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LIQUID-LIQUID EQUILIBRIA OF AQUEOUS TWO-PHASE SYSTEMS CONTAINING POLY (ETHYLENE GLYCOL) OF DIFFERENT MOLECULAR WEIGHT AND POTASSIUM CITRATE

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ABSTRACT

The phase diagrams corresponding to systems of potassium citrate and poly (ethylene glycol) of different molecular weights (4000, 6000 and 10000) were determined at varying temperature. Moreover, the medium pH (5, 7 and 9) effect was also studied. The binodal curves were described using a three parameter equation and the tie-lines were satisfactorily described using the Othmer-Tobias and Bancroft equations. An increase in pH caused the expansion of the two-phase area. The binodal curves became more asymmetric and close to the origin with an increase in poly (ethylene glycol) molecular weight.

Key words: Aqueous two phase system, Poly ethylene glycol, Potassium citrate.

INTRODUCTION

Aqueous two-phase systems (ATPS) are formed when two mutually incompatible water-soluble polymers (e.g., dextran and polyethyleneglycol, (PEG)) or one polymer and one inorganic salt (e.g., PEG and potassium phosphate) are dissolved in water above a critical concentration.

ATPS are well-known green extraction systems. The extraction mechanism is similar to that of conventional organic systems. Due to the differences in the concentration of polymer and the salt in the top and bottom phases, several solute molecules, such as biological macromolecules will be unevenly distributed in each phase, depending upon their solubility in each phase, which enhances the separation of specific bio molecules from their crude[1]. However, ATPS have many advantages: quick separation, easy operation, clear phase boundary, and no emulsification and, most importantly, the systems avoid organic

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solvents which are volatile, flammable, or explosive. So ATPS now have been a widely used extraction and separation technique both in experimental and in industrial applications[2-4]. Aqueous polymer-salt systems have several advantages over the polymer-polymer systems due to the larger differences in density, greater selectivity, lower viscosity, less cost, etc. In recent years, ATPS, especially poly (ethylene glycol) + inorganic salt + water systems, have found widespread applications, mostly because of their use in separation of macromolecules from the mixture of biological materials[5,6].

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 behaviour using PEG with different molecular weights and inorganic salts. The most common inorganic salts employed by different research groups on the aqueous two-phase extraction technique 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 have been investigated citrates as a substitute for inorganic salts. Citrate is biodegradable and nontoxic and could be discharged into biological wastewater treatment plants[7]. Very limited experimental work on the PEG + Potassium citrate + water system has been done, and not much data have been reported.

Zafarani-Moattar et al.[8] have determined liquid–liquid equilibrium data for the aqueous PPG 400 + tripotassium citrate system at different temperature (293.15, 298.15, 308.15 and 318.15) K. Zafarani-Moattar and Hamidi[9] reported data on binodal curves and tie lines for PEG 6000 + potassium citrate + water systems at 25, 35, and 45°C. Jayapal et al.[10] reported data on binodal curves and tie lines for PEG 2000 + potassium citrate+ water systems 25, 30, 35, 40 and 45°C. Sadeghi[11] has determined data for aqueous two-phase systems containing poly(vinylpyrrolidone) (PVP) and potassium citrate at various temperatures of 298.15, 308.15 and 318.15 K.

The goal of this work is to obtain a complete set of measurements on the phase equilibrium for PEGs of increasing molecular weights and potassium citrate at different pH values and temperatures.

EXPERIMENTAL

Materials

Poly (ethylene glycols) of the following average molecular masses: 4000 (PEG4000), 6000 (PEG 6000), and 10000 (PEG 10000) were purchased from Merck was used for the present work. Purified tri-potassium citrate monohydrate 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.

