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Liquid−Liquid Equilibria for Random Copolymer of Ethylene Oxide + Propylene Oxide + Salt + Water

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Liquid−liquid equilibria of aqueous two-phase systems composed of aqueous solutions of random copolymer of ethylene oxide + propylene oxide and various salts were measured. The salts used were Na_2SO_4, K_2HPO_4, KH_2PO_4, and the mixture of K_2HPO_4 + KH_2PO_4. The effect of temperature on liquid−liquid equilibria is discussed.

Introduction

Aqueous two-phase systems (ATPS) have been considered for almost four decades, especially in the separation of various biological products (Albertsson, 1986; Walter and Brooks 1985). There are basically two kinds of ATPS: polymer−polymer ATPS, e.g., poly(ethylene glycol) (PEG) + dextran + water systems; and polymer-salt ATPS, e.g., PEG + potassium phosphate + water systems. The common problem encountered with these two kinds of ATPS is the difficulty in separating target biomolecules from the polymer solution. Since separation of the phase-forming polymers and biomolecules after the primary extraction is an important step in both laboratory- and industrial-scale processes, expensive and time-consuming methods, such as ultrafiltration, electrophoresis, and chromatography, have been employed to separate bioproducts from polymer solutions. The separation is far from being satisfactory (Li, 1996). If the polymer could be easily removed and recycled without costly ultrafiltation or chromatography, these ATPSs would be more cost-efficient. Therefore, a polymer that has the characteristics of temperature-induced phase formation needs to be considered. This is because the ethylene oxide + propylene oxide random copolymer (symbolized as EOPO), which is linear and nonionic, has a decreased solubility in water at higher temperatures. When heated, it can be separated from the aqueous solution. A two-phase system composed of an EOPO bottom phase and an aqueous top phase is thus formed. The temperature at which this phenomenon occurs is known as the cloud point of the polymer. A 10 mass % EOPO (1:1) solution, for example, has a cloud point of 70 °C (Li et al., 1997), while a 10 mass % PEG (M_w = 20 000) solution has a cloud point of 111.7 °C (Harris et al., 1991). The cloud point can be lowered by increasing the amount of the added sodium sulfate concentrations. The 0.2 M sodium sulfate lowered the cloud point by 22 °C for the EOPO (1:1) solution, which is favorable for most biological substances to be separated and purified. Thus, the combination of a cheap aqueous two-phase system with the temperature-induced phase formation offers a simple and efficient solution to the problem of polymer removal and recycling (Harris et al., 1991; Alred et al., 1992, 1994; Modlin et al., 1994; Li et al., 1997).

Work in our laboratory and others has shown that ATPS composed of EOPO and hydroxypropyl starch is useful for enzyme purification and amino acid separation (Harris et al., 1991; Jahansson et al., 1995; Li, 1996; Li et al., 1997). However, there are, to our knowledge, no reports on the application of EOPO + aqueous salt two-phase systems. Many phase diagrams for aqueous PEG + salt two-phase systems have been reported (Snyder et al., 1992), while liquid−liquid equilibrium (LLE) data on EOPO + aqueous salt two-phase systems are scarce, thus limiting the potential application of such systems to biotechnology. The starting point for any ATPS is the phase diagram, which can then be used to design and operate aqueous two-phase extraction processes. Furthermore, phase diagram data are necessary for the development of models that can predict phase separation. Thus, this work presents the LLE for EOPO + salt + water systems. The salts used are Na_2SO_4, K_2HPO_4, KH_2PO_4, and the mixture of K_2HPO_4 + KH_2PO_4.

Experimental Section

1. Materials. EOPO (1:1) (monobutyl ether of ethylene oxide + propylene oxide random copolymer, EO−PO molar ratio 1:1) was produced by Zhejiang University Chemical Factory (Hangzhou, P. R. China). The average molecular weight and the molecular weight distribution were determined by gel permeation chromatography (GPC, Waters 150C). Three-column system (Ultrastyragel linear, 500A and 100A) was used, and the column temperature was set to 30 °C. The solvent was tetrahydrofuran. The flow rate was 1.0 mL/min. The average number- and weight-molecular weight of EOPO were 4200 and 4650, respectively. The polydispersity of EOPO was 1.108, which indicated that EOPO sample used in this study had a narrow molecular weight distribution.

