Liquid–Liquid Equilibrium Data for Aqueous Solutions of Surfactant Brij 58 with (NH$_4$) H$_2$PO$_4$, (NH$_4$)$_2$HPO$_4$, and KH$_2$PO$_4$

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Liquid–liquid equilibrium data of three systems containing surfactant polyoxyethylene cetyl ether (with abbreviation name Brij 58) and three salts, (NH$_4$)$_2$HPO$_4$, NH$_4$H$_2$PO$_4$, and KH$_2$PO$_4$, were measured at 298.15 K. Then the binodal curves were described using two three-parameter equations and the tie lines were described using four two-parameter equations. The best equations to correlate the binodal curves and the tie lines for these systems have been reported. Additionally, the effects of the salt type on the phase diagrams have been discussed.

Introduction

Liquid–liquid extraction utilizing aqueous two-phase systems (ATPS) has been used to separate and purify biological products from the complex mixtures.[1–3] There are three kinds of ATPSs containing polymer–polymer ATPS, polymer−salt ATPSs, and surfactant−salt ATPSs that have been used for partitioning of biological materials. The two first kinds of ATPSs are very well-known,[4–18] but surfactant−salt ATPSs scarcely are found in the literature. Recently, Xie et al.[19] have studied the phase equilibria for surfactant Triton-X-100−salt aqueous two-phase systems for partitioning membrane proteins. It has been shown that surfactant ATPSs have some advantages in comparison with other ATPSs such as higher content of water in phases, lower interface tension, lower cost, experimental conveniences, and ease of waste disposal and consequently shorter time for phase separation, and due to their hydrophobic property, they are useful for separation membrane proteins.[20]

In the present work, the liquid–liquid equilibrium (LLE) data of surfactant polyoxyethylene cetyl ether (Brij 58) and three salts, (NH$_4$)$_2$HPO$_4$, NH$_4$H$_2$PO$_4$, and KH$_2$PO$_4$, as new surfactant−salt ATPSs are presented. The obtained LLE data can be used to develop thermodynamic models. Furthermore, the effect of salt type on the LLE for these systems is investigated.

Experimental Section

Materials. The nonionic surfactant, Brij 58 (polyoxyethylene cetyl ether), was purchased from Sigma-Aldrich Co. and used without further purification. The average ethylene oxide (EO) chain length and the average molecular weight of surfactant are 20 and 1124 g·mol^{-1}, respectively. Also, the critical micelle concentration (CMC), the hydrophilic lypophilic balance (HLB), and the melting point are 0.077 mg·L^{-1}, 16, and 311.15 K, respectively.[21] The salts NH$_4$H$_2$PO$_4$, (NH$_4$)$_2$HPO$_4$, and KH$_2$PO$_4$ were obtained from Merck. The salts were dried at 383.15 K for 24 h. All chemicals were used without further purification.

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Table 1. Coefficients of Equation 1 for System 1 (Aqueous Solution of Brij 58 + (NH$_4$)$_2$HPO$_4$), System 2 (Aqueous Solution of Brij 58 + NH$_4$H$_2$PO$_4$), and System 3 (Aqueous Solution of Brij 58 + KH$_2$PO$_4$) at 298.15 K

system no.	a_0	a_1	a_2
1	1.3320	0.1310	0.0013
2	1.3320	0.1309	0.0018
3	1.3320	0.1311	0.0015

Apparatus and Procedure. A glass vessel, with a volume of 50 cm^3, was used to carry out the phase equilibrium determinations. The glass vessel was provided with an external jacket in which water at a constant temperature was circulated using a thermostat. The temperature was controlled to within ± 0.01 K. The binodal curves were determined by a titration method. A salt solution of known concentration was titrated with the Brij 58 solution or vice versa, until the solution turned turbid. For determination of the tie lines, feed samples (about 20 cm^3) were prepared by mixing appropriate amounts of Brij 58, salt, and water in the vessel. The thermostat was set at a desired temperature, and the sample was stirred for 30 min. Then the mixture was allowed to settle for 48 h. After separation of the two phases, the concentration of (NH$_4$)$_2$HPO$_4$ and NH$_4$H$_2$PO$_4$ in the top and bottom phases was determined by the phenate method.[22] The concentration of KH$_2$PO$_4$ was determined by flame photometry as a spectroscopy method. The concentration of Brij 58 in both phases was determined from refractive index measurements using a refractometer (ABBE 60 model, England) with a precision of ± 0.0001. The relation between the refractive index n_D and the mass fractions of Brij 58 (w_1) and salt (w_2) is given by

