Phase Diagrams of Ammonium Sulfate + Ethanol/1-Propanol/2-Propanol + Water Aqueous Two-Phase Systems at 298.15 K and Correlation

Yun Wang, Yongsheng Yan,* Shiping Hu, Juan Han, and Xiaohui Xu

School of Chemistry and Chemical Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang, 212013, People's Republic of China

---

Binodal data for the ethanol/1-propanol/2-propanol + ammonium sulfate + water systems were determined at 298.15 K. On the basis of an empirical equation and lever rule, the liquid−liquid equilibrium compositions of the ethanol + ammonium sulfate + water system were directly calculated by Matlab. The Othmer−Tobias equation and Bancroft equation proved the reliability of the calculation method and the corresponding tie-line data. The phase-separation abilities of hydrophilic alcohols were compared by the effective excluded volume (EEV) of salt and the binodal curves plotted in molality. The phase-separation abilities of the investigated alcohols are in the order of 1-propanol > 2-propanol > ethanol > methanol. The mechanism of phase separation and the effects of alcohols, salts, and pH values are also discussed. The results show that the increase in the amount of phase-forming substance and the decrease in pH values (from 9.03 to 3.58) are both advantageous to the formation of the aqueous two-phase system (ATPS).

Introduction

In comparison with traditional liquid−liquid extraction, aqueous two-phase extraction (ATPE) has shown great potential for the efficient extraction and purification of biological products due to its biocompatibility.[1−3] The application of ATPE is free of toxic organic solvents, which can address the increasing challenge of environmental protection and product safety. There are two main types of aqueous two-phase systems (ATPS's): (1) polymer−polymer and (2) polymer−salt. Ionic liquid (IL)−salt systems have been investigated as novel ATPS's in recent years.[4−6] Unfortunately, the practical application of an ATPS is limited because of the high cost of polymers and ILs and the poor understanding of the phase-separation mechanism. An efficient approach to address the cost problem is the development of low-cost ATPS's. Hydrophilic organic solvents, such as methanol, ethanol, 1-propanol, and 2-propanol, seem to be a preferable option. Compared with a polymer or IL-based ATPS, this kind of ATPS has the advantage of low cost. Meanwhile, it is easier to recover the product from the alcohol-rich phase and recycle the phase-forming alcohol.

It is generally believed that the formations of polymer/IL/hydrophilic alcohol + salts + water ATPS's are due to the "salting-out" effect of salts.[7−9] In other words, the competition between salts and other phase-forming substances for water molecules leads to the formation of two phases. In further analysis of the investigated systems in this paper, the difference in the acting force between an "ion−water" pair and an "alcohol−water" pair will lead to the exclusion of alcohol or the crystallization of salt. In fact, we have found that besides the interaction of "ion−water" and "alcohol−water", the interaction between alcohol molecules themselves is also very important for phase separation. On the basis of the intermolecular forces mentioned above, we can either recover salts or carry out ATPE. Therefore, the solubility data of salts in component solvents and the binodal data of ATPS are both very important for designing these two processes.

Ammonium sulfate shows weak acidity in aqueous solutions, which is extremely appropriate to separate bioproducts stable in a weak acid environment. Katayama and Miyahara[10] reported that aqueous ethanol solution would separate into two phases with addition of K_2HPO_4 or NaH_2PO_4, while the addition of $(NH_4)_2SO_4$ caused no phase separation. In fact, we found that an ATPS could also be formed by adding $(NH_4)_2SO_4$ to aqueous ethanol solution at 298.15 K. In the paper, the phase diagrams of ethanol/1-propanol/2-propanol + $(NH_4)_2SO_4$ + water at 298.15 K were plotted. A nonlinear equation was proposed to correlate the binodal data. With the obtained equation and lever rule, the phase equilibrium compositions were calculated by Matlab. We have also discussed the phase-separation mechanism, especially the phase-separation abilities of hydrophilic alcohols. The effective excluded volume (EEV) of the salts and the locations of binodal curves plotted in molality were used to evaluate the phase-separation abilities of the investigated alcohols. Moreover, the effect of alcohols, salts, and pH values are also discussed. The increase in the addition of alcohols and salts, as well as the decrease in pH values, are advantageous to the exclusion of alcohols from the salt-rich phase to the alcohol-rich phase.

Experimental Section

Materials. Ammonium sulfate, methanol, ethanol, 1-propanol, and 2-propanol were supplied by the Sinopharm Chemical Reagent Co., Ltd. with a minimum purity of 99.0 %, 99.5 %, 99.7 %, 99.0 %, and 99.7 %, respectively. All chemicals were used without further purification. Double distilled and deionized water was used throughout the entire experiment.

