Partitioning of L-Lysine Monohydrochloride in Aqueous Two-Phase Systems of Poly(ethylene glycol) and Dipotassium Hydrogen Phosphate or Trisodium Citrate 5-Hydrate

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The partition constants of L-lysine HCl were measured in polymer−salt aqueous two-phase systems. These systems contain poly(ethylene glycol) with a nominal molecular weight of 4000 or 10000 and two different salts (dipotassium hydrogen phosphate or trisodium citrate 5-hydrate). The experimental data were obtained at temperatures of (293.15, 298.15, and 303.15) K. The effects of temperature, pH, polymer and salt concentrations, polymer molecular weight, and salt type on the partitioning of L-lysine HCl were also studied. The results showed that salt concentration has a significant effect on the partition constant while temperature has less effect. The Diamond and Hsu model was used to correlate the experimental partition constants of L-lysine HCl.

Introduction

L-Lysine is an essential amino acid that is not produced by the body naturally and, hence, needs to be obtained externally from diet. L-Lysine is significant in healthy protein synthesis, nitrogen balance, and immune function.

The driving force for research and development in bioseparation has been derived from the encountered complexity in the downstream processing of pharmaceutical and biological products. These products are often presented at low concentrations in complex mixtures containing other similar materials. Accordingly, as much as (50 to 90) % of the production cost of a typical biological product resides in its purification.[1] One method for purifying such products is via extraction in aqueous two-phase systems (ATPS).

ATPS arise in aqueous mixtures of different water-soluble polymers or a single polymer and a specific salt.[2] Both polymer−polymer and polymer−salt ATPS have advantages over conventional extraction using organic solvents. Since water is the shared part of each phase's bulk, ATPS form a gentle environment for biomaterials.[3] Aqueous two-phase extraction has the potential to achieve the desired purification and concentration of the product in a single step.[4]

Albertsson used ATPS for the separation and purification of biomolecules, cell organelles, membranes, and cells.[4] Zaslavsky et al. measured partitioning of glycine, lysine, aspartic acid, and oligopeptides in an ATPS containing dextran and poly(ethylene glycol) (PEG 8000).[5]

Grossmann et al. measured partition constants of particular amino acids such as glycine, L-glutamic acid, L-phenylalanine, and L-lysine and low molecular weight peptides in ATPS of K_2HPO_4 and PEG with molecular weights of about (6000 and 35000) $g \cdot mol^{-1}$ at 293 K.[6]

Additionally, Haghtalab et al. investigated partitioning of lysozyme, bovine serum albumin, and *R*-amylase in ATPS of PEG + K_2HPO_4 + water and PEG + Na_2SO_4 + water at 298 K.[7] Moreover, Salabat et al. also measured and correlated partition constants of three amino acids, L-tryptophan, L-phenylalanine, and L-tyrosine, in ATPS PEG 6000 and $MgSO_4$, Na_2SO_4, and $(NH_4)_2SO_4$ at 298 K.[8]

Khederlou et al. measured and modeled partition constants of the antibiotic cephalexin in ATPS containing PEG 4000 (or 10000) and K_2HPO_4 or Na_3 citrate at three different temperatures, (300.2, 307.2, and 310.2) K.[9]

Recently Pazuki et al. measured and modeled partition constants of the antibiotic penicillin G acylase in ATPS containing PEG 20000 (or 35000) and KH_2PO_4 or Na_3 citrate from (301.2 to 310.2) K.[10]

In this work, the partition constants of L-lysine HCl were measured in ATPS of PEG + K_2HPO_4 + water and PEG + Na_3 citrate + water at three different temperatures, (293.15, 298.15, and 303.15) K. The effects of temperature, pH, polymer molecular weight, type of salt, polymer, and salt concentrations on the partition constants of L-lysine HCl were investigated. The correlation of Diamond and Hsu was used for predicting the partition constants. The expression relates the partition constant of the biomolecule to the polymer concentration difference between the phases.[11]

Experimental Section

Materials. Poly(ethylene glycol) with a nominal molecular weight of (4000 and 10000) $g \cdot mol^{-1}$, dipotassium hydrogen phosphate with 99.5 % purity, trisodium citrate 5-hydrate with 99 % purity, and L-lysine monohydrochloride with 99 % purity were supplied by Merck Chemicals. Double-distilled and deionized water was used in all of the controlled experiments.