Na_2SO_4, K_2HPO_4, and KH_2PO_4 were analytical regent grade (Shanghai Chemical Reagent Corporation, P. R. China). For the potassium phosphate systems, the Henderson−Hasselbach equation was used to determine the ratio of mono- and dibasic salts necessary to make the pH equal to 6.8. The salts were dried overnight above 100 °C. Water was singly distilled. All other reagents were of analytical grade.

2. Experimental Procedure. The systems were prepared by mass from stock solutions, 60 mass % EOPO, 20 mass % Na_2SO_4, 40 mass % K_2HPO_4, and 20 mass % KH_2PO_4. Known masses of these solutions and water were weighted into a test tube to have the desired initial overall compositions. The total amount was 20 g. After the

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Table 1. Phase Compositions as Mass Fraction for EOPO (1:1) + Salt + H_2O

tie line no.	(mass %)									STL
	total system			bottom phase			top phase			
	EOPO	salt	H_2O	EOPO	salt	H_2O	EOPO	salt	H_2O	
				EOPO (1:1) + Na_2SO_4 + H_2O (25 °C)						
1	15.50	7.00	77.50	0.41	11.32	88.27	41.83	0.17	58.00	−3.71
2	14.50	6.50	79.00	0.62	9.65	89.73	37.70	0.67	61.63	−4.13
3	13.50	6.01	80.49	0.94	8.48	90.58	36.34	0.57	63.09	−4.47
4	12.50	5.50	82.00	1.28	7.90	90.82	31.65	1.11	67.24	−4.47
5	11.50	5.00	83.50	2.23	6.88	90.89	27.14	1.26	71.60	−4.43
				EOPO (1:1) + K_2HPO_4 + H_2O (25 °C)						
1	18.00	10.52	71.48	0.21	16.29	83.50	47.93	0.70	51.37	−3.06
2	17.49	9.99	72.52	0.31	15.88	83.81	45.01	0.79	54.20	−2.92
3	16.51	8.99	74.50	0.21	14.45	85.34	43.41	0.86	55.73	−3.19
4	15.49	7.99	76.52	0.28	12.53	87.19	40.37	1.08	58.55	−3.51
5	14.52	7.00	78.48	0.53	11.12	88.35	33.00	1.48	65.52	−3.37
6	13.50	6.00	80.50	1.03	9.11	89.86	30.56	1.71	67.73	−3.98
				EOPO (1:1) + KH_2PO_4 + H_2O (25 °C)						
1	19.50	9.50	71.00	1.00	15.18	83.82	48.50	1.47	50.03	−3.47
2	18.50	9.00	72.50	0.97	14.79	84.24	39.60	2.22	58.18	−3.07
3	17.51	8.50	73.99	1.09	14.14	84.77	34.35	2.92	62.73	−2.96
4	15.50	7.50	77.00	2.16	11.54	86.30	29.33	3.46	67.21	−3.36
5	14.50	6.99	78.51	3.94	9.96	86.10	26.53	3.67	69.80	−3.59
				EOPO (1:1) + xK_2HPO_4 + KH_2PO_4 + H_2O ($x = 1.68$, pH = 6.8) (25 °C)						
1	16.01	5.15	78.84	0.43	13.68	85.89	39.81	1.57	58.62	−3.26
2	15.03	4.55	80.42	0.69	11.31	88.00	35.12	1.91	62.97	−3.66
3	14.18	3.94	81.88	1.73	9.69	88.58	30.28	2.45	67.27	−3.95
4	13.52	3.64	82.84	2.88	8.56	88.56	26.98	2.69	70.33	−4.11
				EOPO (1:1) + K_2HPO_4 + H_2O (2 °C)						
1	18.01	10.52	71.47	0.10	18.22	81.68	39.16	1.41	59.58	−2.32
2	16.52	8.99	74.49	0.52	15.40	84.08	32.98	2.05	64.97	−2.43
3	15.48	7.99	76.53	0.96	13.36	85.68	30.14	2.42	67.44	−2.67
4	14.53	7.00	78.47	2.31	11.38	86.31	23.95	3.30	72.75	−2.68

