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Development and Evaluation of PEG-Lithium Citrate Salt Based Aqueous Two Phase System and Its Application in Partitioning of Proteins from Fish Industry Effluent

Regupathi Iyyaswami, Prasanna D. Belur, Basavaraju Girish, and Vishwanatha H. Nagaraj

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A aqueous two phase system (ATPS) comprising of PEG (Average mol. Wt: 4000, 6000, 8000) – lithium citrate salt-water systems were studied. The basic studies like binodal curve data generation and equilibrium studies were carried out. Furthermore, the binodal model and Othmer-Tobias and Bancroft models for phase equilibria were used for reproducing the experimental binodal data and phase equilibrium composition data, respectively. Good agreement was obtained with the experimental binodal data and tie line data with the models. The effective excluded volume values were obtained from the binodal model for the present ATPS. The tie line length was determined through the phase equilibrium composition data. This system was used to partition crude proteins of the fish industry effluent. The effects of PEG and salt weight fraction in terms of tie line length and effective excluded volume on partitioning coefficient of crude protein were studied in detail. From the results it was observed that, the crude proteins present in the fish effluent were partitioned in the PEG rich phase and the maximum partition coefficient of 7.82 was obtained. The results are discussed in the context of practical potential of this citrate based ATPS in separating crude proteins from fish industry effluent.

Keywords aqueous two-phase system; fish protein; protein partition

INTRODUCTION

Aqueous two phase systems (ATPSs) of polymer with salt have been recognized as an economical and efficient downstream processing method in the separation and purification of biomolecules such as proteins, enzymes, viruses, chloroplasts, and nucleic acids in industrial biotechnology (1,2). Polyethylene glycol (PEG) is a water-soluble and highly biocompatible non-toxic polymer and is often used as an ATPS phase-forming component in combination with another hydrophilic polymer such as dextran or inorganic salts. The ATPS present myriad environmental and process advantages such as low toxicity, minimal flammability, short process time, low energy consumption, relative reliability in scale-up and a biocompatible environment due to high water content (80 to 90 w/w %) in each of the equilibrium phases and have similar densities and low interfacial tensions (3). A wide variety of applications of ATPS have been reported that included the separation of whey proteins (4,5), alkaline xylanase (6), and cell particles (7). Verity of phosphate and sulfate salts were extensively studied in the literature for the separation of biomolecules. Moreover, these salts characteristics, equilibrium, and physical properties of the two phases were analyzed with respect to the separation of biomolecules (8–11). However, only a very limited amount of research work has been reported using citrate salt-PEG system.

Fish waste is a good source of protein (12), but a huge amount of the waste is still being discarded without much effort to recover its protein (13). Production of fish protein ingredients is growing throughout the world, which demands for traditional raw materials for production of fish protein ingredients (14). Treated fish waste/extractives like the crude proteins has found many applications among which the most important are natural pigments, dietic products (chitosan), food-packaging applications (chitosan), cosmetics (collagen), enzyme isolation, trypsin extraction (15), and moisture maintenance in foods (hydrolysates) (16). Apart from that the fish waste can be utilized for the production of short-chain fatty acids, amino acids, and gelatin, which were consumed/utilized for several therapeutic products and nutrient products (17).

Fish processing consists of various operations like sorting, dressing, cutting, eviscerating, skinning, pre-cooking, breading, blanching, filleting, salting, and packing. Washing is one of the most important steps in each phase. This washing water represents 60% of the total effluent volume (18,19). Wastewaters from fish processing plants are usually high in proteinaceous compounds and oils and it contains an average of 6 g of soluble protein per liter (18,20).

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However, protein concentration is often too low to be economically recovered by classical processes such as evaporation or spray drying (19). The recovery of proteins from the fish industry effluents by membrane separation processes has also been studied by several authors (20,21). Though membrane separation looks promising, the process suffers from severe fouling and hence frequent cleaning is required (20). Protein partitioning in ATPSs is a good alternative method to be employed as a first purification step. ATPS protein purification from the biological suspensions eliminates the contaminants (e.g., RNA, carbohydrates, lipids) in the bottom phase present. Further, ATPS for the recovery of products from biological suspensions require:

i. well characterized operating conditions that can be applied to a wide range of processes and
ii. an understanding of the process disadvantages attributed to two-phase partitioning (22).