Methods. ATPS were made from stock solutions of $w = 0.45$ PEG and $w = 0.30$ salt. A specified amount of each solution was taken and was mixed in a 100 cm^3 glass cell connected to a thermostat. A specified amount of L-lysine HCl was measured and added to this solution. The resulting solution was mixed

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Table 1. Parameters of the Refractive Index Equation

component	α_0	α_1	α_2
water	1.3324		
PEG 4000		0.1490	
PEG 10000		0.1475	
Na_3 citrate			0.1424
K_2HPO_4			0.163

for 20 min on a magnetic stirrer, and then the pH of the solution was measured. To achieve final phase equilibrium, the solution was kept for 24 h at the desired temperature. After 24 h the samples from the top and bottom phase were taken and kept for further analysis.

Quantitative Analysis. The mass fraction of salt was determined by flame photometry (Corning 410, from Jenway, England).

The mass fraction of PEG was determined by refractive index measurements using an Erma optical refractometer (model 17101, from Erma optical, Japan). The correlation between the refractive index (n_D) and the weight fractions of polymer (w_P) and salt (w_S) can be obtained as follows:

$$n_D = a_0 + a_1 w_P + a_2 w_S \quad (1)$$

The parameters of eq 1 are presented in Table 1 for different polymer−salt ATPS. This correlation was used for the top and bottom phases.

The mass fraction of L-lysine HCl was determined by UV−vis spectrophotometry at 216 nm using a spectrophotometer (M501, from Camspec, England). The absorbance of the top-phase and bottom-phase solutions was measured using a blank solution of polymer, salt, and water. The concentration of L-lysine HCl in the top and bottom solutions was determined from the correlation between the absorbance and the concentration of standard solutions of L-lysine HCl.

The pH of aqueous solutions was measured with a pH meter (model 744, from Deutsche Metrohm, Filderstadt, Germany).

The relative uncertainty in the mass fractions of salt and polymer was less than 3 %, while the relative uncertainty in the mass fractions of L-lysine HCl was less than 7.5 %.

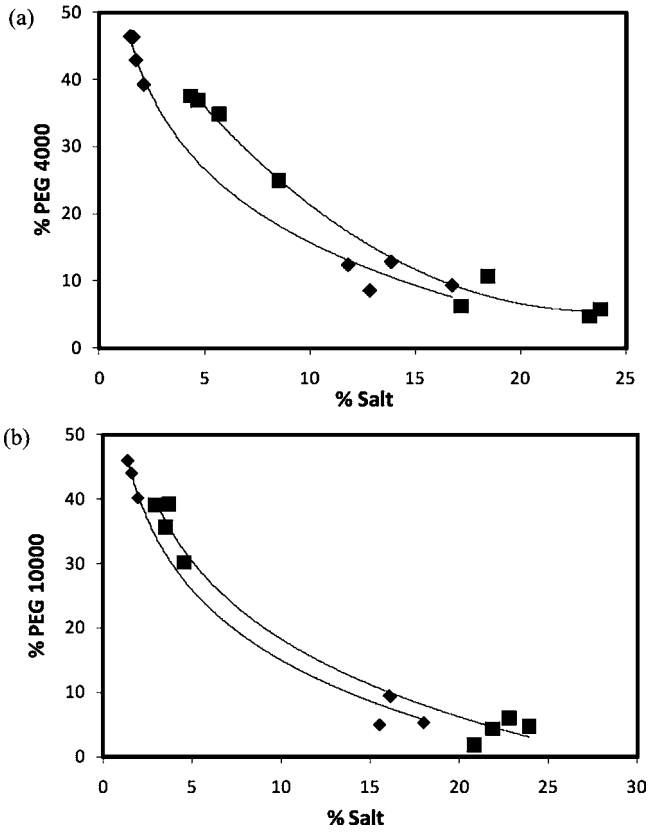

Figure 1. Effect of pH on the binodal curves of PEG and salt systems for (a) PEG 4000: ■, pH 7.98, salt Na_3cit; ♦, pH 9.61, salt K_2HPO_4; and (b) PEG 10000: ■, pH 7.59, salt Na_3cit; ♦, pH 9.45, salt K_2HPO_4.