solution was mixed in a closed test tube by inverting it upside down 50 times, phase separation was speeded up by centrifugation at 3000 rpm for 10 min. Then the tube was placed in a water bath at (25 ± 0.05) °C or (2 ± 0.05) °C for 48 h, waiting for proper phase equilibration and separation, as indicated by the absence of turbidity in both top and bottom phases.

Samples of the top phase were carefully withdrawn first, with care being taken to leave a layer of solution at least 0.5 cm thick above the interface. Samples of the bottom phase were then withdrawn by using a plastic syringe with a long needle. A tiny bubble of air was retained on the needle tip and expelled once in the bottom phase to prevent contamination from top phase material, with special attention given to avoid disturbing the equilibrium systems in the subsequent analysis (Li et al., 1996).

3. Determination of Binodal and Tie Lines. The binodal curve represents the borderline between one and two phases. Detailed description of the determination has been given elsewhere (Albertsson, 1986). The tie line describes the compositions of the two phases when they are in equilibrium. After the concentrations of polymer and salt were determined, the tie line was constructed.

4. Analytical Procedure. The concentration of EOPO was determined by the improved method as described by Wang et al. (1997). EOPO was first precipitated with $K_4[Fe(CN)_6]$. The excess of $K_4[Fe(CN)_6]$ was then titrated with $ZnCl_2$. This improved method is more accurate and easy-handling than the original one (Tang and Shen, 1990). The average relative deviations were 0.71% for the improved method and 2.1% for the original method, respectively. The presence of potassium phosphate or sodium sulfate had no effect on the determination of EOPO.

In the presence of EOPO, on the other hand, the accuracy of the potassium phosphate determined by the Chinese standard method (the quinoline phosphomolybdate gravimetric method, GB 6008-85) was greatly reduced. It was therefore necessary to remove EOPO by adding a slight excess of $K_4[Fe(CN)_6]$ to the sample solution. EOPO was precipitated and filtrated off from the solution. Nitric acid was then added to the filtrated stock solution. After that, the solution was heated gently to boiling in order to oxidize $[Fe(CN)_6]^{4-}$ to $[Fe(CN)_6]$.$^{3-}$ The latter anion had no influence on the determination of potassium phosphate. Finally the potassium phosphate concentrations can be determined using GB 6008-85.

The concentration of water was determined by drying samples at 70 °C for 24 h until the sample reached constant mass. This method was only suitable for EOPO + Na_2SO_4 + water system, but not for EOPO + potassium phosphate + water. This is because the water hydration is more strongly bound to potassium phosphate. The concentration of H_2O in these systems was calculated by difference of EOPO and potassium phosphate concentrations.

As for EOPO + Na_2SO_4 + H_2O system, the concentration of Na_2SO_4 was calculated by difference of EOPO and water concentrations.

The estimated uncertainties in the reported mass %, based on determinations with samples of known composition, and duplications of five runs are as follows:

EOPO, ±0.71%; potassium phosphate, ±0.30%; water, ±0.30%.