$$n_D = a_0 + a_1 w_1 + a_2 w_2 \qquad (1)$$

The values of coefficients a_0, a_1, and a_2 were listed in Table 1. The precision of the mass fraction of Brij 58 achieved using eq 1 is less than 0.002. It should be noted that this equation is only valid for dilute solutions of Brij 58 solution and salt, for obtaining the linear calibration plots.

Results and Discussion

For aqueous systems containing Brij 58 and three salts (KH$_2$PO$_4$, NH$_4$H$_2$PO$_4$, and (NH$_4$)$_2$HPO$_4$), the binodal and tie

Table 2. Binodal Data as Weight Fractions for System 1 (Aqueous Solution of Brij 58 + (NH₄)₂HPO₄), System 2 (Aqueous Solution of Brij 58 + NH₄H₂PO₄), and System 3 (Aqueous Solution of Brij 58 + KH₂PO₄) at 298.15 K

system 1		system 2		system 3	
w_1	w_2	w_1	w_2	w_1	w_2
0.0893	0.1123	0.0253	0.0896	0.0591	0.0512
0.0721	0.1128	0.0241	0.0911	0.0463	0.0593
0.0483	0.1137	0.0217	0.0977	0.0421	0.0641
0.0342	0.1144	0.0193	0.1033	0.0320	0.0786
0.0099	0.1159	0.0161	0.1119	0.0162	0.1145
0.0049	0.1169	0.0091	0.1379	0.0126	0.1282
0.0023	0.1180	0.0062	0.1503	0.0072	0.1533
0.0019	0.1183	0.0020	0.1843	0.0035	0.1712
0.0009	0.1208	0.0016	0.1938	0.0014	0.1841
0.0004	0.1223	0.0012	0.1985	0.0009	0.1962
0.0003	0.1249	0.0010	0.2031	0.0005	0.2118

Table 3. Tie Line Data as Weight Fraction for System 1 (Aqueous Solution of Brij 58 + (NH₄)₂HPO₄), System 2 (Aqueous Solution of Brij 58 + NH₄H₂PO₄), and System 3 (Aqueous Solution of Brij 58 + KH₂PO₄) at 298.15 K

top phase		bottom phase	
w_1	w_2	w_1	w_2
		System 1	
0.0623	0.1135	0.0005	0.1237
0.0534	0.1132	0.0006	0.1216
0.0342	0.1144	0.0007	0.1203
		System 2	
0.0221	0.0964	0.0110	0.2102
0.0209	0.0986	0.0012	0.1982
0.0192	0.1012	0.0010	0.1970
		System 3	
0.0391	0.0692	0.0009	0.2013
0.0359	0.0714	0.0006	0.1910
0.0341	0.0716	0.0011	0.1783

line data at 298.15 K were given in Tables 2 and 3, respectively. The binodal curves for these aqueous systems were shown in Figures 1 to 3. Also the tie lines of three systems were given in Figures 4 and 6. The binodal curves were fitted using two equations: a nonlinear expression of Mistry et al.[23]