Apparatus and procedure

The binodal curves were determined by the cloud point method[12]. Potassium 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 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 AR 2140) 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 (Systronics 128 flame photometer), respectively. For dilute aqueous solutions containing a polymer and a salt, the relation between the refractive index (η_D) and the mass fractions of PEG (W_1) and salt (W_2) is given by

$$\eta_D = a_0 + a_1W_1 + a_2W_2 \quad \ldots(1)$$

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.[13] for the poly (propylene glycol) + NaCl + H_2O system, which was later successfully applied by Zafarani-Moattar et al.[9] and Jayapal et al.[10] for the PEG 6000 and PEG 2000 + potassium citrate + water system. Hence the same method was adopted for the present system. The calibration plot was drawn between refractive index versus PEG (10 to50 % 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 + potassium citrate + water system. The estimated errors are within 1.0 %. The tie line lengths (TLL) for the different compositions were calculated according to

$$\text{TLL} = [(w_1^T - w_1^B)^2 + (w_2^T - w_2^B)^2]^{1/2} \quad \ldots(2)$$

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.

RESULTS AND DISCUSSION

Effect of PEG molecular weight

The binodal curves for ATPS of potassium citrate and PEGs of different molecular weight at 30°C, pH 7, are shown in Fig. 1. The binodal curve describes the border between the single-phase area and the two-phase area. The area above the binodal describes all compositions giving rise to two phase systems. It is found that as the polymer molecular weight increases the binodal curve shifts down and close to the origin, it's indicating a clear increase in the two-phase region. A similar behaviour was observed for the traditional PEG + phosphate ATPS[14]. If increase in PEG molecular weight, thus requiring lower concentrations for phase separation. This may be caused by the increase in the incompatibility between the system components due to the more hydrophobical character of PEGs of higher molecular weight[15].

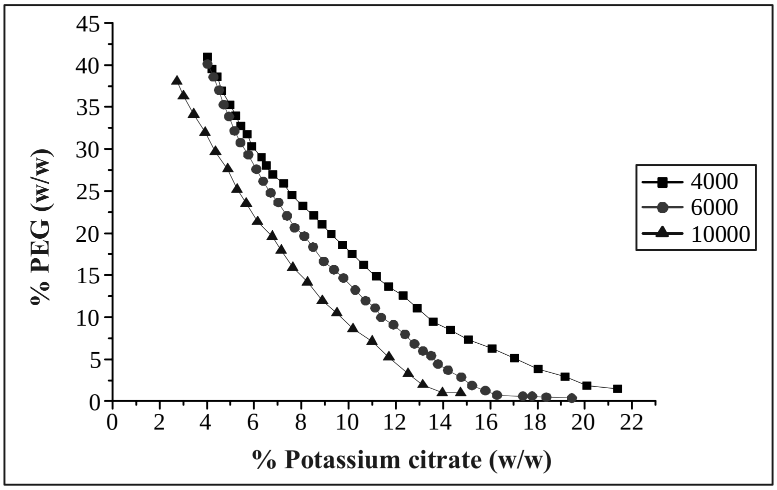

Fig. 1: Binodal curves for PEG (1) of varying molecular weight + potassium citrate (2) + water (3) at 30^{o}C and pH 7

Effect of pH

The effect of pH on binodal curve is shown in Fig. 2, which summarizes the phase diagrams corresponding to ATPS of PEG 4000 at different pH values. It's found that the two-phase area is expanded with an increase pH which is attributed to the decrease in phase-forming ability in the studied systems. The binodal curves showed similar shape for the different pH values (5, 7 & 9) and tend to superimpose at high PEG or Citrate concentrations, thus indicating that either the exclusion or the salting out effect respectively prevail in phase separation. When PEG and citrate concentrations adopt intermediate values, a smaller concentration of citrate is needed for two-phase formation at basic pH values with higher ratios between trivalent and divalent citrate ions. The ratio between trivalent and divalent citrate ions increases with pH and since trivalent ions are more effective in phase separation a smaller concentration of citrate is needed for two phase formation. Such effect is more clearly seen for lower PEG concentration. Which is good agreement with the reported experimental results for the other citrate salt based aqueous two phase system[16,17].

