Liquid−Liquid Phase Equilibria of (Ethanol or Methanol + Water) Containing either Dipotassium Hydrogen Phosphate or Sodium Dihydrogen Phosphate

Hirotake Katayama* and Masahito Miyahara

Department of Materials Chemistry, Hosei University, Koganei, Tokyo, Japan 184-8584

Liquid−liquid equilibria (LLE) for (ethanol + water + K_2HPO_4), (ethanol + water + NaH_2PO_4), and (methanol + water + K_2HPO_4) were measured at temperatures of (288.15, 298.15, and 308.15) K. A nonlinear equation was used to describe the binodal curves, in terms of the mass fraction of phosphate versus that of ethanol, and the tie lines were successfully correlated with the Othmer−Tobias and Bancroft equations. LLE results were predicted using the binodal and Othmer−Tobias equations as well as by the binodal and Bancroft equations. Both predictions showed close agreement with experimental values, and the average deviations of the former pair of equations were 0.41, 0.24, and 0.19 for (ethanol +water + K_2HPO_4), (ethanol + water + NaH_2PO_4), and (methanol + water + K_2HPO_4), respectively.

Introduction

An aqueous solution of 1-propanol, 2-propanol, or acetone easily separates into two liquid phases upon addition of a salt such as sodium chloride, but aqueous ethanol cannot be phase-separated by an ordinary salt. We found that aqueous ethanol separated into two phases upon addition of either K_2HPO_4 or NaH_2PO_4. An aqueous methanol solution was separable in two phases upon addition of K_2HPO_4 but not NaH_2PO_4.

K_2HPO_4 is a base in aqueous solution, but NaH_2PO_4 is an acid. Aqueous solutions containing both K_2HPO_4 and NaH_2PO_4 work as buffers. The addition of KH_2PO_4 and Na_2HPO_4 to aqueous ethanol was found to produce no phase separation. The solubilities of KH_2PO_4 and Na_2HPO_4 in water at a temperature of 298.2 K are 20.0 wt % and 17.1 wt %, respectively,[1] and both are less soluble than K_2HPO_4 or NaH_2PO_4. The addition of ammonium chloride, ammonium sulfate, sodium sulfate, or sodium acetate also caused no phase separation of the ethanol solution.

Liquid−liquid-phase equilibria of aqueous ethanol containing cesium sulfate have been investigated by Hu et al.[2] However, to our knowledge, liquid−liquid equilibria (LLE) of methanol + water + salt have not been reported.

In the present work, LLE for (ethanol + water + K_2HPO_4), (ethanol + water + NaH_2PO_4), and (methanol + water + K_2HPO_4) were measured at temperatures of (288.15, 298.15. and 308.15) K, and their phase diagrams were determined.

Proteins are liable to undergo denaturation in organic solvents. According to Scopes,[3] the stability of the native conformation of glyceraldehyde-3-phosphate dehydrogenase decreased in the following organic solvents (in the order listed): methanol, ethanol, 2-propanol, 1-propanol, 1-butanol, and 1-pentanol. The enzyme is the most stable in methanol, then in ethanol, etc. (except for water). The knowledge of the phase equilibria measured will be applied to the separation and purification of biomolecules such as proteins and amino acids and of pharmaceutical intermediates by liquid−liquid extraction.

Experimental Section

Materials. Methanol, ethanol, K_2HPO_4, and Na_2HPO_4 were purchased from Wako Pure Chemical Industries Ltd. These chemicals minimum purities were stated as 99.5, 99.8, 99.0, and 99.0 mass %, respectively, and were used without further purification. *N*-Methylformamide, used as a gas chromatography standard, was supplied from Tokyo Kasei Industry Co. Ltd. (stated to have a minimum purity of 99.0 mass %), and it was used after dehydration with 0.3 nm molecular sieves. Distilled water was used throughout.