Results and Discussion

The experimental liquid−liquid equilibrium results for the aqueous two-phase systems, EOPO (1:1) + Na_2SO_4, EOPO (1:1) + K_2HPO_4, EOPO (1:1) + KH_2PO_4, EOPO (1:1) + xK_2HPO_4 + KH_2PO_4 (x is the mole ratio of K_2HPO_4 to KH_2PO_4, $x = 1.68$, pH = 6.8), are given in Table 1 and, as

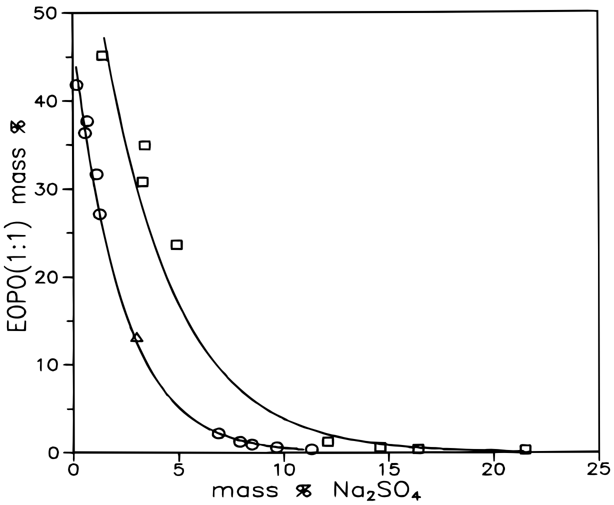

Figure 1. Phase diagrams for EOPO (1:1) + Na_2SO_4 + H_2O: (○) points obtained by analysis of separate phases; (△) plait point; (□) PEG3350 + Na_2SO_4 + H_2O. The data for the system with PEG3350 are taken from Snyder et al. (1992).

Table 2. Plait Point Compositions as Mass Fraction

system	salt (mass %)	EOPO (mass %)
EOPO (1:1) + Na_2SO_4 + H_2O (25 °C)	3.0	13.2
EOPO (1:1) + K_2HPO_4 + H_2O (25 °C)	4.2	13.1
EOPO (1:1) + KH_2PO_4 + H_2O (25 °C)	6.0	13.8
EOPO (1:1) + xK_2HPO_4 + KH_2PO_4 + H_2O^a (25 °C)	4.7	12.8
EOPO (1:1) + K_2HPO_4 + H_2O (2 °C)	6.6	11.3

[a] ($x = 1.68$, pH = 6.8).

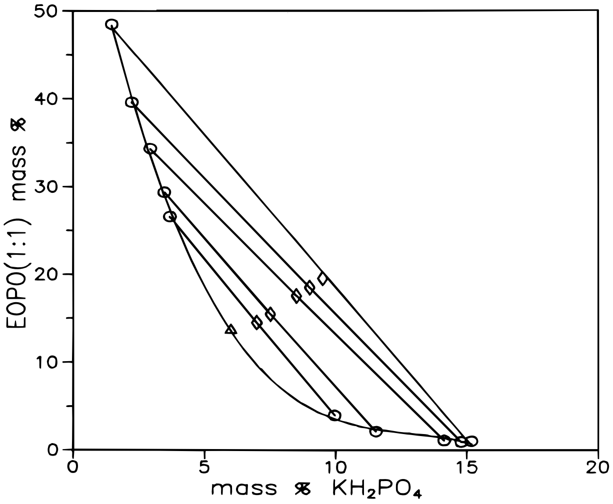

Figure 2. Phase diagrams for EOPO (1:1) + KH_2PO_4 + H_2O: (◇) points obtained by mixing of polymer; (○) points obtained by analysis of separate phases; (△) plait point.