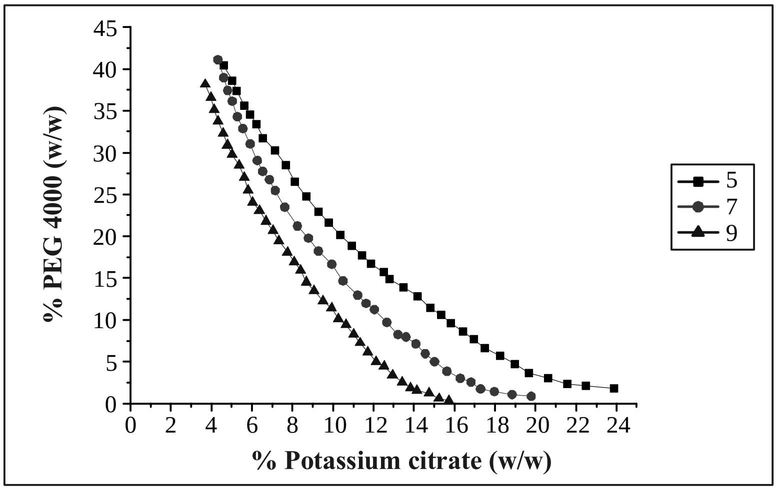

Fig. 2: Effect of pH on Binodal curves of ATPS of PEG 4000 (1) + Potassium citrate (2) + water (3) at 30°C

Effect of temperature

The phase system for the PEG 4000 + potassium citrate + water is described for three different temperatures, (20, 30 and 40°C), and the corresponding values are presented in Table 1. The binodal curve and the tie lines for the PEG 4000 + potassium citrate + water is show in Fig. 3. and The effect of temperature on binodal curve is show in Fig. 4. The figure shows that the increase in temperature from 20°C to 40°C causes an increase of the area of two-phase region. It is found that as temperature increases the salt solubility also increases and hence the two-phase region expands. Therefore the binodal curve shifts down. According to the model proposed by Kjellander and Florin[18], 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). At higher temperatures, provided that the structure of water in the PEG hydration shell does not break down too rapidly with increasing temperature, the unfavorable entropy contribution becomes preminent and the system phase separates itself. This trend also agrees with experimental results from the literature for other ATPS[19,20].

Table 1: Binodal data for PEG 4000 (100 W_1) + Potassium citrate (100 W_2) + water (100 W_3) system at different temperature

20°C		30°C		40°C	
100W_1	**100W_2**	**100W_1**	**100W_2**	**100W_1**	**100W_2**
40.37	4.61	40.93	4.02	39.4	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
31.7	6.55	32.78	5.43	30.3	5.29
30.29	7.11	31.75	5.7	29.32	5.55
28.48	7.68	30.33	5.9	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	9.8	25.93	7.25	22.77	6.92
20.2	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	12.8	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

Cont…

20°C		30°C		40°C	
$100W_1$	$100W_2$	$100W_1$	$100W_2$	$100W_1$	$100W_2$
11.42	14.79	14.83	11.16	10.97	10.48
10.64	15.36	13.66	11.69	9.68	11.05
9.6	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	14.3
4.7	18.99	6.29	16.08	3.16	15.18
3.66	19.68	5.12	17.02	2.3	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		