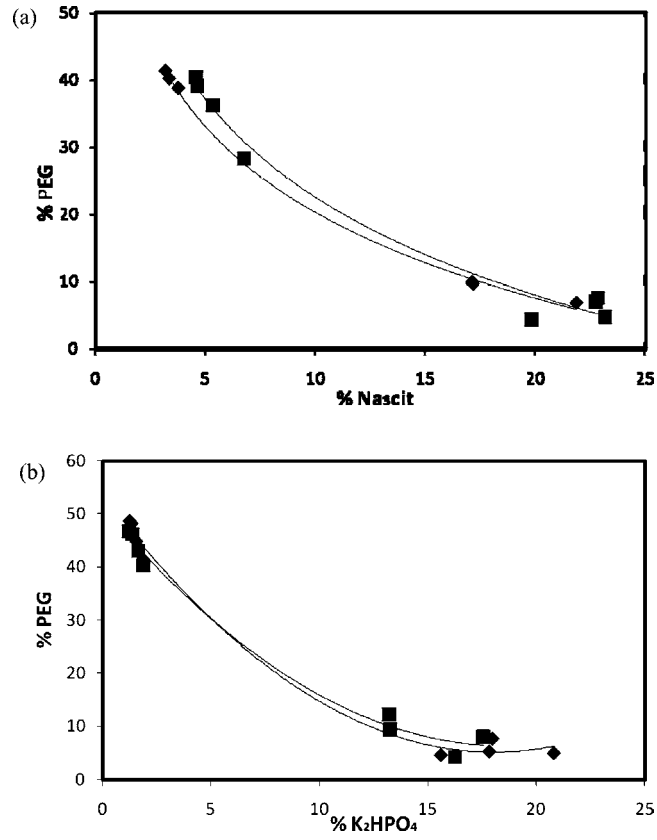

Figure 2. Effect of PEG molecular weight on phase diagrams for (a) Na_3cit: ■, PEG 4000; ♦, PEG 10000; and (b) K_2HPO_4: ■, PEG 4000; ♦, PEG 10000.

Results and Discussion

Effects of pH on the Phase Diagrams. The phase diagrams for the PEG 4000 and PEG 10000 systems at different pH values are depicted in Figure 1. It is evident that acidity has similar effects on binodal curves despite the different PEG molecular weights. With increasing pH values, the binodal curves shift toward lower polymer and salt concentrations, and therefore, the area of phase separation region expands.

Effects of PEG Molecular Weight on the Phase Diagrams. The effects of PEG molecular weight on the binodal curves are shown in Figure 2. By increasing the PEG molecular weight, the phase separation region expands, and the binodal curve shifts towards a lower concentration of polymer and salt.

Effect of Temperature on the Phase Diagrams. Effects of temperature on the binodal curves are shown in Figure 3. It is apparent that a drop in temperature causes a binodal shift towards lower polymer and salt concentrations. This means that, by decreasing temperature, the area of phase separation expands.

Composition of ATPS and Partitioning Values. The mass fractions of polymer, salt, and L-lysine HCl in the feed, top, and bottom phases as well as the partition constants of L-lysine HCl at different temperatures are presented in Tables 2 and 3. The experimental data for the partitioning of L-lysine HCl in PEG + Na_3 citrate + water at different pH values are