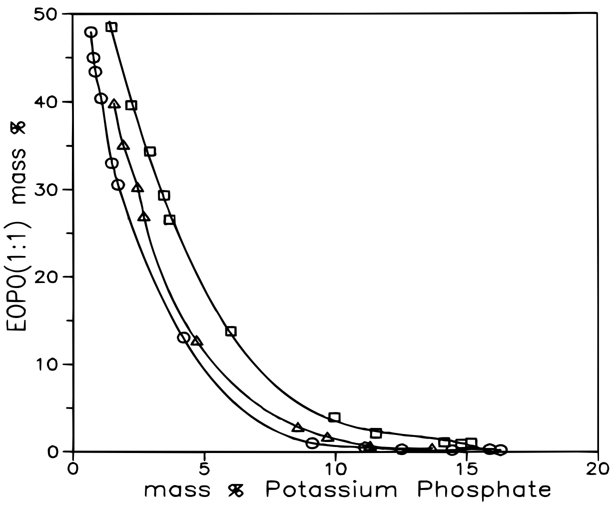

Figure 3. Comparison of binodals for EOPO (1:1) + various potassium phosphate + H_2O at 25 °C: (○) EOPO (1:1) + K_2HPO_4 + H_2O; (△) EOPO (1:1) + xK_2HPO_4 + KH_2PO_4 + H_2O ($x = 1.68$, pH = 6.8); (□) EOPO (1:1) + KH_2PO_4 + H_2O.

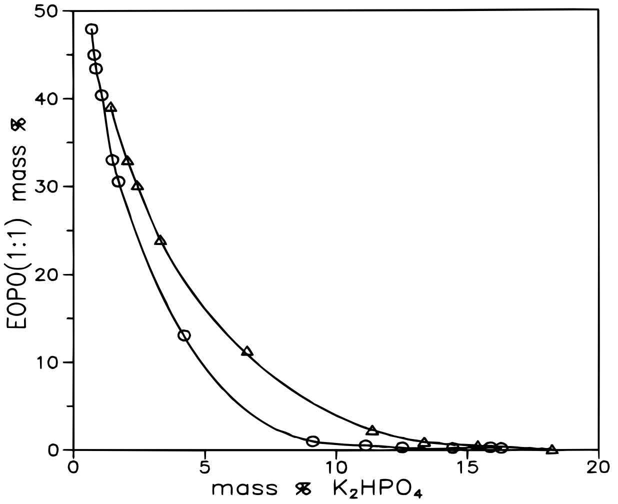

Figure 4. Influence of the temperature on the binodals for EOPO (1:1) + K_2HPO_4 + H_2O: (△) 2 °C; (○) 25 °C.

an example, graphically in Figure 1, along with the binodal curve of the system PEG3350 + Na_2SO_4 for comparison. The phase diagram for the EOPO (1:1) + Na_2SO_4 is similar to that for PEG3350 + Na_2SO_4, but there is a parallel displacement between the two as shown in Figure 1. Hydrophobicity difference between the two phases is considered as the driving force of phase separation. Since EOPO is more hydrophobic in character than PEG, the two-phase region of EOPO (1:1) + Na_2SO_4 is much broader than that of PEG3350 + Na_2SO_4.

An estimation of the plait point compositions was obtained by drawing a straight line connecting the midpoints of all experimental tie lines and extrapolating it to the point of intersection with the binodal curve. The compositions of the plait points are listed in Table 2.

The tie lines for each mixture were constructed by plotting the best line that could fit the three points of total compositions, of the top and the bottom phases. The linear correlation factor (r^2) in most cases is greater than 0.9989, which indicates that the analytical determination of the constituents of the two equilibrium liquid phases was accurate and consistent.

The slope of tie line (STL) is defined as the ratio as following

$$\mathrm{STL} = (\Delta\mathrm{EOPO}/\Delta\mathrm{salt})$$

where Δ is the difference between the concentrations of a given polymer and a salt in the two coexisting phases. Zaslavsky (1995) pointed out that STL value of an aqueous polymer–polymer system is almost constant in contrast to many nonaqueous polymer systems and solvent two-phase systems. However, as shown in Figure 2, the STL value of the EOPO + KH_2PO_4 system decreases with the tie line length, which indicates that the slope becomes flatter when the tie line length becomes larger. This is also the case for the other EOPO + salt + water systems as shown in Table 1. No satisfactory explanation can be given to the mechanism of this phenomena.

Figure 3 shows that the salt type has a strong influence on the LLE.

The temperature affects phase diagrams as shown in Figure 4, which changes the position of the binodal curve, tie line length, and distribution of polymer and salt in each phase. Increasing temperature not only moves the binodal curve toward lower concentrations but also increases the length of the tie line. The effect observed is similar to that described by Mishima when PEG and K_2HPO_4 are used to form a phase system (Mishima et al., 1995).

