

Liquid-Liquid Equilibria of Aqueous Two-phase Systems Containing Polyethylene Glycol 4000 and Two Different Salts of Ammonium

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Abstract: The aim of this study was to survey on phase diagrams and Liquid-Liquid Equilibrium (LLE) data of the aqueous PEG₄₀₀₀ - (NH₄)₂HPO₄ and PEG₄₀₀₀ - (NH₄)₂SO₄ systems experimentally at 298.15 K. The salting-out effect was also discussed on the basis of the Gibbs free energy of hydration of ions. The experimental binodal data were satisfactorily correlated with the Merchuk equation. Tie line compositions were correlated using the Othmer-Tobias and Bancroft equations, and the parameters have also reported. Good agreement was obtained with the experimental data with the model equations.

Key words: Liquid-liquid equilibrium, polyethylene glycol 4000, salts of ammonium

INTRODUCTION

Presently, industrial demands and economic downstream processes for extraction and purification of biomolecules with high yield purity of the product are growing fast (Haghtalab and Joda, 2009; Amaresh *et al.*, 2008). The Aqueous Two Phase System (ATPS) provides a powerful method to separating mixtures of biomolecules by extraction in down stream processing. Aqueous two-phase systems are formed by soluble polymers in water by using two polymers or an inorganic salt and a polymer (Albertsson, 1971). Using ATPS as a practical process allows one to integrate clarification, concentrating, and partial purification of biomolecules in one step. Also polymer-salt aqueous two-phase systems have several advantages such as low price, low viscosity, and short time for phase separation. One of the major polymers for this purpose is Polyethylene Glycol (PEG), which is a nontoxic, inflammable and low in cost (Imani *et al.*, 2009). Aqueous Two-Phase Systems (ATPSs) can be applied successfully for the separation of biological materials, dyes, drug molecules, proteins, and nucleic acid (Azevedo *et al.*, 2009; Da Silva and Meirelles, 2000; Karakatsanis and Liakopoulou-Kyriakides, 2007). On the other hand, a liquid-liquid extraction process requires knowledge of the phase behavior of the system for engineering design and process optimization.

This work is devoted to obtaining LLE data for aqueous PEG₄₀₀₀ - (NH₄)₂HPO₄ and PEG₄₀₀₀ - (NH₄)₂SO₄ systems at 298.15 K. Suitable equations were used to correlate the binodal and the tie-line data for the investigated systems.

MATERIALS AND METHODS

Materials: The salts and other chemicals used were of analytical grade. The salts were dried in an oven at about 393.15K for 24 h before use. All chemicals were used without further purification.

Apparatus and procedure: The experimental apparatus employed is essentially similar to the one used previously (Khayati *et al.*, 2009). The bimodal 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; which indicated the formation of two liquid phases. In accordance to with the amount titrant added until turbidity was observed. The composition of the mixture was determined by mass using an analytical balance with a precision of ± 0.0001 g. For the determination of the tie-lines, feed samples 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 2-3 h. Then the mixture was allowed to settle for 24 h. Portions of solutions in both phases were taken out carefully for analysis.

The concentration of PEG was determined by refractive index measurements at 298.15K using a Kruss Abbe refractometer AR3D. Since the refractive index of phase samples depends on PEG and salt concentration, calibration plots of refractive index versus polymer concentration were prepared for different concentration of salt (Cheluget *et al.*, 1994). The relation between the refractive index, n_D , and the mass fractions of polymer, w_1 and w_2 salt, is given by:

Table 1: Values of parameters of Eq. (1) for PEG₄₀₀₀ (1) + salt (2) + H₂O (3) systems at 298.15 K

System	α_1	β_2
PEG ₄₀₀₀ + (NH ₄) ₂ HPO ₄ + H ₂ O	0.2556	0.1426
PEG ₄₀₀₀ + (NH ₄) ₂ SO ₄ + H ₂ O	0.1722	0.1394

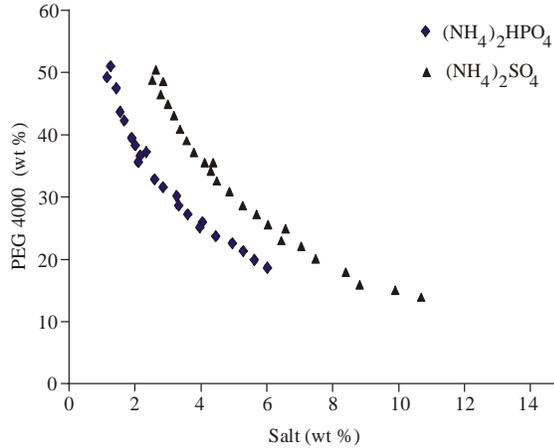


Fig. 1: Experimental binodal data for the PEG₄₀₀₀ (1) + salt (2) + H₂O (3) systems at 298.15 K

$$n_D = n_D^0 + a_1 w_1 + a_2 w_2 \quad (1)$$

where n_D is the refractive index of pure water for which the value 1.3325 was measured at 298.15 K and a_1 and a_2 are constants. The values of coefficients and n_D^0 for the applied systems are given in Table 1.

All of experiments were done in engineering faculty laboratory of Guilan University.

RESULTS AND DISCUSSION

Experimental binodal data of the aqueous two-phase systems: PEG₄₀₀₀ - (NH₄)₂HPO₄ and PEG₄₀₀₀ - (NH₄)₂SO₄ determined at 298.15 K are given in Fig. 1. The tie-line data for these systems are given in Table 2.