Apparatus and Procedures. The binodal curves were determined by the titration method (cloud point method). A $(NH_4)_2SO_4$ solution of known concentration was titrated with alcohol until the clear solution turned turbid. The compositions of the mixture were determined by a Sartorious analytical balance (model BS 124S) with a precision of 0.0001 g. A conical flask (50 cm^3) was used to carry out the experiment, and the

---

* Corresponding author. E-mail: gaojian-wy@163.com.

10.1021/je900504e CCC: $40.75

Table 1. Binodal Data for the Ethanol/1-Propanol/2-Propanol (1) + Ammonium Sulfate (2) + Water (3) Systems at 298.15 K

ethanol + $(NH_4)_2SO_4$ + H_2O				1-propanol + $(NH_4)_2SO_4$ + H_2O				2-propanol + $(NH_4)_2SO_4$ + H_2O			
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
68.95	0.48	26.28	15.16	70.27	0.39	8.25	15.27	75.79	0.21	38.74	5.78
63.84	0.89	24.80	16.23	65.27	0.62	6.84	17.49	74.23	0.23	37.83	6.16
58.28	1.75	22.79	17.74	63.07	0.74	6.93	17.57	71.13	0.34	34.15	7.56
58.06	1.80	21.98	18.38	58.30	1.08	6.39	18.49	70.88	0.36	29.08	9.68
54.28	2.58	20.62	19.43	54.65	1.37	6.41	18.50	64.02	0.75	28.07	10.06
53.89	2.66	20.51	19.69	52.24	1.61	6.47	18.84	63.63	0.76	26.89	10.60
51.73	3.00	17.53	22.07	47.89	2.10	5.56	20.20	61.92	0.90	25.77	11.06
51.35	3.15	17.19	22.14	43.55	2.63	5.17	20.82	58.48	1.21	25.73	10.96
49.82	3.44	15.20	24.04	43.19	2.74	5.12	21.40	58.27	1.24	23.48	11.92
49.31	3.54	15.09	24.38	41.15	2.96	4.15	23.51	57.59	1.30	15.75	15.96
48.02	4.01	14.75	24.49	38.57	3.40	4.21	23.86	54.43	1.78	12.60	17.66
46.61	4.48	13.94	25.06	37.31	3.58	3.41	25.99	53.03	1.99	11.05	18.95
45.21	4.77	12.79	26.26	31.49	4.63	3.33	29.75	51.08	2.26	9.29	20.80
41.35	6.18	12.19	26.75	27.70	5.34	3.25	30.28	49.81	2.52	8.75	21.34
39.72	7.13	11.87	27.24	25.10	5.91	2.36	32.58	49.38	2.65	6.99	23.74
39.07	7.49	10.27	29.59	21.90	6.68	2.46	33.98	47.22	3.23	6.18	24.98
37.21	8.32	9.35	31.01	17.44	8.21	2.15	36.43	46.23	3.32	4.02	29.95
37.28	8.35	8.18	32.30	14.79	9.47	2.09	36.79	45.20	3.55	3.55	31.08
35.78	9.15	7.62	33.50	14.35	9.72			43.31	4.25	3.11	32.63
33.82	10.25	7.32	33.90	13.67	10.00			42.54	4.40	2.92	33.73
32.47	10.88	7.26	34.12	13.50	10.16			42.34	4.48	2.35	35.93
31.70	11.42	6.09	35.77	10.94	12.11			41.81	4.65	1.55	40.36
29.26	13.07	4.71	38.26	9.19	13.95			40.44	5.12	1.15	41.38
28.14	13.84	4.57	38.27	9.20	13.98			39.02	5.60		
27.48	14.34	3.74	39.30	9.08	14.26			38.98	5.70		
26.64	14.90	2.78	40.65	8.06	15.22			38.92	5.76		

temperature was maintained within ± 0.1 K in a water bath. The binodal data were then fitted to the following equation

$$w_1 = a_1 \exp(-w_2/b_1) + a_2 \exp(-w_2/b_2) + c \qquad (1)$$

where w_2 is the mass fraction of $(NH_4)_2SO_4$, w_1 is the mass fraction of alcohol, and a_1, a_2, b_1, b_2, and c are fitting parameters.

The phase equilibrium experiments were carried out in a separating funnel (60 mL). The feed samples were prepared by mixing an appropriate mass of alcohol (m_1), ammonium sulfate (m_2), and water (m_3) in a vessel (50 mL), and the mass of each composition was determined by the same Sartorious analytical balance mentioned above. After sufficient mixing, the samples were placed in a water bath (298.15 K) for more than 6 h until it separated into two clear phases. After the phase separation, the mass of the bottom phase (m_b) was also determined, and the mass of the top phase (m_t) was obtained by the subtraction method. Then the equilibrium compositions were calculated by Matlab, using eqs 2, 3, 4, and 5 as follows