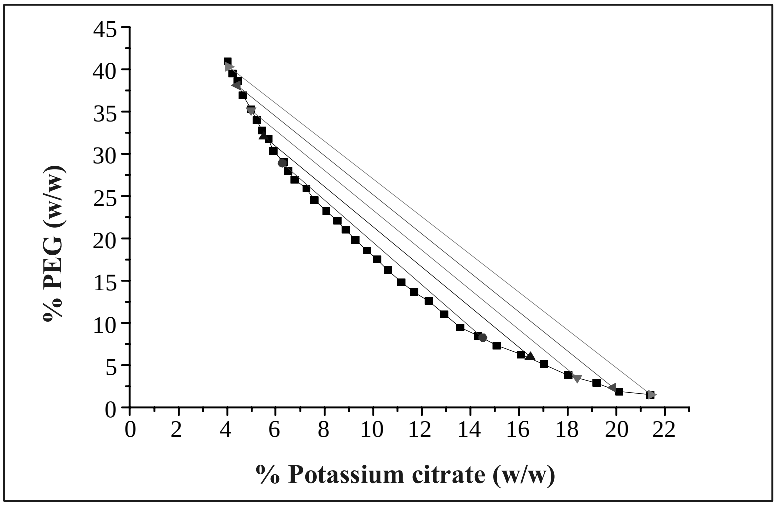

Fig. 3: Binodal curve and the tie lines for the PEG 4000 (1) + potassium citrate (2) + water (3) at 30°C

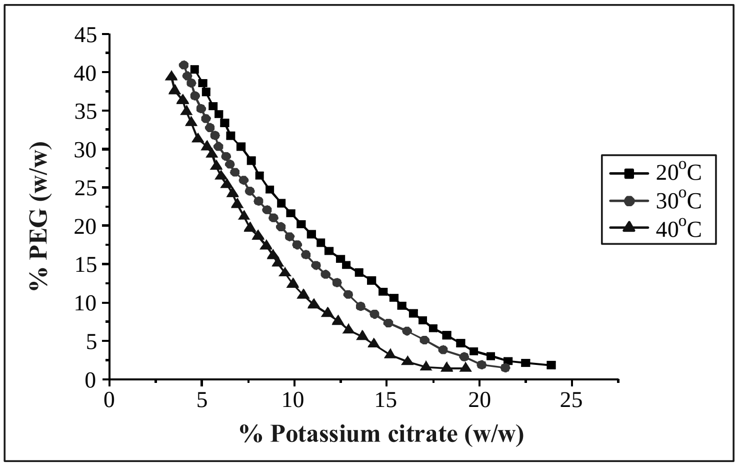

Fig. 4: Effect of Temperature on Binodal curves of ATPS of PEG 4000 (1) + potassium citrate (2) + water (3) at pH 7

Correlation of tie line

The tie line compositions for PEG 4000 + potassium citrate + water were correlated using the suggested relations of Othmer-Tobias (Eq. 3) and Bancroft (Eq. 4) as given below,

$$[(1 - w_1^t)/w_1^t] = k\,[(1 - w_2^b)/w_2^b]^n \qquad \ldots(3)$$

$$(w_3^b / w_2^b) = k_1\,[(w_3^t / w_1^t)]^r \qquad \ldots(4)$$

which were earlier successfully applied for other polymer-salt systems[21] 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 3. Subscript 1, 2, and 3 stands for, polymer, salt and water respectively. A linear dependency of the plots log $[(1 - w_1^t)/w_1^t]$ against log $[(1 - w_2^b)/w_2^b]$ and log (w_3^b/w_2^b) against log (w_3^t/w_1^t) indicated an acceptable consistency of the results.

Table 2: Tie line data for PEG 4000 ($100W_1$) + Potassium citrate ($100\ W_2$) + Water ($100W_3$) system at different temperature

Top phase		Bottom phase	
$100\ W_1$	**$100\ W_2$**	**$100\ W_1$**	**$100\ W_2$**
	T = 20°C		
29.93	7.14	8.77	16.26

Cont…

Top phase		Bottom phase	
100 W_1	**100 W_2**	**100 W_1**	**100 W_2**
32.26	6.45	6.56	17.53
35.28	5.66	4.23	19.34
37.96	5.16	2.60	21.18
39.71	4.96	2.14	22.15
	T = 30°C		
28.89	6.27	8.30	22.17
32.02	5.51	5.98	28.24
35.17	4.99	3.53	34.36
38.07	4.41	2.36	38.91
40.29	4.06	1.55	42.45
	T = 40°C		
25.86	6.17	5.98	13.19
28.19	5.71	3.99	14.63
30.52	5.21	2.25	16.11
32.84	4.56	1.67	17.44
35.52	4.09	1.33	19.10