In the present work, it was proposed to develop data on binodal and tie lines at 25°C, by conducting the experiments using a novel PEG + Lithium citrate hydrate +water system. The equilibrium phase compositions of the tie-lines were verified through the Othmer-Tobias and Bancroft equations (23) for the two phase system. Further, this ATPS was applied to partition the crude protein present in the fish industry effluent and the effects of PEG molar mass (PEG 4000, 6000 and 8000) and tie line length (TLL) of the ATPS were investigated.

Materials

Analytical grade Poly ethylene glycol of various average molecular weights (4000, 6000, and 8000) (Merck-Schuchardt, Hohenbrunn bei Mu nchen, Germany) and Lithium citrate hydrate – $Li_3C_6H_5O_7$, (Sigma Aldrich Inc. Germany) with a minimum purity of 99% were used for the formation of ATPSs. Double distilled, deionized water was used for the present experiments. Bradford Reagent (sigma Aldrich Inc, Germany) was used to estimate the protein content of the equilibrium phases and effluent. The fish effluent used in these experiments was obtained from a fish processing industry located at Mangalore, India.

APPARATUS AND PROCEDURE

Binodal Curve

The solutions of known concentrations for the formation of aqueous two phase systems were prepared by mass in 500 cm^3 capped, graduated flasks, using an analytical balance (OHAUS-Essae-Teraoka Ltd., Japan, model AR2140) with an accuracy of ±0.0001 g. Stock solutions were kept in a Schott-Gerate CT 52 (Germany) thermostatic bath to maintain the temperature at 298 K with an uncertainty of ±0.05°C. To construct the binodal curves for the present systems, all the experiments were carried out using a glass vessel with a working volume of 200 cm^3. The temperature of the working vessel was maintained by circulating water through an external jacket using a thermostat (Schott-Gerate CT 52, Germany). The titration method (cloud point method) was used to determine the phase equilibrium concentrations for establishing binodal curves (24). To ensure the uniform concentration of the constituents of ATPS in the jacketed vessel, constant stirring was applied by using a magnetic stirrer. A salt solution of known concentration was titrated against the polymer solution or vice versa, until the clear solution turned turbid. An analytical balance with a precision of ±0.1 mg was used to determine the composition of the mixture.

Equilibrium Study

Aqueous two-phase systems of varied PEG and salt compositions were prepared by mixing appropriate amounts of a 50% (w/w) PEG 4000, 6000, 8000 solution, 30% (w/w) Lithium citrate hydrate solution and water to adjust to 50 g by mass in graduated glass centrifuge tubes with stoppers. The accuracy of the weight fraction of PEG, salt, and protein was ±0.0001 g.The systems were mechanically shaken for 2 hr and equilibrated at 298 K overnight in a thermostatic water bath. Immediately after centrifugation for 20 min at 5600 × g, the volumes of two coexisting phases were measured.

The PEG concentration in both the phases was determined through measuring the refractive index of the phases using Abbe refractometer (range 1.3 to 1.7 n_D). Extensive calibration was carried out by preparing different concentrations of salt with different polymer concentrations. 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

$$n_D = a_0 + a_1 w_p + a_2 w_s \quad (1)$$

where a_0, a_1, and a_2 are the fitting parameters and the values were presented in Table 1 for the individual system. For phase analysis, the above equation was originally suggested

TABLE 1
The fitting parameters for the Eq. (1) with AARD

System	a_0	a_1	a_2	AARD(%)
PEG 4000-lithium citrate	1.3332	0.00133	0.0013	0.08
PEG 6000-lithium citrate	1.3332	0.00138	0.0014	0.07
PEG 8000-lithium citrate	1.3332	0.00142	0.0015	0.09

by Cheluget et al. (25) for the poly (propylene glycol) + NaCl + H_2O system, which was later successfully applied by various researchers (17,24,26). Hence the same method was adopted for the present system. The calibration plot was drawn between the refractive index versus PEG (10 to 50% w/w) for the different concentrations of Lithium citrate (1 to 20% (w/w)). The concentrations of Lithium Citrate salt present in both the phases were estimated through a Flame photometer (Range 1–100 ppm, model CL-378, Elico ltd, India).