$$w_1^t = a_1 \exp(-w_2^t/b_1) + a_2 \exp(-w_2^t/b_2) + c \qquad (2)$$

$$w_1^b = a_1 \exp(-w_2^b/b_1) + a_2 \exp(-w_2^b/b_2) + c \qquad (3)$$

$$\frac{m^t}{m^b} = \frac{\sqrt{(w_2^b - w_2)^2 + (w_1^b - w_1)^2}}{\sqrt{(w_2^t - w_2)^2 + (w_1^t - w_1)^2}} \qquad (4)$$

$$\frac{w_1 - w_1^t}{w_2 - w_2^t} = \frac{w_1 - w_1^b}{w_2 - w_2^b} \qquad (5)$$

where w_1^t, w_1^b, w_2^t, and w_2^b represent the equilibrium compositions (in mass fraction) of alcohol (1) and ammonium sulfate (2), in the top, t, and bottom, b, phases, respectively. w_1 and w_2 represent the total compositions (in mass fraction) of alcohol (1) and ammonium sulfate (2), respectively.

The obtained bottom phase was concentrated by evaporation, and then put in an air-dry oven at 353.15 K until the mass was constant. Subsequently, the mass fraction of ammonium sulfate in the bottom phase can be experimentally determined.

Results and Discussion

Phase Diagrams and Correlation. The aqueous solution of methanol does not separate into two aqueous phases with the addition of ammonium sulfate because of the high affinity of methanol for water molecules. For the ethanol/1-propanol/2-propanol + ammonium sulfate + water systems, the binodal data determined at 298.15 K are listed in Table 1, and the binodal curves are plotted in Figure 1. For the investigated systems, the binodal data were correlated by a five-parameter equation (eq 1). The fitting parameters, coefficient of determination (R^2), and standard deviations (sd) are listed in Table 2. The fitting results and the reproduced binodal curves in Figure 1 both indicate that eq 1 can be satisfactorily used to correlate the binodal data.

On the basis of the binodal data fitting equation and lever rule, the liquid−liquid equilibrium (LLE) compositions of the ethanol + ammonium sulfate + water system were directly calculated by Matlab using eqs 2, 3, 4, and 5. The results are given in Table 3.

The Othmer−Tobias equation (eq 6) and Bancroft equation (eq 7)[11,12] were used to evaluate the reliability of the calculation

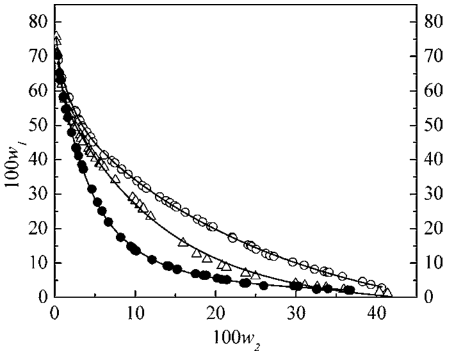

Figure 1. Binodal curves for the ethanol/1-propanol/2-propanol (1) + $(NH_4)_2SO_4$ (2) + H_2O (3) systems at 298.15 K. ○, ethanol; △, 2-propanol; ●, 1-propanol; solid line, reproduced by eq 1.

Table 2. Values of Parameters of Equation 1 for the Alcohols (Ethanol/1-Propanol/2-Propanol) + $(NH_4)_2SO_4$ + H_2O Systems at 298.15 K[a]

alcohol	a_1	a_2	b_1	b_2	c	R^2	100 sd
ethanol	0.18829	0.65946	0.01900	0.26103	−0.10858	0.9998	0.29
2-propanol	0.63426	0.21091	0.12992	0.00608	−0.02260	0.9991	0.67
1-propanol	0.24171	0.48750	0.11239	0.03556	0.01296	0.9994	0.50

[a] sd = $(\sum_{i=1}^{n}(w_1^{\text{cal}} - w_1^{\text{exp}})^2/N)^{0.5}$, where w_1 and N represent the mass fraction of ethanol/1-propanol/2-propanol and the number of binodal data, respectively. w_1^{exp} is the experimental mass fraction of alcohols listed in Table 1; w_1^{cal} is corresponding data calculated using eq 1, and the fitting parameters are listed in Table 2.

Table 3. Phase Equilibrium Compositions for the Ethanol (1) + Ammonium Sulfate (2) + Water (3) System at 298.15 K

	total composition		ethanol-rich phase		$(NH_4)_2SO_4$-rich phase		
no.	100 w_1	100 w_2	100 w_1^{t}	100 w_2^{t}	100 w_1^{b}	100 w_2^{b}	100 $w_2^{\text{b}a}$
①	11.62	29.81	45.69	4.74	8.28	32.30	32.26
②	11.54	31.03	52.34	2.88	6.89	34.27	34.21
③	11.48	32.49	57.87	1.82	5.40	36.55	
④	11.44	32.39	57.75	1.84	5.54	36.32	
⑤	11.45	32.41	57.27	1.92	5.49	36.41	
⑥	11.42	32.50	56.62	2.03	5.36	36.62	

[a] These values were determined by evaporation and the air blast drying method, where ①, ②, and ③ were ATPS's without adjusting pH values and the pH value of ③ was 3.58, while the pH values of ④, ⑤, and ⑥ were adjusted to 6.53, 7.00, and 9.03, respectively. The pH values here refer to the pH values of ammonium sulfate solutions before adding ethanol.