Table 3: Parameters of Equations 3 and 4 for PEG 4000 (100 W_1) + Potassium citrate (100 W_2) + Water (100 W_3) system at different temperature

T/ °C	k	n	R^2	k_1	r	R^2
20	0.4079	1.115	.992	2.606	.7397	.997
30	0.3745	1.082	.997	2.679	.7875	.997
40	0.3633	1.037	.999	2.803	.8880	.998

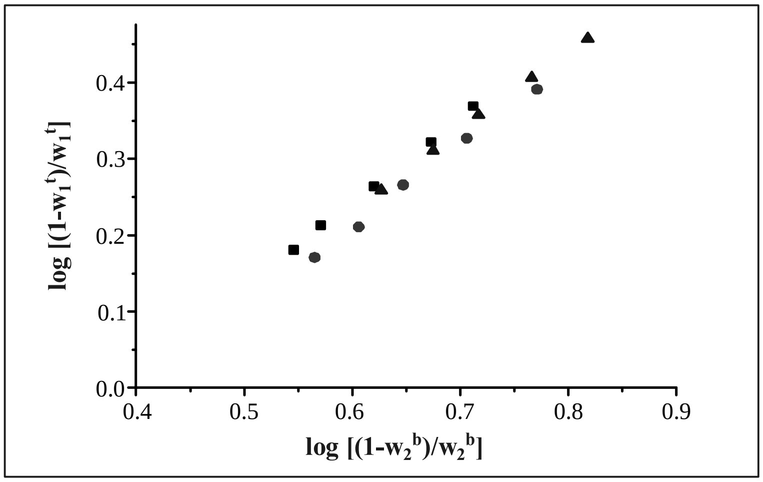

Fig. 5: Linear dependency of the Othmer-Tobias equation: (■) 20°C (●) 40°C (◀) 30°C

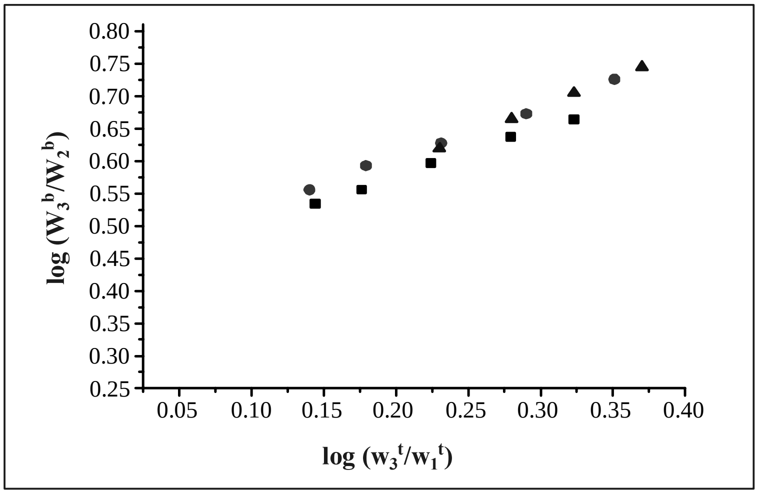

Fig. 6: Linear dependency of the Bancroft equation: (■) 20°C (●) 40°C (◀) 30°C

CONCLUSION

The aqueous two-phase partitioning method of liquid-liquid extraction is useful for separating material of biological origin. PEG + Potassium citrate salt could from environmentally safe aqueous two phase system, which is more suitable for the extraction of protein. We analyzed the effect of different medium conditions such as temperature, pH, and PEG molecular weight on the liquid-liquid equilibrium of these PEG + Potassium citrate systems. The phase diagrams of PEG + Potassium citrate ATPS were determined. The phase formation proved to be both temperature and pH-dependent. An increase in temperature, pH and PEG molecular weight caused the expansion of the two-phase area.

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