$$\ln w_1 = a + bw_2^{0.5} + cw_2^3 \qquad (2)$$

and another equation which is

$$w_1 = a + bw_2^{0.5} + cw_2 \qquad (3)$$

Subscripts 1 and 2 were denoted for surfactant and salt, respectively. Recently, eq 3 has been used to correlate the
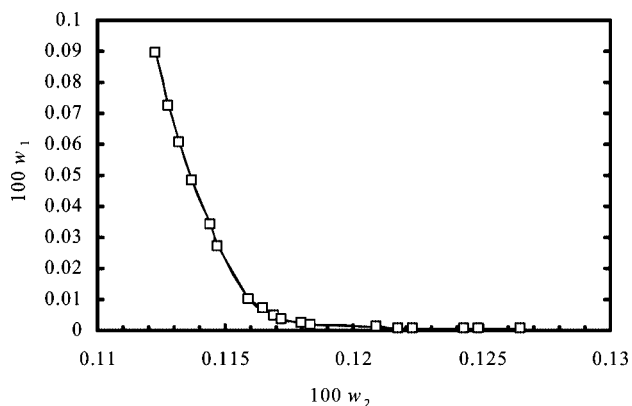

Figure 1. Binodal curve for system 1 (Brij 58 + (NH₄)₂HPO₄ + H₂O). w_1 and w_2 are mass weight fractions for Brij 58 and (NH₄)₂HPO₄, respectively, at 298.15 K.
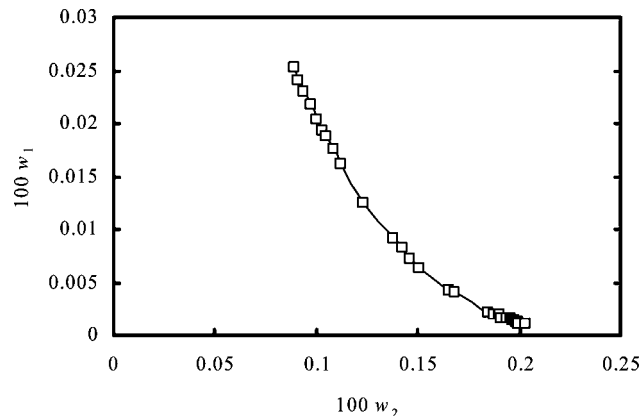

Figure 2. Binodal curve for system 2 (Brij 58 + NH₄H₂PO₄ + H₂O). w_1 and w_2 are mass weight fractions for Brij 58 and NH₄H₂PO₄, respectively, at 298.15 K.
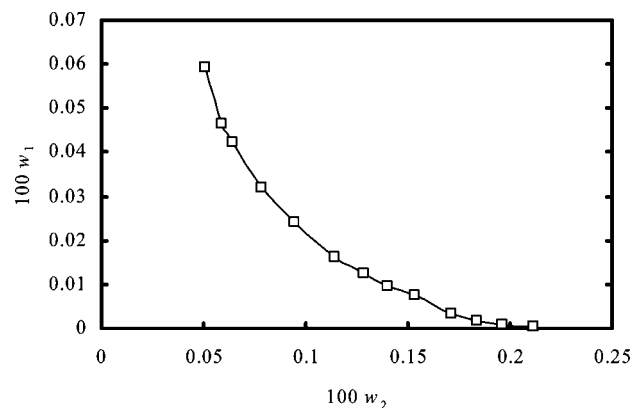

Figure 3. Binodal curve for system 3 (Brij 58 + KH₂PO₄ + H₂O). w_1 and w_2 are mass weight fractions for Brij 58 and KH₂PO₄, respectively, at 298.15 K.
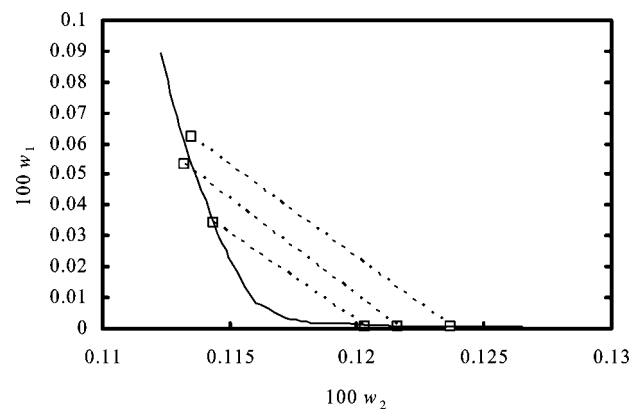

Figure 4. Binodal curve for system 1 (Brij 58 + (NH₄)₂HPO₄ + H₂O). w_1 and w_2 are mass weight fractions for Brij 58 and (NH₄)₂HPO₄, respectively, at 298.15 K. □, tie line data; -, binodal curve; ..., tie lines.

binodal curve data of polyethylene glycol with some salts.[24] The coefficients of eqs 2 and 3 along with the corresponding standard deviations for the investigated systems were given in Tables 4 and 5, respectively.