Table 4. Values of Parameters of Equation 6 for the Ethanol (1) + $(NH_4)_2SO_4$ (2) + H_2O (3) Systems at 298.15 K

alcohol	k_1	n	R	100 sd[a]
ethanol	0.23438	2.0658	0.9987	0.21

[a] sd = $(\sum_{i=1}^{N}(w_1^{\text{t,cal}} - w_1^{\text{t}})^2/N)^{0.5}$, where N represent the number of tie-lines. w_1^{t} is the mass fraction of ethanol in the top phase without adjusting pH value in Table 3; $w_1^{\text{t,cal}}$ is the corresponding data calculated by eq 6.

method and the calculated tie-line data. Equations 6 and 7 have been widely used in the correlation of LLE compositions of poly(ethylene glycol)−salt systems,[11,13] IL−salt systems,[14,15] and hydrophilic alcohol−salt systems.[10]

$$\frac{1 - w_1^{\text{t}}}{w_1^{\text{t}}} = k_1\left(\frac{1 - w_2^{\text{b}}}{w_2^{\text{b}}}\right)^n \tag{6}$$

$$\frac{w_3^{\text{b}}}{w_2^{\text{b}}} = k_2\left(\frac{w_3^{\text{t}}}{w_1^{\text{t}}}\right)^r \tag{7}$$

where w_1^{t}, w_1^{b}, w_2^{t}, w_2^{b}, w_3^{t}, and w_3^{b} represent the equilibrium compositions (in mass fraction) of alcohol (1), salt (2), and water (3) in the top, t, and bottom, b, phases, respectively. k_1, k_2, n, and r are fitting parameters. The fitting parameters of eq 6 for the ethanol + ammonium sulfate + water system are given in Table 4. A linear dependence of $\log((1 - w_1^{\text{t}})/w_1^{\text{t}})$ against $\log((1 - w_2^{\text{b}})/w_2^{\text{b}})$ as well as $\log(w_3^{\text{b}}/w_2^{\text{b}})$ against $\log(w_3^{\text{t}}/w_1^{\text{t}})$ also indicates good fitting properties of the tie-line compositions to eqs 6 and 7. The linear correlation coefficient of eq 7 for the investigated system is 0.9984. The results proved the reliability of the calculation method and the corresponding tie-line data.

Effective Excluded Volume Theory and Phase-Separation Abilities of Hydrophilic Alcohols. In the paper, the EEV of $(NH_4)_2SO_4$ in different hydrophilic alcohol−water component solvents was calculated using the model developed by Guan et al.[16] It is based on the concept that macroscopically any molecule species in a solution is distributed at random and every system composition on the binodal curve is a geometrically saturated solution of one solute in the presence of another. The binodal model developed by Guan et al. was originally used in polymer−polymer systems, while we extended the application of this model to a hydrophilic alcohol−salt system. As for an hydrophilic alcohol (1)/salt (2)/water (3) ternary system, the probability $[P(\underline{V} \geq V_{213})]$ of there being no salt species in an arbitrarily located volume V_{213} is also given by applying the Poisson distribution as to a binary system

$$P(\underline{V} \geq V_{213}) = \frac{(\upsilon_1 V_{213})^k}{k!}e^{-\upsilon_1 V_{213}} = e^{-\upsilon_1 V_{213}} \qquad k = 0 \tag{8}$$

where V_{213} is the EEV of salt and υ_1 is the number density of the alcohol. The volume of the solution (V) can be divided into two parts

$$V = V(\underline{V} \geq V_{213}) + V(\underline{V} \leq V_{213}) \tag{9}$$

where $V(\underline{V} \geq V_{213})$ is the effective available volume of salt and $V(\underline{V} \leq V_{213})$ is the effective unavailable volume of salt in the ternary system. If $P(\underline{V} \geq V_{213})$ is equal to the volume fraction of the effective available volume of salt, then

$$P(\underline{V} \geq V_{213}) = \frac{V(\underline{V} \geq V_{213})}{V} \tag{10}$$

In combination of eqs 8 and 10, the following equation can be deduced

$$e^{-\upsilon_1 V_{213}} = \upsilon_2 V_{213} \tag{11}$$

where υ_2 is the number density of salt. Considering the existence of space after the tight assembly of molecules, eq 11 can be expressed by

$$e^{-\upsilon_1 V_{213}} = \upsilon_2 V_{213} + f_{213} \tag{12}$$

where f_{213} is the volume fraction of unfilled effective available volume after tight packing of salt into the network of the hydrophilic alcohol.