Apparatus and Procedure. The experimental method and apparatus were similar to those described by Katayama and Ichikawa.[4] The temperature was measured using an F25 platinum resistance thermometer (supplied by Automatic System Laboratories, Ltd.) with a stated accuracy of ± 0.03 K and a stated resolution of 0.001 K. The temperature fluctuations of the water bath were within ± 0.08 K. Each 50 cm^3 aliquot of the {(ethanol or methanol) + water + phosphate)} was poured into six flasks sealed with glass stopcocks, and the flasks were immersed in the bath. The mixtures were agitated for 4 h and then allowed to settle for more than 12 h.

First, 1 cm^3 samples were withdrawn from each phase using long-needle syringes, and the contents of ethanol (or methanol) and water were analyzed by gas chromatography. Next, 10 cm^3 and 3 cm^3 amounts were removed from the upper and lower layers, respectively, and dried for more than 2 h at a temperature of 423 K in an oven, after which the K_2HPO_4 or NaH_2PO_4 contents were determined gravimetrically. The crystals of K_2HPO_4 and NaH_2PO_4 are in an anhydrous state at temperatures higher than 321.5 and 330.6 K, respectively. Phase compositions were deduced from gas chromatography (the techniques to be described below) and gravimetric analysis.

For (ethanol + water + K_2HPO_4) and (ethanol + water + NaH_2PO_4), the contents of ethanol and water were determined from a calibration curve between the area and mass ratios of water-to-ethanol, using a Shimadzu GC-8A gas chromatograph fitted with a thermal conductivity detector and a stainless steel column of 2 m long with and 3 mm i.d., packed with Porapack-Q. The samples were kept warm until injection into the unit. A material balance in a sample gives the following relation:

$$\text{sample (mass)} = \text{water (mass)} + \text{ethanol or methanol (mass)} + \text{phosphate (mass)}$$

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* Corresponding author. Fax: +81-42-387-6140. E-mail: katayama@k.hosei.ac.jp.

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Table 1. Liquid−Liquid Equilibria for the System Ethanol (1) + Water (2) + K_2HPO_4 (3)

	alcohol-rich phase		phosphate-rich phase	
T/K	w_1^t	w_3^t	w_1^b	w_3^b
288.15	0.672	0.0017	0.0041	0.481
	0.609	0.0034	0.0059	0.455
	0.533	0.0077	0.0087	0.429
	0.480	0.0132	0.0128	0.410
	0.465	0.0152	0.0139	0.404
	0.410	0.0236	0.0184	0.385
	0.334	0.0443	0.0286	0.352
	0.251	0.0887	0.0523	0.301
	0.247	0.0884	0.0515	0.302
298.15	0.679	0.0016	0.0038	0.476
	0.628	0.0033	0.0064	0.451
	0.548	0.0078	0.0109	0.419
	0.483	0.0146	0.0163	0.393
	0.412	0.0267	0.0245	0.365
	0.341	0.0491	0.0390	0.329
	0.311	0.0605	0.0442	0.315
	0.263	0.0865	0.0622	0.285
	0.235	0.1087	0.0771	0.264
308.15	0.689	0.0017	0.0048	0.472
	0.664	0.0023	0.0054	0.462
	0.578	0.0061	0.0099	0.422
	0.534	0.0095	0.0133	0.403
	0.504	0.0125	0.0162	0.391
	0.466	0.0179	0.0208	0.373
	0.414	0.0280	0.0284	0.349
	0.337	0.0527	0.0458	0.300

Table 2. Liquid−Liquid Equilibria for the System Ethanol (1) + Water (2) + NaH_2PO_4 (3)

	alcohol-rich phase		phosphate-rich phase	
T/K	w_1^t	w_3^t	w_1^b	w_3^b
288.15	0.561	0.0208	0.0231	0.475
	0.517	0.0260	0.0273	0.456
	0.473	0.0338	0.0320	0.437
	0.449	0.0398	0.0352	0.420
	0.429	0.0444	0.0376	0.413
	0.402	0.0531	0.0436	0.396
	0.349	0.0751	0.0537	0.355
298.15	0.603	0.0156	0.0214	0.466
	0.486	0.0344	0.0377	0.393
	0.450	0.0454	0.0505	0.376
	0.433	0.0469	0.0511	0.373
	0.350	0.0830	0.0768	0.330
	0.333	0.0896	0.0802	0.316
	0.245	0.1431	0.1257	0.263
308.15	0.456	0.0437	0.0439	0.399
	0.435	0.0504	0.0512	0.384
	0.413	0.0582	0.0561	0.368
	0.372	0.0700	0.0656	0.335
	0.324	0.0933	0.0860	0.302