Partition Coefficient Study

To study the partitioning coefficient of the fish industry effluent through the present ATPS, a number of solution mixtures were prepared using the same compositions (PEG 4000, 6000, 8000 + Lithium citrate + water) for the equilibrium study with 5 mL of fish industry effluent (contains 0.46 g/ml of total protein) to adjust to 50 g by mass in graduated glass centrifuge tubes with stoppers. The accuracy of the weight fraction measurement for all the phase forming components and proteins was ± 0.0001 g. The systems were mechanically shaken for 2 hr and equilibrated at 298 K overnight in a thermostatic water bath. Equilibrated sample solution was centrifuged for 20 min at 5600 × g. The volumes of two coexisting phases were measured and further both the phases were analyzed for their total protein content. Determination of total protein in both equilibrium phases was done by the Bradford method. Calibration graph was developed using BSA (Bovine serum albumin) with readily available Bradford reagent (Sigma).

RESULTS AND DISCUSSION

Phase Diagram

Initially the phase diagrams/binodals for different PEG grades and lithium citrate salt were prepared. The binodal curve describes the border between the single-phase area and the two-phase area. The area above the binodial describes all compositions giving rise to two-phase systems. The binodal curves for PEG 4000 + Lithium citrate, PEG 6000 + Lithium citrate and PEG 8000 + Lithium citrate systems were developed at 25°C and are presented in Fig. 1. The developed binodal data were analysed for the effects of molecular mass of PEG (PEG 4000, 6000, 8000) and the weight fraction of PEG and salts. Further, the experimental binodal data for all the three proposed systems were fitted by modifying the constants and coefficients of the expressions available in the literature (17,25,26). The binodal equation for the aqueous polymer-salt systems can be written as

$$w_P = a_1 + b_1 w_S^{0.5} + c_1 w_S \qquad (2)$$

where w_p and w_s are the mass fractions of PEG (4000, 6000, 8000) and lithium citrate, respectively. The present experimental binodal data of the poly(ethylene glycol) + lithium citrate +water systems were also fitted to Eq. (2) by regression analysis, and the constants and coefficients are listed in Table 2 along with Arithmetic Average Relative Deviation (AARD).

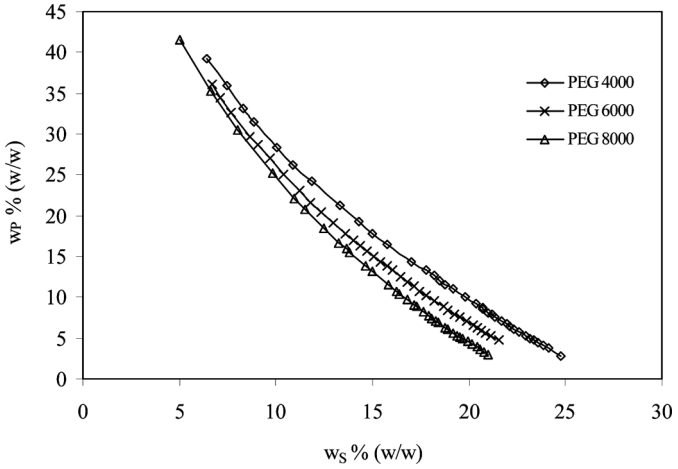

FIG. 1. Effect of PEG average molecular weight on binodal curve for PEG + lithium citrate + water systems at 25°C.

It was found from the literature that, the effect of increasing polymer molecular weight is to move the binodals towards lower polymer concentrations (1,27). This was found here without exception; nevertheless the magnitude of the changes depends on the average molecular weights of the polymers used (Fig. 1). Further, the two phase region in the binodal diagram of any ATP system depends on the nature of the salt associated with the polymer. This phenomenon can be explained with the help of salting-out strength of the salt present in the system.