On the basis of obtained standard deviations, we have concluded that eq 2 for system 1 (Brij 58 + (NH₄)₂HPO₄ + H₂O) and eq 3 for systems 2 and 3 (Brij 58 + NH₄H₂PO₄ + H₂O and Brij 58 + KH₂PO₄ + H₂O) can be satisfactorily used to correlate the binodal curves.
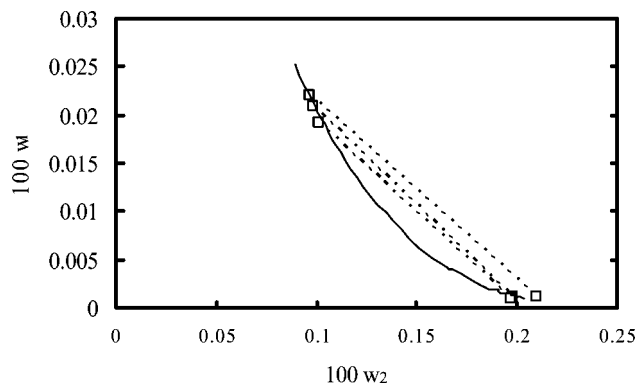
Figure 5. Binodal curve for system 2 (Brij 58 + $NH_4H_2PO_4$ + H_2O). w_1 and w_2 are mass weight fractions for Brij 58 and $NH_4H_2PO_4$, respectively, at 298.15 K. □, tie line data; -, binodal curve; . . ., tie lines.
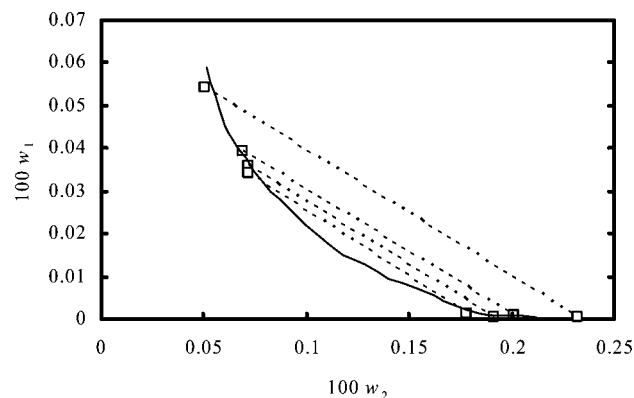

Figure 6. Binodal curve for system 3 (Brij 58 + KH_2PO_4 + H_2O). w_1 and w_2 are mass weight fractions for Brij 58 and KH_2PO_4, respectively, at 298.15 K. □, tie line data; -, binodal curve; . . ., tie lines.

Table 4. Values of Parameters of Equation 2 for System 1 (Aqueous Solution of Brij 58 + $(NH_4)_2HPO_4$), System 2 (Aqueous Solution of Brij 58 + $NH_4H_2PO_4$), and System 3 (Aqueous Solution of Brij 58 + KH_2PO_4) at 298.15 K

system	a	b	c	SD[a]
1	−19.2430	144.1700	−22108.5000	0.4766
2	−1.8523	−5.4494	−311.9260	0 0.0220
3	−1.6352	−5.5531	−402.0070	1.9678

[a] SD = $((100 w_1^{calcd} - 100 w_1^{exptl})^2/N)^{0.5}$, where N is the number of bimodal data.