These phase diagrams, which have been measured, are advantageous in investigating the partitioning of biomolecules, e.g., erythromycin, in such systems in depth (Li, 1996).

Literature Cited

Albertsson, P. A. *Partition of Cell Particles and Macromolecules,* 3rd ed.; John Wiley & Sons: New York, 1986.

Alred, P. A.; Tjerneld, F.; Kozlowski, A.; Harris, J. M. Synthesis of dye conjugates of ethylene oxide-propylene oxide copolymers and application in temperature-induced phase partitioning. *Bioseparation* **1992**, *2*, 363−373.

Alred, P. A.; Kozlowski, A.; Harris, J. M.; Tjerneld, F. Application of temperature-induced phase partitioning at ambient temperature for enzyme purification. *J. Chromatogr. A* **1994**, *659*, 289−298.

GB 6008-85 Chinese Standard Method for Determination of Phosphorus.

Harris, P. A.; Karlstrom, G.; Tjerneld, F. Enzyme purification using temperature-induced phase formation. *Bioseparation* **1991**, *1*, 237−246.

Johansson, H.; Korlstrom, G.; Mattiasson, B.; Tjerneld, F. Effect of hydrophobicity and counter ions on the partitioning of amino acids in thrmoseparating UCON-water two-phase systems. *Bioseparation* **1995**, *5*, 269−279.

Li, M. Fundamental and Engineering Study on New Aqueous Two-Phase Systems: Partitioning, Modeling, and Technical Aspects of Erythromycin and Amino Acids. Ph.D. Thesis, Zhejiang University, 1996.

Li, M.; Zhu, Z. Q.; Mei, L. H. Liquid−Liquid Equilibria for Hydroxypropyl Starch + Polyethylene Glycol + Water at 25 °C. *J. Chem. Eng. Data* **1996**, *41*, 500−503.

Li, M.; Zhu, Z. Q.; Mei, L. H. Partitioning of Amino Acids by Aqueous Two-Phase Systems Combined with Temperature-induced Phase Formation. *Biotechnol. Prog.* **1997**, *13*, 105−108.

Mishima, K.; Nakatani, K.; Nomiyama, T.; Matsuyama, K.; Nagatani, M.; Nishikawa, H. Liquid−liquid equilibria of aqueous two-phase systems containing polyethylene glycol and dipoatssium hydrogenphosphate. *Fluid Phase Equilib.* **1995**, *107*, 269−276.

Modlin, R. F.; Alred, P. A.; Tjerneld, F. Utilization of temperature-induced phase separation for the purification of ecdysone and 20-hydroxyecdysone from spinach. *J. Chromatogr. A* **1994**, *668*, 229−236.

Snyder, S. M.; Cole, K. D.; Szlag, D. C. Phase Compositions, Viscosities, and Densities for Aqueous Two-Phase Systems Composed of Polyethylene Glycol and Various Salts at 25 °C. *J. Chem. Eng. Data* **1992**, *37*, 7(2), 268−274.

Tang, T.; Shen, Z. Determination of Polyethylene Glycol in Biomaterials and Foods. *Food Ferment. Ind.* (China) **1990**, *4*, 44−48.

Walter, H.; Brooks, D. *Partition in Aqueous Two-Phase Systems: Theory, Methods, Uses and Applications in Biotechnology;* Academic Press: Orlando, FL, 1985.

Wang, Y. Q.; Li, M.; Zhu, Z. Q. An Improved Method for Volumetric Determination of Ethylene Oxide Adducts in Aqueous Solutions. Submitted to *Zhejiang Daxue Xuebao* (J. Zhejiang University).

Zaslavsky, B. Yu. *Aqueous Two-Phase Partitioning: Physical Chemistry and Bioanalytical Applications;* Marcel Dekker: New York, 1995.

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