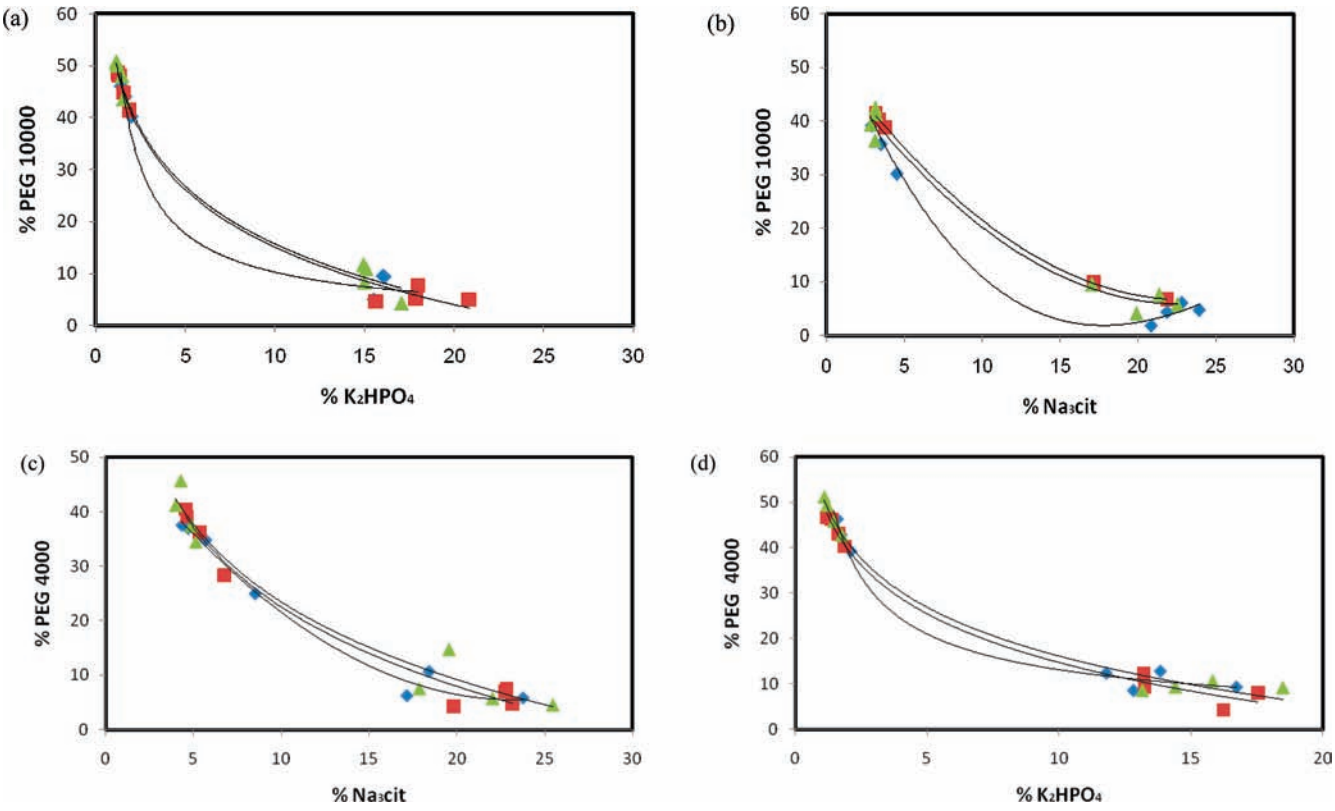

Figure 3. Effect of temperature change on binodal curves for different systems for (a) PEG 10000 and K_2HPO_4, (b) PEG 10000 and Na_3cit, (c) PEG 4000 and Na_3cit, and (d) PEG 4000 and K_2HPO_4. ♦, $T = 293.15$ K; ■, $T = 298.15$ K; ▲, $T = 303.15$ K.

Table 2. Mass Fractions of PEG (1), Na_3 Citrate (2), and L-Lysine HCl (3) in the Top and Bottom Phases and the Partition Constants