The mass ratio of phosphate to sample (=α) (i.e., phosphate mass fraction) was determined from the gravimetrical analysis, and the mass ratio of ethanol to water or methanol to water (=β) was determined from gas chromatographic analysis. From the relation above, the ratio of water to sample (i.e., water mass fraction) was $(1 - \alpha)/(1 + \beta)$, and the ratio of ethanol to sample (ethanol mass fraction) was $\beta(1 - \alpha)/(1 + \beta)$.

For (methanol + water + K_2HPO_4), 1 cm^3 of the sample was put into a small bottle and weighed on an electronic balance with an accuracy of ± 0.0001 g. Then 1 cm^3 of an aqueous solution containing 20 wt % *N*-methylformamide (MFA) was put into the bottle and weighed on the balance as well. The analysis of the sample was carried out with the GC-8A unit having a stainless steel column with 2 m length, 3 mm i.d., and containing PEG 20M (about 10 %) coated on Shin-carbon A. The mass ratio of methanol to MFA was obtained from the calibration curve using the area and mass ratios of methanol to MFA.

Table 3. Liquid-Liquid Equilibria for the System Methanol(1) + Water(2) + K_2HPO_4(3)

	alcohol-rich phase		phosphate-rich phase	
T/K	w_1^t	w_3^t	w_1^b	w_3^b
288.15	0.551	0.0302	0.0340	0.514
	0.504	0.0460	0.0421	0.485
	0.498	0.0475	0.0438	0.482
	0.493	0.0534	0.0497	0.473
	0.492	0.0523	0.0488	0.475
	0.470	0.0581	0.0525	0.467
	0.435	0.0731	0.0628	0.448
	0.433	0.0752	0.0604	0.445
	0.400	0.0941	0.0717	0.425
	0.384	0.0991	0.0712	0.420
298.15	0.502	0.0455	0.0448	0.481
	0.494	0.0464	0.0437	0.479
	0.467	0.0593	0.0509	0.461
	0.465	0.0589	0.0506	0.462
	0.448	0.0660	0.0543	0.453
	0.442	0.0672	0.0576	0.450
	0.391	0.0955	0.0751	0.418
308.15	0.579	0.0313	0.0358	0.511
	0.564	0.0318	0.0380	0.511
	0.563	0.0316	0.0360	0.511
	0.483	0.0596	0.0539	0.460
	0.482	0.0559	0.0504	0.466
	0.478	0.0544	0.0513	0.468
	0.464	0.0597	0.0536	0.460
	0.423	0.0805	0.0655	0.433
	0.395	0.0977	0.0807	0.415

Table 4. Values of Parameters of Equation 1

T/K	a	b	c	d	dev(1)[a]	R^2
			Ethanol (1) + Water (2) + K_2HPO_4 (3)			
288.15	−0.1553	−6.138	8.926	−23.513	0.35	0.9998
298.15	−0.1456	−5.998	9.045	−24.493	0.43	0.9998
308.15	−0.1851	−4.979	5.352	−19.218	0.25	0.9999
			Ethanol (1) + Water (2) + NaH_2PO_4 (3)			
288.15	−0.0528	−3.942	1.401	−7.420	0.38	0.9999
298.15	−0.0453	−4.075	2.884	−10.876	0.38	0.9987
308.15	0.0729	−4.517	2.625	−8.747	0.27	0.9998
			Methanol (1) + Water (2) + K_2HPO_4 (3)			
288.15	−0.0006	−4.037	4.383	−10.373	0.41	0.9996
298.15	0.0967	−4.894	4.383	−12.197	0.21	0.9998
308.15	0.0216	−3.874	3.447	−8.911	0.47	0.9996