Using the transformation relationship between molecular number density and mass fraction of components

$$\upsilon_s = \frac{\rho N_{\text{a}} w_s}{M_S} \qquad s = 1, 2 \tag{13}$$

where w_s is the mass fraction of component s, ρ is the density of solution, N_{a} is Avogadro's constant, and M_s is the molar mass of components. The density of solution is treated as a constant, and the scaled EEV (V_{213}^*) defined by Guan et al. is expressed as

$$V_{213}^* = \rho N_{\text{a}} V_{213} \tag{14}$$

By applying eqs 13 and 14 in eq 12 or 11, two equations can be given, respectively

$$\ln\left(V_{213}^* \frac{w_2}{M_2} + f_{213}\right) + V_{213}^* \frac{w_1}{M_1} = 0 \tag{15}$$

$$\ln\left(V_{213}^* \frac{w_2}{M_2}\right) + V_{213}^* \frac{w_1}{M_1} = 0 \tag{16}$$

In the original application, eq 16 was used to correlate binodal data of polymer−polymer systems because of the marked difference in size between the two components. The f_{213} value will be very small and consequently can be neglected. The EEV

Table 5. Values of Parameters of Equation 15 or 16 for the Alcohol (1) + $(NH_4)_2SO_4$ (2) + Water (3) Systems at 298.15 K

alcohol	$10^2\ V^*_{213}/(\mathrm{g\cdot mol^{-1}})$	$10^{-2}\ f_{213}$	R	100 sd[a]
ethanol	2.3129	2.1795	0.9971	1.60
2-propanol	3.0931	1.7357	0.9966	1.93
1-propanol	3.8602		0.9889	3.53

[a] sd $= (\sum_{i=1}^{N}(w_1^{\mathrm{cal}} - w_1^{\mathrm{exp}})^2/N)^{0.5}$, where N represent the number of binodal data. w_1^{exp} is the experimental mass fraction of alcohol listed in Table 1; w_1^{cal} is corresponding data calculated using eq 15 or 16.

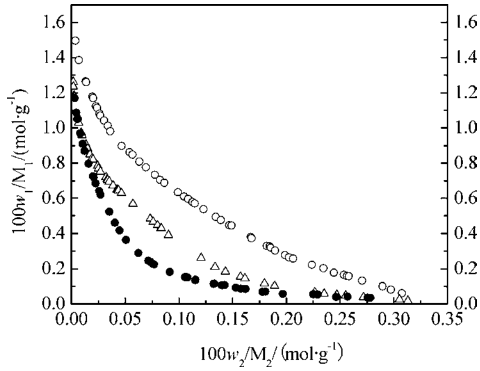

Figure 2. Effect of type of alcohols on the binodal curves plotted in molality for the ethanol/1-propanol/2-propanol (1) + $(NH_4)_2SO_4$ (2) + H_2O (3) systems at 298.15 K. ○, ethanol; △, 2-propanol; ●, 1-propanol.

represents the smallest spacing of an individual alcohol which will accept an individual salt, so it reflects the compatibility of components in the same system. The scaled EEV of different salts in the same component solvent have been used to evaluate the salting-out abilities of salts.[13,17,18] In this paper, the scaled EEV of the same salt in different hydrophilic alcohol−water component solvent was used to evaluate the phase-separation abilities of the investigated alcohols. The phase-separation abilities of alcohols increase with an increase of the EEV.

As for the ethanol + $(NH_4)_2SO_4$ + water system and the 2-propanol + $(NH_4)_2SO_4$ + water system, the parameter f_{213} is not so small as to be neglected, and eq 15 shows a much higher accuracy in binodal data fitting than eq 16. Nevertheless, as for the 1-propanol + $(NH_4)_2SO_4$ + water system, there is no significant difference between these two equations in binodal data fitting, so a simplified equation (eq 16) can be used. As shown in Table 5, the scaled EEV of $(NH_4)_2SO_4$ varies in different alcohol−water solvents due to the difference in size, shape, and interaction of components. The scaled EEV of $(NH_4)_2SO_4$ in aqueous 1-propanol, 2-propanol, and ethanol solutions is in a decreasing order, which indicates that the phase-separation abilities of the investigated alcohols are in the order of 1-propanol > 2-propanol > ethanol. The phase-separation abilities of alcohols can also be compared by the binodal curves plotted in molality in Figure 2. The increase in EEV is reflected by a decrease in the concentration of alcohol required for the formation of the ATPS. So, in comparison of the locations of binodal curves plotted in molality, it can also be concluded that the phase-separation abilities of alcohols are in the same order as the EEV.