T		feed			top phase			bottom phase			
K	pH	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	K_{Lys}
					PEG 4000 + Na_3 Citrate						
293.15	7.92	26.4685	11.3960	0.0183	37.5181	4.3526	0.0098	5.7937	23.7833	0.0098	0.9956
293.15	7.89	26.4709	11.3971	0.0089	36.9121	4.7057	0.0076	10.6404	18.4310	0.0411	0.1841
293.15	8.24	27.0589	9.4194	0.0187	34.8153	5.7059	0.0037	4.6838	23.2592	0.0066	0.5667
293.15	7.86	16.7012	12.9098	0.0208	24.9419	8.5228	0.0118	6.2100	17.1680	0.0189	0.6227
298.15	8.37	26.4685	11.3960	0.0182	40.4617	4.5730	0.0077	7.5720	22.8355	0.0247	0.3098
298.15	8.45	26.4709	11.3971	0.0089	39.1369	4.6250	0.0063	6.9865	22.7459	0.0457	0.1374
298.15	8.34	27.0589	9.4194	0.0187	36.2321	5.3470	0.0007	4.7089	23.1628	0.0107	0.0660
298.15	8.02	16.7013	12.9098	0.0207	28.3236	6.7401	0.0147	4.2767	19.8229	0.0973	0.1515
303.15	7.93	26.4685	11.3960	0.0182	45.6423	4.2788	0.0159	14.7199	19.5698	0.0867	0.1830
303.15	8.20	26.4709	11.3971	0.0089	41.1488	3.9945	0.0101	4.5768	25.4779	0.0271	0.3735
303.15	8.40	27.0589	9.4194	0.0187	37.3604	4.8687	0.0173	5.7758	22.0464	0.0325	0.5308
303.15	8.24	16.7013	12.9098	0.0207	34.4175	5.1390	0.0277	7.5453	17.8775	0.0735	0.3773
					PEG 10000 + Na_3 Citrate						
293.15	7.37	26.4685	11.3960	0.0182	39.0883	2.9106	0.0053	4.7722	23.9192	0.0593	0.0891
293.15	7.65	26.4709	11.3972	0.0089	39.2084	3.6992	0.0030	6.0574	22.7987	0.0705	0.0419
293.15	7.83	27.0589	9.4194	0.0187	35.6686	3.5034	0.0152	4.3845	21.8630	0.0247	0.6156
293.15	7.50	16.7012	12.9098	0.0207	30.1350	4.5301	0.0020	1.8457	20.8410	0.1863	0.0107
298.15	7.41	26.4685	11.3960	0.0182	41.4090	3.1754	0.0031	6.7688	21.8511	0.0597	0.0523
298.15	7.50	26.4710	11.3971	0.0089	49.5093	0.000	0.0190	26.2727	0.2442	0.1807	0.1053
298.15	7.93	27.0589	9.4194	0.0187	38.8065	3.7644	0.0072	9.8908	17.1426	0.0256	0.2808
298.15	7.82	16.7012	12.9098	0.0207	40.2215	3.3520	0.0116	9.5991	17.1638	0.2580	0.0449
303.15	7.3900	26.4684	11.3960	0.0182	41.3861	3.1991	0.0046	5.8559	22.5159	0.0463	0.0991
303.15	7.4000	26.4709	11.3971	0.0089	42.4519	3.1485	0.0142	7.6533	21.3563	0.0822	0.1726
303.15	7.5700	27.0590	9.4195	0.0187	39.4109	2.8574	0.0038	9.5815	17.0416	0.0334	0.1144
303.15	7.5800	16.7013	12.9097	0.0207	36.3683	3.1298	0.0120	4.1806	19.8972	0.1172	0.1026

accordingly reported in Table 2. The experimental data for the partitioning in PEG + K_2HPO_4 + water at different pH values are reported in Table 3. Values reported in the aforementioned tables mark that the top phase is rich in PEG while the bottom phase is rich in salt. Binodal curves, the tie lines for systems PEG 10000 and K_2HPO_4 at 298.15 K and PEG 4000 and K_2HPO_4 at 303.15 K are shown in Figure 4.

Partitioning Behavior. Partition constants of L-lysine HCl in the PEG 4000 + Na_3 citrate system diminish by decreasing salt and polymer concentrations in the feed, and yet for the PEG 4000 + K_2HPO_4 system, the reverse behavior was noticed.

For the PEG 10000 + Na_3 citrate system by decreasing salt concentration in the feed, the partition constants increased, and yet by decreasing the polymer concentrations in the feed, the

Table 3. Mass Fractions of PEG (1), K_2HPO_4 (2), and L-Lysine HCl (3) in the Top and Bottom Phases and the Partition Constants