[a] The root-mean-square deviation, dev(1), is defined as follows:

$$\mathrm{dev}(1) = 100.0 \times \frac{\sqrt{\sum_{n=1}^{N}\sum_{k=1}^{2}(w_{1,k,n,\mathrm{cal}} - w_{1,k,n,\mathrm{exp}})^2}}{2N}$$

where k is the number of phases (1, 2); n is N, the number of LLE data (1 to N = 5−10), and 1 stands for the first component, ethanol.

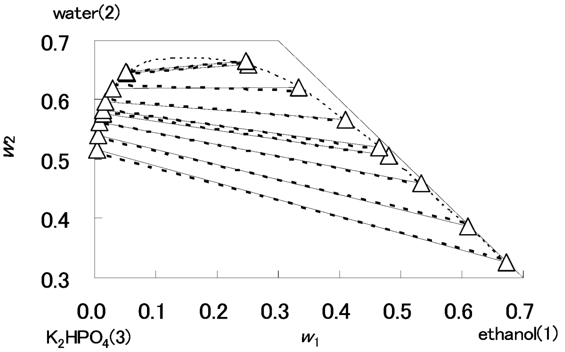
Compositions of the sample for (methanol +water + K_2HPO_4) were determined as follows. The ratio of sample-to-MFA is expressed as

$$\frac{\text{sample (mass)}}{\text{MFA (mass)}} = \frac{\text{water (mass)}}{\text{MFA (mass)}} + \frac{\text{methanol (mass)}}{\text{MFA (mass)}} + \frac{K_2HPO_4\text{ (mass)}}{\text{MFA (mass)}}$$

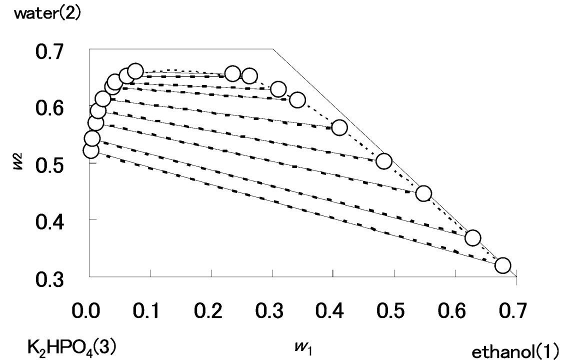
where since the mass ratio of K_2HPO_4 to sample (i.e., mass fraction of K_2HPO_4) (=α) is obtained from gravimetric analysis, the above relation is converted to

$$\frac{\text{water (mass)}}{\text{MFA (mass)}} + \frac{\text{methanol (mass)}}{\text{MFA (mass)}} = (1 - \alpha)\frac{\text{sample (mass)}}{\text{MFA (mass)}}$$

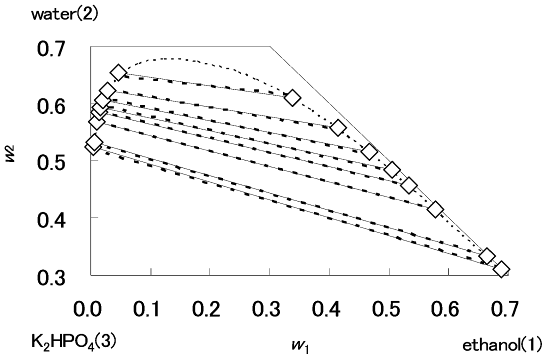
where K_2HPO_4 (mass)/MFA (mass) = α × sample (mass)/MFA (mass).