Table 5. Values of Parameters of Equation 3 for System 1 (Aqueous Solution of Brij 58 + $(NH_4)_2HPO_4$), System 2 (Aqueous Solution of Brij 58 + $NH_4H_2PO_4$), and System 3 (Aqueous Solution of Brij 58 + KH_2PO_4) at 298.15 K

system	a	b	c	SD[a]
1	150.3000	−875.8500	1275.9000	6.5235
2	0.1778	−0.7440	0.7797	0.0161
3	0.1941	−0.8152	0.8502	0.1939

[a] SD = $((100 w_1^{calcd} - 100 w_1^{exptl})^2/N)^{0.5}$, where N is the number of bimodal data.

The correlation equations given by Othmer−Tobias and Bancroft[25] for the tie lines (eqs 4 and 5, respectively) were used to correlate the tie line compositions

$$\left(\frac{1 - w_1^t}{w_1^t}\right) = k_1 \left(\frac{1 - w_2^b}{w_2^b}\right)^n \tag{4}$$

$$\left(\frac{w_3^b}{w_2^b}\right) = k_2 \left(\frac{w_3^t}{w_1^t}\right)^f \tag{5}$$

Table 6. Values of the Parameters of Equations 4 to 7 for System 1 (Aqueous Solution of Brij 58 + $(NH_4)_2HPO_4$), System 2 (Aqueous Solution of Brij 58 + $NH_4H_2PO_4$), and System 3 (Aqueous Solution of Brij 58 + KH_2PO_4) at 298.15 K

eq no.	parameter	system 1	system 2	system 3
4	k_1	0.0000	4.2712	2.1294
	n	19.8280	1.7662	1.7562
5	k_2	6.2237	0.4305	0.6190
	f	0.0498	0.5873	0.5977
6	k_3	1.1238	6.3601	5.4388
	j	−0.0080	0.0699	0.0630
7	k_4	4.1963	3.7380	4.0281
	r	−17.6500	−16.4174	−18.4011

Table 7. SD Tie Line Data for System 1 (Aqueous Solution of Brij 58 + $(NH_4)_2HPO_4$), System 2 (Aqueous Solution of Brij 58 + $NH_4H_2PO_4$), and System 3 (Aqueous Solution of Brij 58 + KH_2PO_4) at 298.15 K[a]

eq no.	SD	system 1	system 2	system 3
4	1	0.2830	0.1774	0.4464
	2	0.1444	0.8060	1.1907
5	1	1.2930	0.1740	0.1131
	2	0.1566	1.0270	1.4310
6	1	0.3563	0.1101	0.0189
	2	0.0602	0.6550	0.0954
7	1	2.8300	0.1224	0.3738
	2	0.0734	1.2240	0.4370

[a] Where L is the number of tie lines and $j = 1$ and 2. SD_1 and SD_2 represent the weight percent standard deviations for Brij 58 and salt, respectively. $SD_j = \{1/2 L \sum_{j=1}^{L} \sum_{i=1}^{L} [(100 w_{i,j,calcd}^t - 100 w_{i,j,exptl}^t)^2 + (100 w_{i,j,calcd}^b - 100 w_{i,j,exptl}^b)^2]\}^{0.5}$.

Superscripts "t" and "b" stand for surfactant-rich phase and salt-rich phase, respectively, and subscripts 1, 2, and 3 were denoted for surfactant, salt, and water, respectively. k_1, k_2, n, and f represent fit parameters of eqs 4 and 5. The data of the three systems also were correlated with two other equations (eqs 6 and 7)

$$\ln\left(\frac{w_2^t}{w_2^b}\right) = j + k_3^2(w_1^b - w_1^t) \tag{6}$$

where the k_3 term is the salting-out coefficient, and j is a constant related to the activity coefficient, respectively. Recently, Zafarani et al.[26] have used eq 6 to correlate the tie line compositions of systems containing PEG. The last equation has the following expression

$$w_2^t = (r + k_4^2)w_1^t - \frac{\ln(w_2^t/w_3^b)}{k_4^2} \tag{7}$$

where k_4 and r represent fit parameters of eq 7. This equation has been used for correlating the tie line data of LLE for polyvinylpyrrolidone in our previous paper.[27] The fit parameters for eqs 4 to 7 were reported in Table 6.