Effective Excluded Volume (EEV)

The direction of protein partition depends on the effective excluded volume of both the salt and PEG rich phases, which also could be related to the salting out strength of the salt. The EEV for the entire system was calculated

TABLE 2
Values of parameters of Eq. (2) for PEG (4000, 6000, 8000) + lithium citrate + water systems at 25°C

System	a_1	b_1	c_1	AARD(%)
PEG 4000-lithium citrate	84.1938	−19.7081	0.6783	0.95
PEG 6000-Lithium citrate	89.9588	−24.1089	1.2359	0.32
PEG 8000-lithium citrate	96.2324	−27.5420	1.5709	0.59

through the relationship, as proposed by Guan et al. (28), the binodal equation for the aqueous polymer–salt systems is defined as:

$$\ln\left(V_{123}^{*}\frac{w_p}{M_p}\right) + V_{123}^{*}\frac{w_s}{M_s} = 0 \quad (3)$$

where V_{123}^{*} is the EEV, M_P and M_s are molar mass of PEG (g/mol) and salt, respectively. The EEV represents the acceptability of one component by a network constructed of the other component. Since the molecular packing in a solution is compact, in an ensemble average it is impossible for holes of any significant size to exist, which will admit additional molecules, unless the solution adjusts its structure. The EEV is significant during the phase formation in the ATPS. Several theories were developed to explain EEV through salting-out phenomena, keeping ionic strengths of salts and polymers and viscosity effects.

The salting-out effects are additive of the anions and cations present in the salt system. The salting-out ability can also be related to the Gibbs free energy of hydration of the ions. In considering that the salts share a common cation but contain different anions, from the literature it was found that, better salting-out of PEG is when the ions of the salt have a more negative free energy of hydrophobicity. The anions follows the order of HPO_2^{-4} > SO_2^{-4}>$C_4H_4O_2^{-4}$>H_2PO^{-4}>OH^->CHO^-. When comparing salts having the same anion, the one whose cation has a more negative Gibbs free energy of hydration is better at salting-out PEG. The cations follows the order Zn^{2+}>Mg^{2+}>Li^+>Na^+>NH^{+4} (29). It was also observed that the salts having larger values of EEV has higher salting-out strength (30,31). Since the citrate ion and lithium ion having the higher hydration value and EEV, the lithium citrate salt is a good option for the ATP system.

In a study conducted by Zafarani-Moattar (31), salting-out of PEG has been mainly accomplished by the use of either phosphates or sulfates. Harris et al. (32) studied the partitioning of milk proteins using polyethylene glycol 4000-ammonium sulfate and found that the system was problematic because the sulfate rich-phase induced the aggregation of the protein and favored its precipitation in the system interface. These salts, however, lead to high phosphate or sulfate concentration in the effluent streams and therefore to environmental concern. As an alternative approach Vernau and Kula (33) have investigated citrates as a substitute for inorganic salts and found that citrate forms aqueous two-phase system with PEG which is suitable for the biomolecules extraction. In the present work, the effective excluded volume of the of PEG (4000, 6000 and 8000) – Lithium citrate – water systems were estimated by using the model developed by Guan et al. (28), which is based on the statistical geometry methods for aqueous polymer-polymer systems. The present PEG-Lithium citrate systems show higher values of EEV for higher molecular mass of polymer and salt in the system (Table 4), due to the higher salting-out characteristics.

Equilibrium Study

The equilibrium studies of the selected ATPS are essential to effectively apply the new ATPS to a real application (fish industry effluent). At equilibrium the composition of phases were very important in the contest of the partitioning study. To obtain the equilibrium phase composition at the known feed composition of PEG and salt, the Othmer-Tobias (Eq. (4)) and Bancroft (Eq. (5)) relationship is used in the literature. Further, this prediction could be very helpful for applying any ATP system to the real time partition process. In this context, the equilibrium studies were made for the current ATPS (without the presence of fish protein). The two phase systems were formed at several compositions of salt and PEG in the two phase region and the phases were stirred well and allowed to reach equilibrium. Through these equilibrium experiments, a few number of tie lines were created. The tie line compositions were reported in Table 3. The reliability of tie line compositions was ascertained by the equations given by Othmer-Tobias (Eq. (4)) and Bancroft (Eq. (5)) (23).