(a) 288.15 K



(b) 298.15 K



(c) 308.15 K

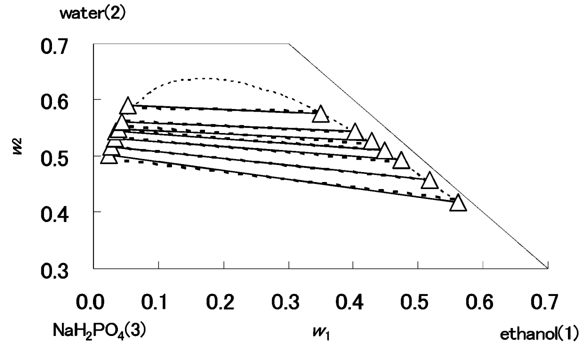
Figure 1. Liquid−liquid equilibria of the system ethanol + water + K_2HPO_4. The symbols △, ○, and ◇: experimental values at 288.15, 298.15, and 308.15 K, respectively. Solid tie lines, experimental values. Dotted binodal curves, values calculated using eq 1. Dotted tie lines, values calculated using eqs 1 and 2.

Since the mass of sample and MFA have been previously measured with the balance, the right side term of the above equation is known before the sample was injected. The mass ratio of water to MFA is determined through deducting the mass ratio of methanol to MFA from the right term. The compositions of the sample were determined from the mass ratios of methanol to MFA, water to MFA, and K_2HPO_4 to MFA.

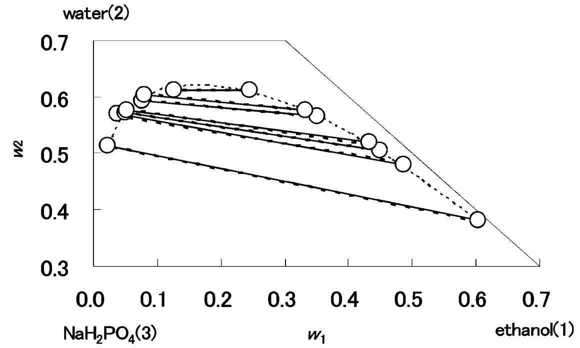
The ethanol (or methanol) and water contents obtained from gas chromatography were measured to mass fractions of 0.0002, and the contents of K_2HPO_4 or NaH_2PO_4 from gravimetry were measured to with mass fractions of 0.0001. However, because of reproducibility during the LLE measurements, the larger mass fractions were estimated to be significant to three decimal places, and those of the smaller mass fractions were estimated to be significant to four decimal places.

Results and Discussions

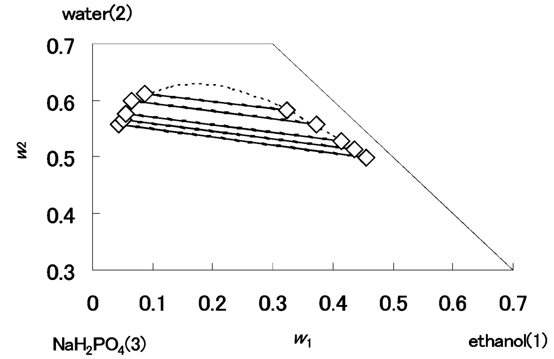
The results of LLE investigation for (ethanol + water + K_2HPO_4), (ethanol + water + NaH_2PO_4), and (methanol + water + K_2HPO_4) are shown in Tables 1, 2, and 3 and in Figures 1, 2 and 3, respectively, in terms of mass fraction of w_1, w_2, and w_3. Subscripts of 1, 2, and 3 denote the components of alcohol, water, and phosphate, respectively, and superscripts t and b denote the top phase (alcohol-rich phase) and the bottom phase (phosphate-rich phase), respectively. It was found by superimposing panels a, b, and c of Figure 1 that the binodal curves at temperatures of (288.15, 298.15, and 308.15) K lay almost on the same line. However, the slopes of their tie lines decreased with decreasing temperature. The reason for this is supported in the following manner. The decrease of temperature causes the decrease of solubility of phosphate (K_2HPO_4) into water. That is, an affinity of the phosphate to water is weakened as temperature is lowered. However, the temperature decrease enhances the intermolecular hydrogen bond force between alcohol and water at the same time. Then, some amounts of water in the lower phase move into the upper phase when the temperature is decreased.