The salting-out ability can also be related to the Gibbs free energy of hydration of the ions (ΔG_{hyd}) (Rogers *et al.*, 1996). In Fig. 1, considering that the salts share a common cation (NH₄⁺) but contain different anions, it is easy to see that the salting-out ability of the anions follows the ordering HPO₄²⁻ > SO₄²⁻. It seems that

Table 2: Experimental phase equilibrium compositions for the PEG₄₀₀₀ (1) + salt (2) + H₂O (3) systems at 298.15 K

PEG ₄₀₀₀ + (NH ₄) ₂ HPO ₄ + H ₂ O				PEG ₄₀₀₀ + (NH ₄) ₂ SO ₄ + H ₂ O			
Top phase		Bottom phase		Top phase		Bottom phase	
100 w ₁	100 w ₂	100 w ₁	100 w ₂	100 w ₁	100 w ₂	100 w ₁	100 w ₂
24.89	4.49	5.78	21.02	35.41	3.99	7.71	22.52
34.52	2.45	4.79	28.23	37.82	3.78	5.73	25.61
36.99	2.19	4.41	29.01	39.84	3.49	5.49	26.85
40.98	1.85	2.69	35.21	54.18	2.42	2.99	36.72

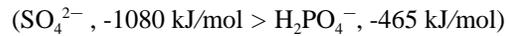
Table 3: Parameters and standard deviations of Eq. (2) for the PEG₄₀₀₀ (1) + salt (2) + H₂O (3) systems at 298.15 K

System	a	b	c	sd*
PEG ₄₀₀₀ + (NH ₄) ₂ HPO ₄ + H ₂ O	1.1828	-7.8070	-530.8290	0.0103
PEG ₄₀₀₀ + (NH ₄) ₂ SO ₄ + H ₂ O	1.8199	-8.0178	-26.2295	0.0081

$$*: sd = \left(\sum_{i=1}^N \frac{(w_1^{cal} - w_1^{exp})^2}{N} \right)^{0.5} \quad \text{where } w_1 \text{ and } N \text{ represent}$$

the concentration (in weight percent) of polymer and the number of binodal data, respectively

better salting-out of PEG is observed when the ions of the salt have a more negative (ΔG_{hyd}) value:



Since the Gibbs energy of hydration value for the HPO₄²⁻ ion was not available in the literature, the standard partial entropy can be used to explain the hydration energy of the ions. However, Shibukawa *et al.* (2000) pointed out that the standard partial entropy of the aqueous HPO₄²⁻ ion (10.9 J/K.mol) is smaller, when compared to that of aqueous SO₄²⁻ (64.5 J/K.mol), indicating that the HPO₄²⁻ ion attracts water molecules more strongly than SO₄²⁻; therefore the salting-out ability of *di-ammonium hydrogen phosphate* is stronger than the ammonium sulfate as can be seen from the Fig. 1.

Correlation:

Correlation of binodal data: For the correlation of binodal data of some aqueous PEG + salt systems the following nonlinear expression developed by Merchuk *et al.* (1998) has been successfully used by Zafarani-Moattar and Tolouei (2008):

$$w_1 = a \exp(bw_2^{0.5} - cw_2^3) \quad (2)$$

Using Eq. (2), the coefficients a, b, and c along with the corresponding standard deviations for the investigated systems were obtained, and the results are collected in Table 3. On basis of obtained standard deviations, we conclude that Eq. (2) can be satisfactorily used to correlate the binodal curves of the investigated systems.

Correlation of tie-line data: For the correlation of LLE data of polymer + salt + water systems, several models

Table 4: Parameters and standard deviations of Eq. (3) for the PEG₄₀₀₀ (1) + salt (2) + H₂O (3) systems at 298.15 K

System	n	k ₁	R ²
PEG ₄₀₀₀ + (NH ₄) ₂ HPO ₄ + H ₂ O	1.0603	0.8641	0.964
PEG ₄₀₀₀ + (NH ₄) ₂ SO ₄ + H ₂ O	1.1562	0.5499	0.984

Table 5: Parameters and standard deviations of Eq. (4) for the PEG₄₀₀₀ (1) + salt (2) + H₂O (3) systems at 298.15 K

System	r	k ₂	R ²
PEG ₄₀₀₀ + (NH ₄) ₂ HPO ₄ + H ₂ O	0.9095	1.1465	0.977
PEG ₄₀₀₀ + (NH ₄) ₂ SO ₄ + H ₂ O	0.7981	1.3344	0.988

have been developed. However, in this work we decided to use a relatively simple two-parameter equation which can be derived from the Othmer-Tobias and Bancroft (1942) equations.

The Othmer-Tobias and Bancroft Eq. (1942) used have the following from:

$$\left(\frac{1 - w_1^{top}}{w_1^{top}} \right) = k_1 \left(\frac{1 - w_2^{bot}}{w_2^{bot}} \right)^n \quad (3)$$

$$\left(\frac{w_3^{bot}}{w_2^{bot}} \right) = k_2 \left(\frac{w_3^{top}}{w_1^{top}} \right)^r \quad (4)$$

where k_1 , n , k_2 and r represent fit parameters. These equations have also been used to assess the reliability of LLE data. The corresponding correlation coefficient values and the values of the fitted parameters are given in Table 4-5. On the basis of the obtained standard deviations given in Table 4-5, we conclude that the performance of Eq. (3) and (4) is fairly good in correlating the tie-line data of the investigated systems. R² values are between 0.964 and 0.988 that indicates the degree of consistency of the related data. In general, the Bancroft correlation provides a better description of this system than does the Othmer-Tobias correlation as shown in the R-squared values.

CONCLUSION

Binodal curves and tie line compositions were measured for the PEG₄₀₀₀ + two different salts of ammonium based ATPS at 298.15 K. The binodal curve and tie-line data were correlated using available empirical equations, and the values of the parameters were reported and salting-out ability of the salt was discussed.

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