The standard deviations for eqs 4 to 7 were given in Table 7. From Table 7, we conclude that eq 6 is the best equation to correlate the tie line data for these systems. The comparison of the binodal curves for three systems has been shown in Figure 7. Three salts, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, and KH_2PO_4, have been chosen for comparing the cation type on the binodal curves displacement.

As Figure 7 shows, the binodal curves for system 2 (Brij 58 + $NH_4H_2PO_4$ + H_2O) and system 3 (Brij 58 + KH_2PO_4 + H_2O) show similar behavior, while system 1 (Brij 58 + $(NH_4)_2HPO_4$ + H_2O) presents a very different phase diagram with respect to systems 2 and 3. It means that the cation type
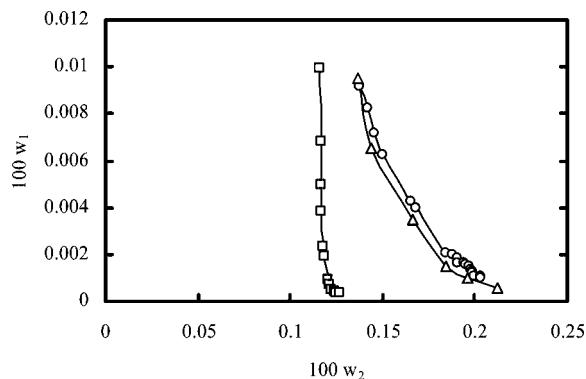
Figure 7. Binodal curves for the Brij 58 + salt + H_2O system. w_1 and w_2 are mass weight fractions for Brij 58 and salt, respectively, at 298.15 K with salts: □, $(NH_4)_2HPO_4$; ○, $NH_4H_2PO_4$; △, KH_2PO_4.

does not play an important role in the bimodal displacement wherein the main differences between three salts arise from the charge value of the anion. Figure 7 shows that increasing the charge of the anion from −1 (for $NH_4H_2PO_4$ and KH_2PO_4) to −2 (for $(NH_4)_2HPO_4$) will cause a decrease of the salt concentration.

Ananthapadmanabhan et al.[28] have observed a similar trend in the case of aqueous PEG–salt systems with three salts, NaOH, Na_2SO_4, and Na_3PO_4. The effectiveness of the anion in forming an aqueous two-phase system with PEG was found to be PO_4^{-3} > SO_4^{-2} > OH^-. They postulated that anions with a higher valence are better salting-out agents than anions with a lower valence because the higher valence anion hydrates more water than the lower valence anion, thus decreasing the amount of water available to hydrate PEG. This trend has theoretically been predicted by Kenkare.[29]

In addition to mentioned salts, two other salts, i.e., NaCl and K_2HPO_4, were examined to obtain a proper ATPS, but none of them were suitable ATPSs. The aqueous system containing Brij 58 and NaCl did not have an obvious turbid point for determination of the bimodal diagram, and also salt K_2HPO_4 was hardly solved in Brij 58 solution and caused error in the bimodal diagram determination.

Conclusions

For three aqueous solution systems containing surfactant Brij 58 with three salts ($(NH_4)_2HPO_4$, $NH_4H_2PO_4$, and KH_2PO_4), the binodal and tie line data were determined at 298.15 K. The binodal and tie line data for these systems were satisfactorily described by proposed equations. There is mainly a difference between phase diagrams of systems containing $NH_4H_2PO_4$ and KH_2PO_4 and the phase diagram of the system containing $(NH_4)_2HPO_4$. It seems that the charge of the anion plays an important role in the displacement of the phase diagram.

Our experimental data for ATPSs containing surfactant Brij 58 with three salts demonstrate that surfactant Brij 58 can be a proper candidate for ATPSs as well as polyethylene glycol and polyvinylpyrrolidone, and it can be considered as a suitable case for separation of biological materials such as membrane proteins.

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