Mechanism of Phase Separation for the Hydrophilic Solvent + Salt + Water ATPS. Methanol, ethanol, 1-propanol, and 2-propanol can dissolve in water in any proportion because of "hydrogen bond" interaction. When phase-separation salts are added to component solvents, the "ion− dipole" interaction leads to the hydration of ions, and then the salts can dissolve in the solvents. Both of the magnitudes of the "hydrogen bond" interaction and the "ion−dipole" interaction are in accordance with the polarity of solvent molecules, while permittivity (ε) is an easily available parameter for evaluating the "polarizing capability" of solvents. Table 6 gives the coefficients of a simple polynomial fitting of permittivity to temperature with an equation[19] of the form

Table 6. Coefficients of Polynomial Fitting of Permittivity to Temperature for Water, Methanol, Ethanol, 1-Propanol, and 2-Propanol[a]

name	T/K	ε_r	a	b	$10^{-5}\ c$	range/K
water	298.15	78.36	249.21	−0.79069	72.997	273 to 372
methanol	298.15	32.61	193.41	−0.92211	128.39	177 to 293
ethanol	298.15	24.85	151.45	−0.87020	195.70	163 to 523
1-propanol	298.15	20.52	98.045	−0.36860	36.422	193 to 493
2-propanol	298.15	19.27	104.16	−0.41011	42.049	193 to 493

[a] a, b, and c are fitting parameters. The quantity tabulated here is the relative permittivity (ε_r), which is the ratio of the actual permittivity to the permittivity of a vacuum. The d value of ethanol is $-0.15512\cdot10^{-5}$, while for the other alcohols, $d = 0$. The third column of the table lists the ε_r values at the temperature specified in the second column. The temperature range of the fit is given in the last column.

$$\varepsilon_r(T) = a + bT + cT^2 + dT^3 \qquad (17)$$

The ε_r values are in the order of water > methanol > 1-propanol > 2-propanol. Solvents with high ε_r values are favorable for the hydration of ions and the weakening of the Coulomb force between the cation and the anion. Therefore, when ammonium sulfate is added to the aqueous alcohol solution, water molecules arrange directionally around ions prior to other hydrophilic solvents. In comparison of ε_r values, it can be concluded that the magnitude of the acting force between the investigated alcohols and the water molecules is in the order of methanol > ethanol > 1-propanol > 2-propanol, and the acting force of 1-propanol is slightly larger than 2-propanol with water molecules. However, the scaled EEV of the salt and the locations of binodal curves plotted in molality both indicate that the phase-separation abilities of the investigated alcohols are in the order of 1-propanol > 2-propanol > ethanol. In fact, the acting force between alcohol molecules always being neglected is also very important for phase separation. "Boiling point" is an easily available and efficient criterion for evaluating the intensity of the acting force (including the van der Waals force and hydrogen force) between alcohol molecules. For the investigated alcohols, the difference in boiling points is mainly determined by the van der Waals force. The enhancement of the van der Waals force will lead to the increase of the boiling point. As for isomers, the existing branch will generate steric hindrance, resulting in the reduction of the van der Waals force. Therefore, the boiling point of 1-propanol is much higher than 2-propanol. The boiling points of methanol, ethanol, 1-propanol, and 2-propanol are (64.6, 78.29, 97.2, and 82.3) °C, respectively.[19] Therefore, the acting force between alcohols themselves is in the order of 1-propanol > 2-propanol > ethanol > methanol. The difference of temperature between 1-propanol and 2-propanol is approximately 15 °C, which reflects that the van der Waals force between 1-propanol molecules is much higher than that of 2-propanol molecules. It leads to an easier exclusion of 1-propanol from the salt-rich phase to the alcohol-rich phase compared with 2-propanol.

Effect of Alcohols and Salts on the Phase Separation. The addition of salts to the aqueous alcohol solution leads to the migration of water molecules away from alcohol molecules to ions of salts. Then, more alcohol molecules will be excluded from the salt-rich phase to the alcohol-rich phase. As shown in Figure 3, the mass fraction of ethanol in the top phase increases

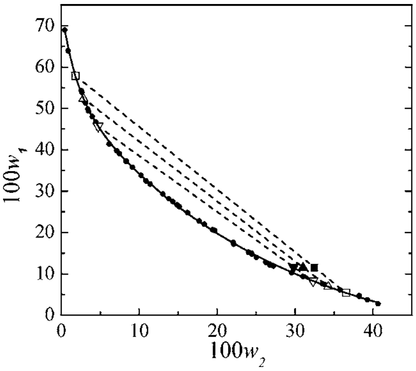

Figure 3. Effect of ammonium sulfate on the equilibrium phase compositions for the ethanol (1) + $(NH_4)_2SO_4$ (2) + H_2O (3) ATPS at 298.15 K. ●, experimental binodal data; solid line, calculated binodal curve from eq 1; ▼, ▲, ■, total compositions of tie line; ▽, △, □, calculated equilibrium compositions; dashed line, tie line; ▼, 0.40 mg·mL^{-1} $(NH_4)_2SO_4$ (50 mL) + ethanol (10 mL); ▲, 0.42 mg·mL^{-1} $(NH_4)_2SO_4$ (50 mL) + ethanol (10 mL); ■, 0.44 mg·mL^{-1} $(NH_4)_2SO_4$ (50 mL) + ethanol (10 mL).