T		feed			top phase			bottom phase			
K	pH	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	100 w_1	100 w_2	100 w_3	K_{Lys}
					PEG 4000 + K_2HPO_4						
293.15	9.9300	26.4685	11.3960	0.0182	46.3093	1.5945	0.0010	9.2671	16.7435	0.0055	0.1871
293.15	9.4600	26.4710	11.3971	0.0090	46.3825	1.4663	0.0008	12.7625	13.8552	0.0068	0.1164
293.15	9.5900	27.0589	9.4194	0.0187	42.8687	1.7336	0.0015	12.3197	11.8059	0.0051	0.2925
293.15	9.4600	16.7012	12.9098	0.0207	39.2427	2.1033	0.0016	8.5056	12.8385	0.0046	0.3477
298.15	9.6600	26.4685	11.3960	0.0182	46.7425	1.1986	0.0009	7.9787	17.5532	0.0057	0.1535
298.15	9.6900	26.4709	11.3971	0.0089	46.2139	1.3750	0.0014	12.2438	13.2250	0.0046	0.2918
298.15	9.7600	27.0589	9.4194	0.0187	43.0288	1.6485	0.0016	9.3242	13.2558	0.0070	0.2237
298.15	9.6800	16.7012	12.9098	0.0207	40.2965	1.8762	0.0002	4.2498	16.2380	0.0007	0.3258
303.15	9.7200	26.4685	11.3960	0.0182	49.4365	1.1900	0.0008	10.5876	15.8432	0.0081	0.0978
303.15	9.7800	26.4709	11.3971	0.0089	51.2261	1.0878	0.0007	9.1469	18.5098	0.0195	0.0361
303.15	9.6400	27.0589	9.4194	0.0187	45.8604	1.4528	0.0008	9.2494	14.4284	0.0025	0.3195
303.15	9.4100	16.7012	12.9098	0.0207	42.7853	1.7484	0.0006	8.4769	13.1714	0.0015	0.4172
					PEG 10000 + K_2HPO_4						
293.15	9.9600	26.4685	11.3960	0.0182	45.9646	1.4124	0.0026	9.4637	16.0374	0.0032	0.8090
293.15	9.1500	26.4710	11.3971	0.0090	46.0005	1.3799	0.0030	9.3204	16.1057	0.0031	0.9718
293.15	9.3300	27.0589	9.4196	0.0187	44.0534	1.6081	0.0003	5.2863	17.9771	0.0025	0.1015
293.15	9.3400	16.7013	12.9098	0.0207	40.2031	1.9634	0.0027	4.9593	15.5123	0.0029	0.9326
298.15	9.4600	26.4685	11.3960	0.0182	48.6066	1.2302	0.0029	4.9829	20.8284	0.0036	0.8043
298.15	9.1800	26.4709	11.3971	0.0089	48.1061	1.3150	0.0040	7.7266	17.9775	0.0049	0.8219
298.15	9.1400	27.0590	9.4194	0.0187	44.8068	1.5399	0.0012	5.2988	17.8431	0.0018	0.6611
298.15	9.2100	16.7012	12.9098	0.0208	41.4081	1.8546	0.0040	4.6508	15.6074	0.0046	0.8602
303.15	9.4700	26.4685	11.3960	0.0182	50.7610	1.1212	0.0023	11.7119	14.9233	0.0027	0.8263
303.15	9.1300	26.4709	11.3972	0.0090	50.0893	1.1769	0.0030	10.9422	15.0677	0.0034	0.8559
303.15	9.0700	27.0589	9.4195	0.0187	47.9632	1.4443	0.0014	8.2583	14.9809	0.0020	0.6903
303.15	9.8300	16.7012	12.9098	0.0206	43.5461	1.5150	0.0038	4.2793	17.0478	0.0039	0.9666

partition constants decreased. For the PEG 10000 + K_2HPO_4 system, however, the reverse behavior was noticed.

For the PEG + Na_3 citrate system, by increasing polymer molecular weight, the partition constants decrease, which in turn means that L-lysine HCl prefers to accumulate more in the salt-rich phase. This behavior can be interpreted as follows: when the polymer molecular weight increases, the length of the polymer chain increases, and there is not enough space for L-lysine HCl in the polymer-rich phase, so it prefers the bottom phase to the top phase. For the PEG + K_2HPO_4 system when the polymer molecular weight increases, the partition constants also increase because in these systems the pH is about 9. At this pH, L-lysine's hydrophobicity increases, and when the polymer molecular weight increases, there are less hydroxyl groups available for the polymer chain. Hence, the hydrophobicity of the polymer chain increases; as a result of such reactions, lysine will transfer more to the top phase, and consequently its partition constant will increase.

L-Lysine HCl prefers to stay in the salt-rich phase because its partition constant in all of the experiments was less than unity. When the temperature of the systems increases the partition constants slightly decrease.