(a) 288.15 K



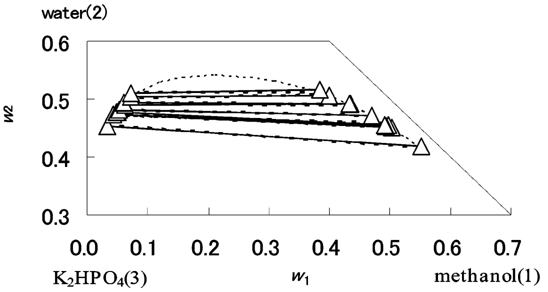
(b) 298.15 K



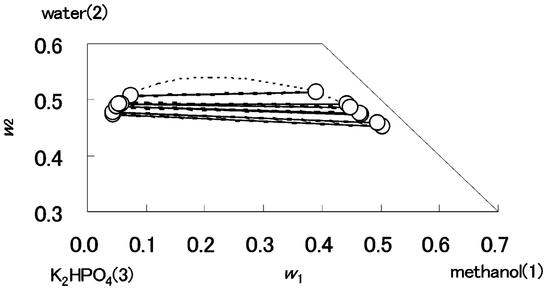
(c) 308.15 K

Figure 2. Liquid−liquid equilibria of the system ethanol + water + NaH_2PO_4. The symbols (△, ○, and ◇) and lines (solid and dotted) are the same as described in the caption of Figure 1.

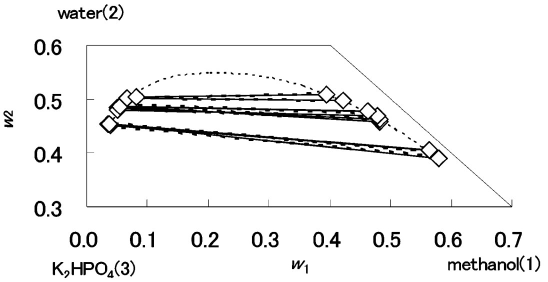
The trends seen in Figures 2 and 3 were similar to those of Figure 1. Comparison of Figures 1 and 3 indicated that the two-phase area of (ethanol + water + K_2HPO_4) was greater than



(a) 288.15 K



(b) 298.15 K



(c) 308.15 K

Figure 3. Liquid−liquid equilibria of the system methanol + water + K_2HPO_4. The symbols (△, ○, and ◇) and lines (solid and dotted) are the same as described in the caption of Figure 1.

that of (methanol + water + K_2HPO_4). This could be explained by the fact that ethanol is less polar than methanol.

The binodal curves were correlated with the following nonlinear equation:[2]

$$\ln w_1 = a + b w_3^{0.5} + c w_3 + d w_3^2 \tag{1}$$

where w_1 and w_3 are the mass fractions of alcohol and phosphate, respectively. The coefficients of eq 1 for three systems investigated, along with the root mean square deviations, dev(1), and the squared correlation coefficients, R^2, for w_1 are given in Table 4. The R^2 values and small deviations confirmed that eq 1 correlated well with the data.

The compositions of the tie lines were correlated with the equations of Othmer−Tobias (eq 2)[5] and of Bancroft (eq 3):[6]

$$\left(\frac{1 - w_1^{\mathrm{t}}}{w_1^{\mathrm{t}}}\right) = k_1\left(\frac{1 - w_3^{\mathrm{b}}}{w_3^{\mathrm{b}}}\right)^n \tag{2}$$

$$\left(\frac{w_2^{\mathrm{b}}}{w_3^{\mathrm{b}}}\right) = k_2\left(\frac{w_2^{\mathrm{t}}}{w_1^{\mathrm{t}}}\right)^r \tag{3}$$

where the values k_1 and n are the fit parameters of eq 2, and k_2 and r are the parameters of eq 3. These parameters, along with R^2 obtained from the method of least squares, are listed in Table 5. The tie lines of the systems investigated were found to be satisfactorily correlated by both eqs 2 and 3.