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

TABLE 3
Tie line data as mass fraction for PEG + lithium citrate +water system at 25°C

Feed		Top phase		Bottom phase			
Wp	Ws	Wp	Ws	Wp	Ws	TLL	K protein
PEG 4000							
0.21	0.12	0.2990	0.0450	0.0007	0.2786	0.38	7.82
0.25	0.15	0.3996	0.0484	0.0045	0.3423	0.49	6.98
0.21	0.17	0.3946	0.0498	0.0021	0.3489	0.49	6.85
0.29	0.12	0.4183	0.0402	0.0012	0.3498	0.52	5.86
0.25	0.17	0.4441	0.0403	0.0048	0.3899	0.56	3.09
0.29	0.15	0.4537	0.0419	0.007	0.3995	0.57	2.69
PEG 6000							
0.21	0.15	0.3373	0.0621	0.0015	0.2899	0.41	6.25
0.25	0.12	0.3667	0.0468	0.0007	0.2943	0.44	5.87
0.25	0.15	0.3890	0.0571	0.0051	0.3118	0.46	4.76
0.29	0.12	0.3979	0.0408	0.0010	0.3123	0.48	3.85
0.29	0.15	0.4817	0.0325	0.0057	0.3515	0.57	1.33
PEG 8000							
0.21	0.12	0.2763	0.0582	0.0025	0.2600	0.34	4.13
0.25	0.12	0.3406	0.0430	0.0026	0.2862	0.41	3.24
0.21	0.15	0.3439	0.0529	0.0033	0.2987	0.42	3.04
0.21	0.17	0.3766	0.0578	0.0043	0.3142	0.45	2.83
0.29	0.12	0.4048	0.0342	0.0017	0.3199	0.49	2.12
0.29	0.15	0.4203	0.0392	0.0011	0.3401	0.52	1.33

TABLE 4
Values of parameters in Othmer-Thobias and Bancroft equation for the PEG (4000, 6000, 8000) + lithium citrate system at at 25°C

	Othmer-Thobias equation			Bancroft equation			EEV g/mol
	K	n	R_2	K_1	r	R^2	
PEG 4000	0.7071	1.2169	0.9869	1.4242	0.7885	0.988	23.56
PEG 6000	0.3181	1.9896	0.9858	1.8258	0.5326	0.995	30.07
PEG 8000	0.4342	1.6935	0.9791	1.7122	0.5838	0.986	33.89

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

where K, n, K_1, and r are the fitting parameters. Superscripts t and b represent the polymer-rich phase (top phase) and the salt-rich phase (bottom phase), respectively. Subscripts p, s, and w stand for PEG, salt, and water respectively.

The present experimental values were fitted to the correlations (Eqs. (4) and (5)) and the coefficients and constants for the current systems were computed and presented in Table 4. Equations (4) and (5) reproduce satisfactorily the equilibrium concentrations for both the phases. The equilibrium data were fitted along with the corresponding binodal curve and represented in Figs. 2–4. Furthermore, the same feed compositions of the PEG- Salt were adopted for the partitioning study of the fish effluent. Since the total crude protein content of the feed effluent is known (0.46 g/mL), two phase mixture were prepared in 50 g basis.

Partition Co-Efficient (K)

Chen (34) suggested that the partition of a protein in PEG/salt ATPS depends on the hydrophobicity of the proteins. Proteins with more polar amino acid residues will show higher affinity for the PEG phase, which is more hydrophobic than the lower salt phase. As reported in the literature, in the present work, it was observed that

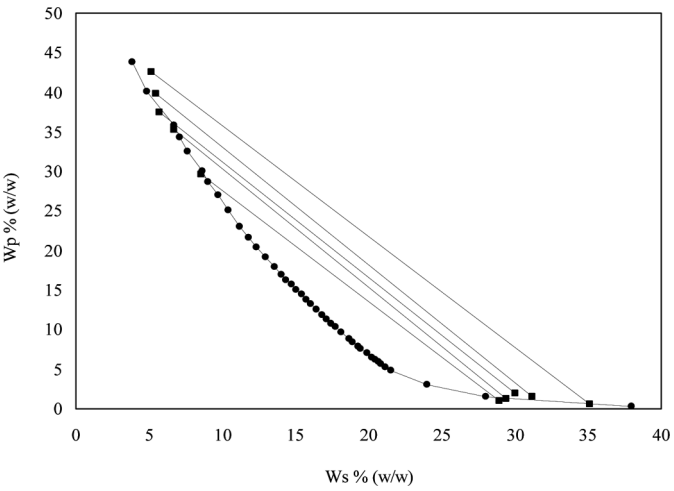

FIG. 3. Binodal curve and tie-lines for PEG 6000 (wP) + lithium citrate (wS) system at 25°C.