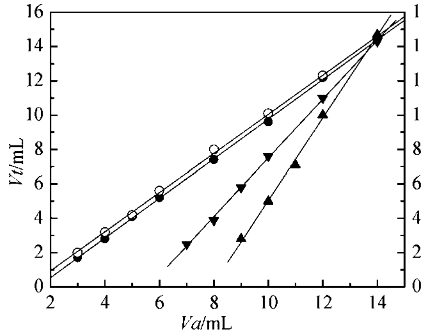

Figure 4. Volume of the alcohol-rich phase as a function of the volume of alcohol added, where Va is the volume of alcohol added and Vt is the volume of alcohol-rich phase. ①, ○, 0.50 mg·mL^{-1} $(NH_4)_2SO_4$ (50 mL) + 1-propanol [(14, 12, 10, 8, 6, 5, 4, 3) mL]; ②, ●, 0.50 mg·mL^{-1} $(NH_4)_2SO_4$ (50 mL) + 2-propanol [(14, 12, 10, 8, 6, 5, 4, 3) mL]; ③, ▼, 0.50 mg·mL^{-1}$(NH_4)_2SO_4$(50 mL) + ethanol [(14, 12, 10, 9, 8, 7) mL]; ④, ▲, 0.40 mg·mL^{-1} $(NH_4)_2SO_4$ (50 mL) + ethanol [(14, 12, 11, 10, 9) mL].

with the addition of ammonium sulfate. Meanwhile, for all of the investigated systems, the volume of the alcohol-rich phase versus the volume of alcohols added was found to be linear, as can be seen in Figure 4. The slope of the straight line was fitted using the linear regression method, and the correlation coefficients of ①, ②, ③, and ④ were 0.9996, 0.9998, 0.9997, and 0.9990, respectively. On the basis of this linear relationship, we can deduce the volume of the alcohol-rich phase by performing a small quantity of experiments. It can be concluded from Figures 3 and 4 that the increasing amounts of phase-forming substance are advantageous to phase separation.

Effect of the pH Value on the Phase Separation. For the ethanol + ammonium sulfate + water ATPS's, the equilibrium compositions are not sensitive to an increase in pH, as shown in Table 3. The mass fraction of ethanol in the top phase slightly decreases with an increase in pH. It be may be that the increase in pH value makes the equilibrium of the reaction

$$NH_4^+ + OH^- \rightleftharpoons NH_3 \cdot H_2O \qquad (18)$$

to the right side, which leads to a decrease in the concentration of NH_4^+. Then, more water molecules will migrate to alcohol molecules. Therefore, less alcohol molecules will be excluded from the bottom phase to the top phase, that is to say, the concentration of ethanol in the alcohol-rich phase slightly decreases when the pH increases.

Conclusion

Binodal data of the ethanol/1-propanol/2-propanol + ammonium sulfate + water ATPS's were obtained at 298.15 K. The experimental binodal data were satisfactorily correlated with a five-parameter equation. On the basis of the obtained equation and lever rule, the equilibrium compositions were calculated by Matlab, which significantly simplify the operation. The Othmer−Tobias equation and Bancroft equation were used to correlate LLE data and proved the reliability of the calculated results. The mechanism of phase separation, especially the phase-separation ability of the hydrophilic solvent was discussed. By combination of the scaled EEV of $(NH_4)_2SO_4$ and the locations of binodal curves plotted in molality, it can be concluded that the phase-separation abilities of the investigated alcohols are in the order of 1-propanol > 2-propanol > ethanol. The effects of alcohols, salts, and pH values were also discussed. The results show that an increase in the addition of a phase-forming substance and the decrease in pH are advantageous to the exclusion of alcohol; moreover, the volume of the alcohol-rich phase versus the volume of alcohol added is found to be linear.