Table 5. Values of Parameters of Equations 2 and 3

T/K	k_1	n	R^2	k_2	r	R^2
			Ethanol (1) + Water (2) + K_2HPO_4 (3)			
288.15	0.439	2.364	0.991	1.402	0.4068	0.991
298.15	0.396	2.203	0.991	1.500	0.4349	0.995
308.15	0.380	2.042	0.988	1.579	0.4690	0.985
			Ethanol (1) + Water (2) + NaH_2PO_4 (3)			
288.15	0.694	1.726	0.985	1.214	0.5717	0.979
298.15	0.512	1.755	0.998	1.420	0.5368	0.996
308.15	0.700	1.300	0.999	1.300	0.7603	0.998
			Methanol (1) + Water (2) + K_2HPO_4 (3)			
288.15	0.881	1.787	0.992	1.036	0.5475	0.989
298.15	0.878	1.747	0.996	1.042	0.5510	0.994
308.15	0.825	1.866	0.987	1.061	0.5087	0.982

Table 6. Deviations of LLE Correlations by Using Equations 1 and 2 and Using Equations 2 and 3

	eqs 1 and 2			eqs 2 and 3		
T/K	dev(1)[a]	dev(2)[a]	dev(3)[a]	dev(1)[a]	dev(2)[a]	dev(3)[a]
			Ethanol (1) + Water (2) + K_2HPO_4 (3)			
288.15	0.61	0.36	0.52	0.60	0.36	0.38
298.15	0.56	0.23	0.51	0.51	0.23	0.38
308.15	0.33	0.38	0.20	0.49	0.38	0.24
	av dev[b]	0.41		av dev	0.40	
			Ethanol (1) + Water (2) + NaH_2PO_4 (3)			
288.15	0.28	0.39	0.22	0.33	0.37	0.25
298.15	0.36	0.22	0.28	0.45	0.26	0.45
308.15	0.06	0.08	0.08	0.14	0.04	0.14
	av dev	0.24		av dev	0.29	
			Methanol (1) + Water (2) + K_2HPO_4 (3)			
288.15	0.10	0.24	0.22	0.30	0.16	0.32
298.15	0.12	0.11	0.15	0.17	0.09	0.19
308.15	0.14	0.33	0.24	0.40	0.26	0.36
	av dev	0.19		av dev	0.26	

[a] The root-mean-square deviation, dev(i), is defined as follows:

$$\mathrm{dev}(i) = \frac{\sqrt{\sum_{n=1}^{N}\sum_{i=1}^{3}\sum_{k=1}^{2}(w_{i,k,n,\mathrm{cal}} - w_{i,k,n,\mathrm{exp}})^2}}{2N} \times 100.0$$

where i is the number of components; k is the number of phases; and n is the number of LLE data (1 to N = 5−10). [b] The average deviation, av dev, is the arithmetic mean values of dev(i) at three temperatures.

Correlation of LLE can be conducted using any two of eqs 1, 2, and 3. The correlation results for these systems using two pairs of equations, namely, eqs 1 and 2 and eqs 2 and 3, are summarized in Table 6. The root mean square deviations from eqs 1 and 2 were almost equal to those from eqs 2 and 3, but the former were slightly superior to the latter. Since the correlation results from the third pair of equations (eqs 1 and 3) were found to be inferior to results from both of the above pairs, they were not listed. In Figures 1, 2, and 3, the calculated binodal curves using eq 1 with the parameters listed in Table 4 are drawn as dotted curves, and the calculated tie lines using eqs 1 and 2 are shown as dotted straight lines.

Figure 4 shows a comparison of LLE for (ethanol + water + K_2HPO_4), (ethanol + water + NaH_2PO_4), and (ethanol + water + Cs_2SO_4).[2] The area of LLE is largest for the system containing K_2HPO_4, the area for the NaH_2PO_4 system is intermediate, and that of the system containing Cs_2SO_4 is the smallest.