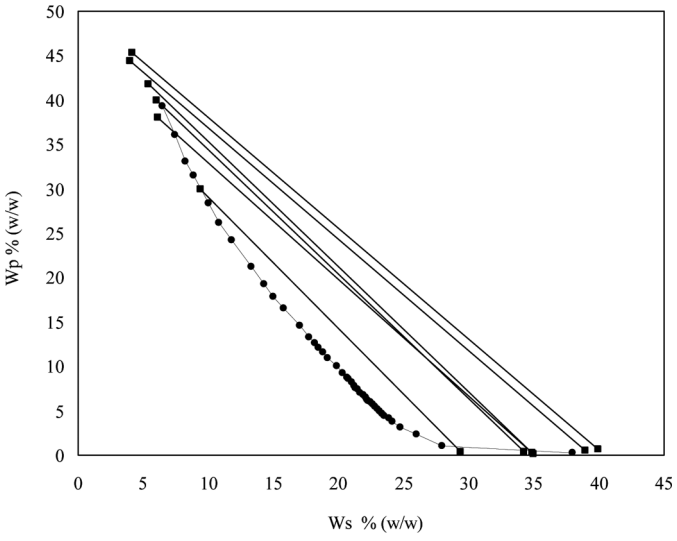

FIG. 2. Binodal curve and tie-lines for PEG 4000 (wP) + lithium citrate (wS) system at 25°C.

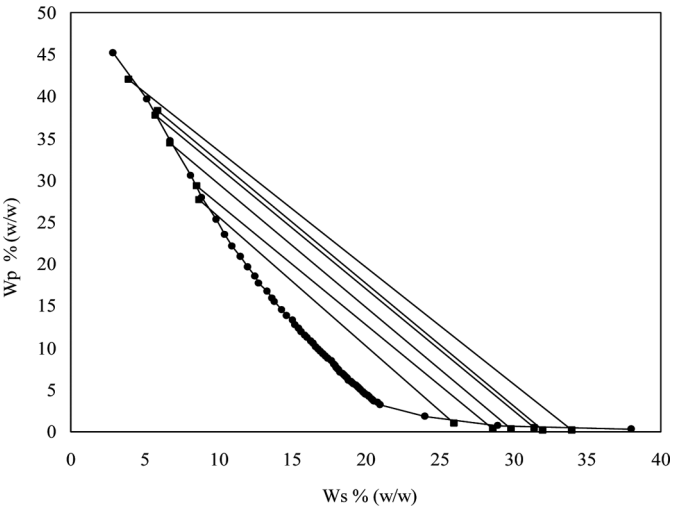

FIG. 4. Binodal curve and tie-lines for PEG 8000 (wP) + lithium citrate (wS) system at 25°C.

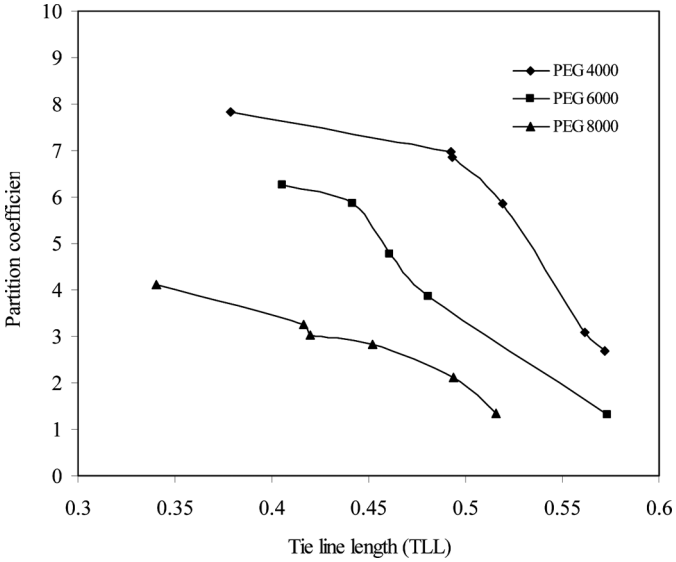

FIG. 5. Effect of TLL on partition coefficient for different molecular weight of PEG.

