. S.; Hu, S. P. Phase Diagrams of Ammonium Sulfate Ethanol/1-Propanol/2-Propanol + Water Aqueous Two-Phase Systems at 298.15 K and Correlation. *J. Chem. Eng. Data* **2010**, *55*, 876−881.

(23) Saravanan, S.; Reena, J. A.; Rao, J. R.; Murugesan, T.; Nair, B. U. Phase Equilibrium Compositions, Densities, and Viscosities of Aqueous Two-Phase Poly(ethylene glycol) + Poly(acrylic acid) System at Various Temperatures. *J. Chem. Eng. Data* **2006**, *51*, 1246−1249.