Literature Cited

(1) Mazzola, P. G.; Lopes, A. M.; Hasmann, F. A.; Jozala, A. F.; Penna, T. C. V.; Magalhaes, P. O.; Rangel-Yagui, C. O.; Jr, A. P. Liquid-Liquid Extraction of Biomolecules: An Overview and Update of the Main Techniques. *J. Chem. Technol. Biotechnol.* **2008**, *83*, 143–157.
(2) Marco, R. P. Practical Application of Aqueous Two-Phase Partition to Process Development for the Recovery of Biological Products. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2004**, *807*, 3–11.
(3) Jorge, B.; Oscar, A.; Blanca, H. L. E.; Marco, R. P. Extraction and Purification of Bioproducts and Nanoparticles Using Aqueous Two-Phase Systems Strategies. *Chem. Eng. Technol.* **2008**, *31*, 838–845.
(4) Jiang, Y. Y.; Xia, H. S.; Guo, C.; Mahmood, I.; Liu, H. Z. Enzymatic Hydrolysis of Penicillin in Mixed Ionic Liquids/Water Two-Phase System. *Biotechnol. Prog.* **2007**, *23*, 829–835.
(5) Soto, A.; Arce, A.; Khoshkbarchi, M. K. Partitioning of Antibiotics in a Two-Liquid Phase System Formed by Water and a Room Temperature Ionic Liquid. *Sep. Purif. Technol.* **2005**, *44*, 242–246.
(6) He, C. Y.; Li, S. H.; Liu, H. W.; Li, K. A.; Liu, F. Extraction of Testosterone and Epitestosterone in Human Urine Using Aqueous Two-phase Systems of Ionic Liquid and Salt. *J. Chromatogr., A* **2005**, *1082*, 143–149.
(7) Zafarani-Moattar, M. T.; Hamzehzadeh, S. Phase Diagrams for the Aqueous Two-Phase Ternary System Containing the Ionic Liquid 1-Butyl-3-Methylimidazolium Bromide and Tri-potassium Citrate at T = (278.15, 298.15, and 318.15) K. *J. Chem. Eng. Data* **2009**, *54*, 833–841.
(8) Sadeghi, R. Aqueous Two-phase Systems of Poly(Vinylpyrrolidone) and Potassium Citrate at Different Temperatures-Experimental Results and Modeling of Liquid-Liquid Equilibrium Data. *Fluid Phase Equilib.* **2006**, *246*, 89–95.
(9) Liu, H. L.; He, C. Y.; Wen, D. W.; Liu, H. W.; Liu, F.; Li, K. A. Extraction of Testosterone and Epitestosterone in Human Urine Using 2-Propanol-Salt-H_2O System. *Anal. Chim. Acta* **2006**, *557*, 329–336.
(10) Katayama, H.; Miyahara, M. Liquid-Liquid Phase Equilibria of (Ethanol or Methanol + Water) Containing either Dipotassium Hydrogen Phosphate or Sodium Dihydrogen Phosphate. *J. Chem. Eng. Data* **2006**, *51*, 914–918.
(11) Zafarani-Moattar, M. T.; Tolouei, S. Liquid-Liquid Equilibria of Aqueous Two-Phase Systems Containing Polyethylene Glycol 4000 and Di-Potassium Tartrate, Potassium Sodium Tartrate, or Di-potassium Oxalate: Experiment and Correlation. *CALPHAD: Comput. Coupling Phase Diagrams Thermochem.* **2008**, *32*, 655–660.
(12) Othmer, D. F.; Tobias, P. E. Liquid-Liquid Extraction Data-Toluene and Acetaldehyde Systems. *Ind. Eng. Chem.* **1942**, *34*, 690–692.
(13) Regupathi, I.; Murugesan, S.; Govindarajan, R.; Amaresh, S. P.; Thanapalan, M. Liquid-Liquid Equilibrium of Poly(ethylene glycol)6000 + Triammonium Citrate + Water Systems at Different Temperatures. *J. Chem. Eng. Data* **2009**, *54*, 1094–1097.
(14) Pei, Y.; Wang, J.; Liu, L.; Wu, K.; Zhao, Y. Liquid-Liquid Equilibria of Aqueous Biphasic Systems Containing Selected Imidazolium Ionic Liquids and Salts. *J. Chem. Eng. Data* **2007**, *52*, 2026–2031.
(15) Deng, Y.; Chen, J.; Zhang, D. Phase Diagram Data for Several Salt + Salt Aqueous Biphasic Syatems at 298.15 K. *J. Chem. Eng. Data* **2007**, *52*, 1332–1335.

(16) Guan, Y.; Lilley, T. H.; Treffry, T. E. A New Excluded Volume Theory and its Application to the Coexistence Curves of Aqueous Polymer Two-Phase Systems. *Macromolecules* **1993**, *26*, 3971–3979.

(17) Huddleston, J. G.; Willauer, H. D.; Rogers, R. D. Phase Diagram Data for Several PEG + Salt Aqueous Biphasic Systems at 25 °C. *J. Chem. Eng. Data* **2003**, *48*, 1230–1236.

(18) Zafarani-Moattar, M. T.; Hamzehzadeh, S. Liquid-Liquid Equilibria of Aqueous Two- Phase Systems Containing Poly(ethylene glycol) and Sodium Succinate or Sodium Formate. *CALPHAD: Comput. Coupling Phase Diagrams Thermochem.* **2005**, *29*, 1–6.

(19) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 87th ed., internet version; 2007. http://www.hbcpnetbase.com (accessed September 18, 2008).

Received for review June 15, 2009. Accepted October 25, 2009. Financial support from the National Natural Science Foundation of China (20777029; 2097607) is gratefully acknowledged.

JE900504E