the protein molecules were accumulated in the PEG phase. In PEG/salt systems, PEG is overall positively charged, due to the polymer's repeating ether oxygen atoms. These are capable of ionic binding with the protein's metallic elements (34). The degree of isolation of protein was expressed as partition coefficient, K and defined as,

$$K = \frac{\text{Amount of protein in PEG rich phase (mg)}}{\text{Amount of protein in salt rich phase (mg)}}$$

The partition coefficient is governed by several factors, some of which can be related to the system parameters like pH, ionic strength, polymer molecular mass, and other factors.

Effect of Tie Line Length (TLL)

In general the PEG molecules present in the ATP systems were mainly responsible for the two phase formation. However, the type and nature of the salt present in the

TABLE 5
Comparison of lithium citrate with literature salts (37–40) systems at 25°C

Feed		Top phase		Bottom phase				
Wp	W_s	W_p	W_s	W_p	W_s	TLL	K protein	% Yield
PEG 4000 + tri-potassium citrate								
0.21	0.12	0.5379	0.0406	0.0195	0.2355	0.55	0.23	26.78
0.21	0.17	0.6807	0.0658	0.1136	0.2505	0.59	0.25	17.93
0.25	0.15	0.6663	0.0064	0.1062	0.2513	0.61	0.27	25.75
0.25	0.17	0.7150	0.0123	0.1495	0.2612	0.62	0.33	29.73
0.29	0.12	0.6103	0.0102	0.1542	0.2593	0.52	0.26	43.54
0.29	0.15	0.7067	0.0056	0.1206	0.2769	0.65	0.28	29.50
PEG 4000 + tri-sodium citrate								
0.21	0.12	0.4320	0.0068	0.0443	0.1933	0.43	0.47	25.48
0.21	0.17	0.3404	0.1504	0.0929	0.2303	0.26	0.51	47.07
0.25	0.15	0.3714	0.1191	0.0927	0.2225	0.30	0.29	28.94
0.25	0.17	0.3878	0.1360	0.1382	0.2180	0.26	0.61	42.06
0.29	0.12	0.4220	0.0877	0.1225	0.2097	0.32	0.24	30.47
0.29	0.15	0.3406	0.1402	0.0930	0.2183	0.26	0.42	48.68
PEG 4000 + sodium sulfate								
0.21	0.17	0.4609	0.1109	0.1090	0.2535	0.38	0.29	22.35
0.25	0.15	0.4443	0.1361	0.0798	0.1949	0.37	2.20	73.07
0.25	0.17	0.4250	0.1613	0.0617	0.2223	0.38	3.15	80.05
0.29	0.12	0.3978	0.1078	0.0878	0.2479	0.34	1.26	83.65
0.29	0.15	0.4642	0.1064	0.0240	0.2638	0.47	2.38	79.62
PEG 4000 + lithium citrate								
0.21	0.12	0.2990	0.0450	0.0007	0.2786	0.38	7.82	94.81
0.25	0.15	0.3996	0.0484	0.0045	0.3423	0.49	6.98	93.32
0.21	0.17	0.3946	0.0498	0.0021	0.3489	0.49	6.85	90.27
0.29	0.12	0.4183	0.0402	0.0012	0.3498	0.52	5.86	91.87
0.25	0.17	0.4441	0.0403	0.0048	0.3899	0.56	3.09	85.62
0.29	0.15	0.4537	0.0419	0.0070	0.3995	0.57	2.69	84.33

system was highly responsible for protein portioning (35). The Tie Line Length (TLL) %, which in turn represents the equilibrium of the ATPS, was utilized to study the combined effect of the weight fraction of PEG and salt. To evaluate the influence of system parameters upon partition behavior, it is suggested to examine the behavior of product recovery from the top phase with respect to TLL. The tie line lengths are estimated using the following relationship (36),