The solubility of K_2HPO_4 in pure water at T = 298.2 K is 62.0 wt %, and crystalline K_2HPO_4 exists as $K_2HPO_4 \cdot 3H_2O$ at

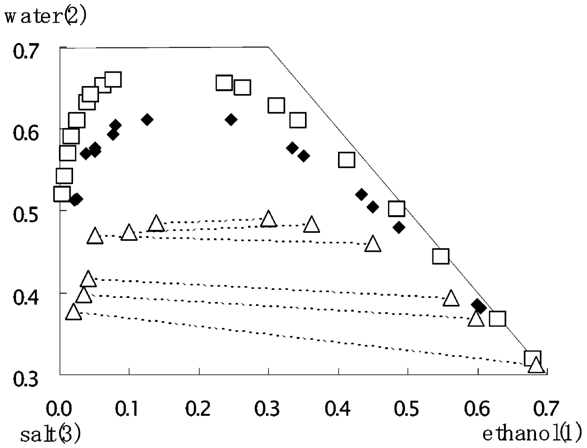


Figure 4. Comparison of liquid−liquid equilibria of the systems ethanol + water + salt (K_2HPO_4, NaH_2PO_4, or Cs_2SO_4). Dotted lines, tie lines of ethanol + water + Cs_2SO_4. The symbols □ and ◆ are the experimental values of (ethanol + water + K_2HPO_4) and (ethanol + water + NaH_2PO_4) at the temperature of 298.15 K, respectively. The symbol △ is the experimental ones of (ethanol + water + Cs_2SO_4) at a temperature of 303.2 K taken from Hu et al.[2]

this temperature.[1] Similarly, at this temperature, the solubility of NaH_2PO_4 is 48.5 wt %, and it exists in the solid form as $NaH_2PO_4{\cdot}2H_2O$ crystals;[1] The solubility of Cs_2SO_4 is 64.54 wt %, and it exists as anhydrous crystals.[1] Thus, the size of the LLE area for (ethanol + water + salt) cannot be determined solely from the solubility of the salt in pure water.

Conclusions

LLE of (ethanol + water + K_2HPO_4), (ethanol + water + NaH_2PO_4), and (methanol + water + K_2HPO_4) were studied at temperatures of (288.15, 298.15, and 308.15) K. The magnitude of the LLE area for these systems was found not to depend only on the solubility of the salt in pure water. The binodal curves of these systems were correlated using a nonlinear equation with four parameters, and the tie lines were satisfactorily described with the Othmer−Tobias and Bancroft equations. LLE calculations could be successfully carried out using any two of these three equations.

Literature Cited

(1) The Chemical Society of Japan. *Chemistry Handbook—Fundamentals*, Part II, 3rd ed.; Maruzen: Tokyo, 1984 (in Japanese).
(2) Hu, M.; Zhai, Q.; Liu, Z.; Xia, S. Liquid−liquid and solid−liquid equilibrium of the ternary system ethanol + cesium sulfate + water at (10, 30, and 50) °C. *J. Chem. Eng. Data* **2003**, *48*, 1561−1564.
(3) Scopes, R. K. *Protein Purification—Principles and Practice*, 3rd ed.; Translated into Japanese by Tukada, K.; Springer-Verlag: Tokyo, 1995.
(4) Katayama, H.; Ichikawa, M. Liquid−liquid equilibria of three ternary systems: methanol−heptane including 1,3-dioxolane, 1,4-dioxane and tetrahydropyran in the range of 253.15 to 303.15 K. *J. Chem. Eng. Jpn.* **1995**, *28*, 412−418.
(5) Othmer, D. F.; Tobias, P. E. Liquid−liquid extraction data—toluene and acetaldehyde systems. *Ind. Eng. Chem.* **1942**, *34*, 690−692.
(6) Gonzalez-Tello, P. G.; Camacho, F.; Blazquez, G.; Alarcon, F. J. Liquid−liquid equilibrium in the system poly(ethylene glycol) + $MgSO_4$ + H_2O at 298 K. *J. Chem. Eng. Data* **1996**, *41*, 1333−1336.

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