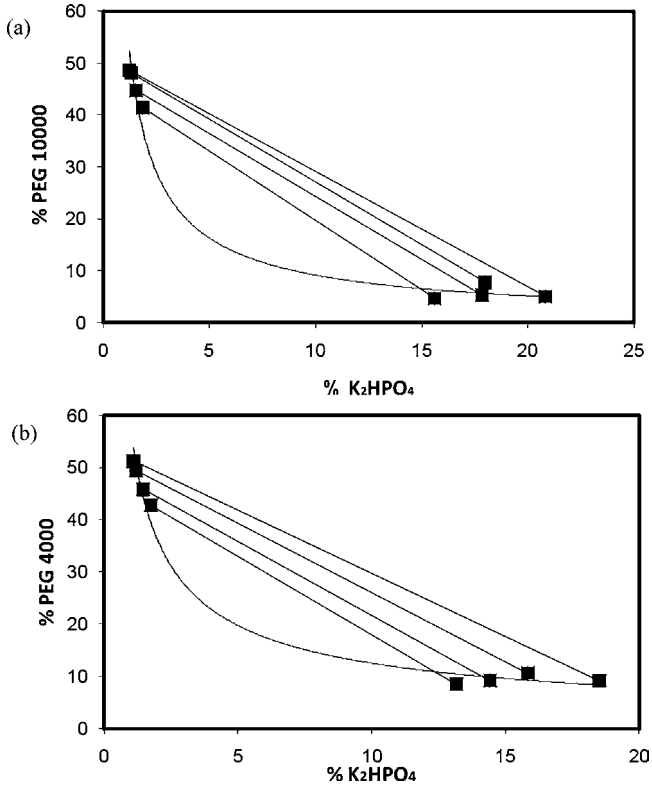

Figure 4. Binodal curve and tie lines for (a) PEG 10000 and K_2HPO_4 at 298.15 K; (b) PEG 4000 and K_2HPO_4 at 303.15 K.

Mathematical Modeling

In this section, the partition constants of L-lysine HCl in the ATPS were correlated using the corresponding experimental data. The partition constant can be defined as follows:[4]

$$K = C_t/C_b \tag{2}$$

In the above equation C_t and C_b are the concentrations of the partitioned substance in the top and bottom phases, respectively.

Diamond and Hsu first introduced a linear correlation between the natural logarithm of the partition constant and the concentration of polymers in the two phases.[12] Then the correlation was improved, and subsequently, the following correlation was obtained:[11]

$$\frac{\ln K}{\Delta W(\mathrm{PEG})} = A + B\cdot\Delta W(\mathrm{PEG}) \tag{3}$$

Equation 3 was used to correlate the partition constant with the weight fraction difference of PEG in the top and bottom phase, which is shown by ΔW(PEG). The adjustable parameters (A and B) of the partition constant correlation were obtained using a linear regression between the experimental partition constants and those obtained from eq 3.

Table 4. Values of *A* and *B* Constants of Equation 3 and rmsd Values in ATPS

system	*A*	*B*	rmsd
PEG 4000 + Na_3 citrate	−6.85	15.20	8.45
PEG 10000 + Na_3 citrate	−12.55	19.88	4.60
PEG 4000 + K_2HPO_4	0.52	−13.14	2.53
PEG 10000 + K_2HPO_4	1.11	−4.57	6.33

Values of the root-mean-square deviation (rmsd) and the adjustable parameters (*A* and *B*) are presented in Table 4. rmsd values can be obtained from eq 4. In this equation, *N* is the number of tie lines.

$$\text{rmsd} = \frac{\sqrt{\sum_{i=1}^{N} (K_i^{\text{exp}} - K_i^{\text{cal}})^2}}{N} \qquad (4)$$

Conclusions

In this research, the partition constants of L-lysine HCl were measured in ATPS of PEG + Na_3 citrate + water and PEG + K_2HPO_4 + water. The partitioning of lysine depends on specific factors that include temperature, pH, polymer molecular weight, and salt and polymer concentrations in the feed. The correlation of Diamond and Hsu was used to correlate the partition constants of lysine in the ATPS. The results indicated that the proposed model can correlate the partition constants of lysine in polymer−salt ATPS with relatively low rmsd values. Moreover, the experimental data indicated that the partitioning of lysine depends on the concentration of salt in the feed. The variations of temperature have a small effect on partitioning.

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