$$TLL = [(w_{p(T)} - w_{p(B)})^2 + (w_{s(T)} - w_{s(B)})^2]^{1/2} \qquad (6)$$

The partitioning coefficient corresponding to the feed composition and the phase composition with tie line length were reported in the Table 3. From Fig. 5 it was observed that the partitioning coefficient increases with decreasing TLL. For the large value of tie line length the equilibrium values of the salt and PEG concentration in both the phases were lying at the top most region of the binodal curve, where the physical properties of the phases like viscosity and density and concentration of either PEG in top phase or salt in the bottom phase were high. However, handling of these phases further for protein recovery may become difficult. But the equilibrium concentration in both the phases for a smaller value of TLL leads to the middle portion of the binodal curve (near by ploit point), where, the concentration of the salt and polymers were low and which is more favorable for the partitioning (35). Conversely, the difficulties with respect to the phase separation are occurred due to the very minimal difference between the individual phase physical properties like density.

Effect of PEG Molar Mass

From the current study it was observed that the partition coefficients of crude fish proteins decreased with increasing PEG molecular mass and concentration. The proteins moved into the salt-rich lower phase, as the PEG molecular mass increased, possibly due to an excluded volume effect (34). Since at the lower molecular weight PEG solution contains a large amount of water, the water soluble proteins easily get accumulated at the polymer phase.

Comparison with Literature ATP Systems

The partition coefficient and % recovery of the crude fish protein for the present ATPS system (PEG 4000 + Lithium citrate hydrate) was compared with tri-potassium citrate (37), tri-sodium citrate (38) and sodium sulphate (39,40) (Table 5) at 25°C. The identical feed compositions of PEG4000 and salt concentrations were maintained for all the system to understand the effect of type of salt over the partitioning coefficient/recovery. Further, Fig. 6 was plotted to study the effect of TLL on the partition coefficient for all the ATPS system considered here for the comparison purpose. It was observed from Fig. 6 that the PEG 4000 + Lithium citrate hydrate system showed a higher

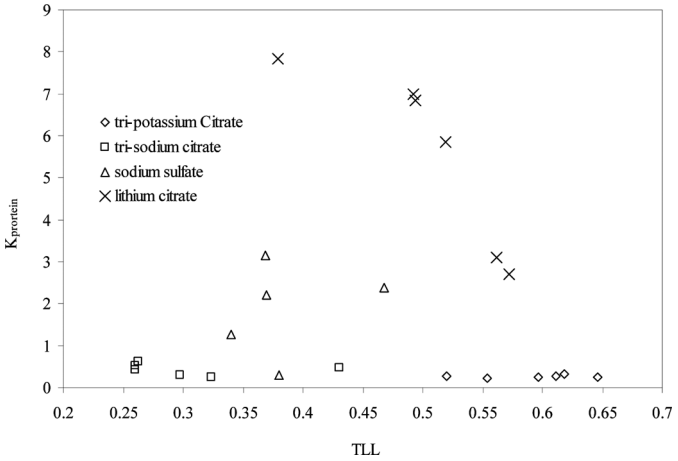

FIG. 6. Comparison of lithium citrate with literature salt (37–40) systems at 25°C.

partition coefficient when compared to all the other systems and it has the ability to recover more amount of protein in the top phase, due to the higher hydration value and EEV of the lithium citrate salt.

CONCLUSION

A novel PEG + Lithium citrate hydrate + water ATPS was taken for the study. The phase diagrams/ binodals for different PEG grades and lithium citrate salt were prepared. The effect of increasing polymer molecular weight is to move the binodals towards lower polymer concentrations as reported by previous studies. The effective excluded volume (EEV) of the systems, which decides the direction of partitioning of the protein, was determined. The present PEG-Lithium citrate systems showed higher values of EEV for higher molecular mass of polymer and salt in the system which suggests the partitioning of proteins to upper PEG rich phase. The equilibrium studies of the selected ATPS were carried out, the experimental values were fitted to the equation given by Othmer-Tobias and Bancroft and verified. The same feed compositions of the PEG- Salt were adopted for the partitioning study of the fish effluent. Partition coefficient of 7.82 was recorded for PEG-4000-lithium citrate-water ATPS. The effect of Tie line length (TLL), which is a system parameter on the partition coefficient was determined. The partition coefficients of crude fish proteins decreased with increasing PEG molecular mass in this ATPS. This study characterizes the novel PEG-lithium citrate-water ATPS and further demonstrates the potential of this system for protein recovery from biological process streams.

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