J. Chem. Eng. Data **2007,** *52,* 859−862

Liquid−Liquid Equilibria of Aqueous Two-Phase Poly(vinylpyrrolidone) and K_2HPO_4/KH_2PO_4 Buffer: Effects of pH and Temperature

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Liquid−liquid equilibrium data of polyvinylpyrrolidone + K_2HPO_4/KH_2PO_4 buffer were measured at (298.15, 308.15, and 318.15) K and pH 5 and pH 7. The binodal curves were described using a three-parameter equation, and the tie lines were described using the Othmer−Tobias equation together with a two-parameter equation.

Introduction

Aqueous two-phase systems (ATPS) have been the subject of much academic interest over past decades, with some studies concerning applications of ATPS for the separation of biological materials such as proteins, enzymes, and nucleic acids. Poly(ethylene glycol) (PEG) is a water-soluble and biocompatible polymer,[1] and ATPS containing PEG have been widely studied. Among the salts, phosphates have been paid more attention because of their important role in environment studies.[2]

PEG and phosphates have been the subject of some recent studies concerning ATPS.[3−6] Peng et al.[3] studied the phase diagram and protein partition coefficient in ATPS containing PEG and K_2HPO_4 + KH_2PO_4 and have described some advantages of this polymer−salt system such as low viscosity and a short duration for phase splitting with respect to polymer−polymer ATPS. Grossmann et al. have studied the partition of low molecular combination peptides in ATPS of PEG and dextran in the presence of small amounts of K_2HPO_4/KH_2PO_4 buffer.[4]

Poly(vinylpyrrolidone) (PVP) is also a water-soluble and biocompatible polymer, but systems containing PVP have been studied sparingly in comparison with PEG. Recently, however, liquid−liquid equilibrium (LLE) data for some ATPS containing PVP and different salts have been reported in the literature.[7−11] Our bibliography search revealed that phase diagrams of ATPS containing PVP and phosphates have not been studied. In this work, the phase diagram and LLE data have been determined for an ATPS containing PVP + K_2HPO_4/KH_2PO_4 buffer solution at two pH values (5 and 7) and at various temperatures (298.15, 308.15, and 318.15) K. These results can be used to develop a thermodynamic model of the system.

Experimental Section

Materials. PVP of molecular weight 24000 was obtained from Merck. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate were obtained from Merck. The polymer and salts were used without further purification.

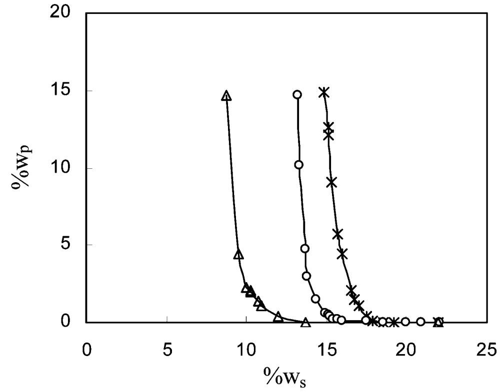

Figure 1. Binodal curves for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at pH 5 and differrent temperatures: ×, 298.15 K; ○, 308.15 K; and △, 318.15 K.

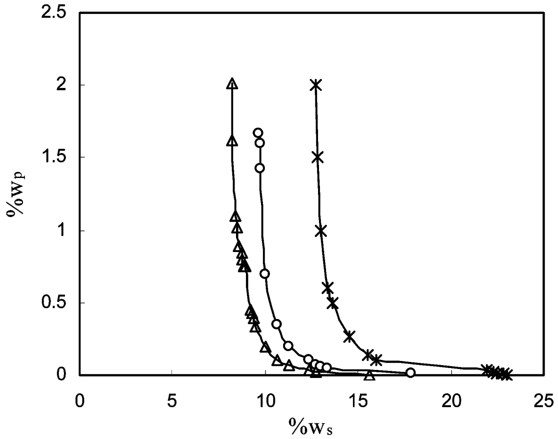

Figure 2. Binodal curve for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at pH 7 and differrent temperatures: ×, 298.15 K; ○, 308.15 K; and △, 318.15 K.

Table 1. Coefficients of Equation 1

temperature/K	a_0	a_1	a_2
298.15	1.3325	0.1354	0.1233
308.15	1.3312	0.1255	0.2259
318.15	1.3298	0.3381	0.2765

Apparatus and Procedure. The experimental apparatus employed is similar to one used previously.[12] A glass vessel was used to carry out the phase equilibrium. The temperature of the vessel and its solution were kept constant by using a thermostat to within ± 0.1 K. To obtain the binodal curves a salt solution of known concentration was titrated with the polymer solution or vice versa, until the solution turned turbid. To determine the compositions of the coexisting phases, feed samples were prepared by mixing appropriate amounts of polymer, salt, and water. After stirring the mixture for 1 h, the

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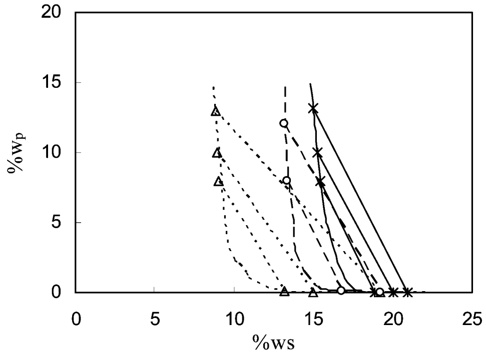

Figure 3. Effect of the temperature on the tie lines at pH 5: —, 298.15 K; – –, 308.15 K; and - - -, 318.15 K.

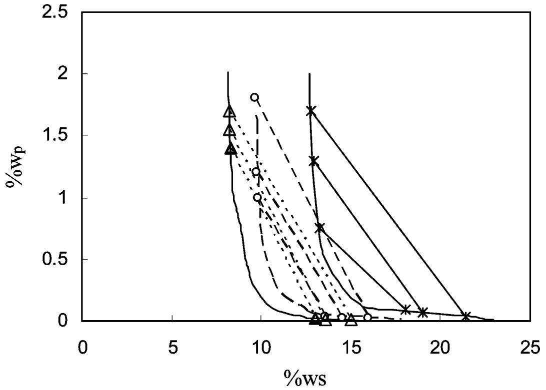

Figure 4. Effect of the temperature on the tie lines at pH 7: —, 298.15 K; – –, 308.15 K; and - - -, 318.15 K.

mixture was allowed to settle for 120 h. After separation of the two transparent phases, the concentrations of the salts in the top and bottom phases were determined by titration. The salts have not been determined separately, and the total salt concentration has been measured by titration of the phosphate ion. The standard deviation of the salt mass percent of the method was ± 0.05 %. The K_2HPO_4/KH_2PO_4 ratio in the phases may be different than in the feed solution; however, the total salt in each phase has been considered in our calculations.

The water content of PVP was determined by Karl Fisher titration using Metrohm equipment. The amount of water ranges from (0.01 to 0.02) mass %, and it was ignored in calculating the water concentration of the solutions. Because of the insignificant water content in the PVP, it was used without further purification in all experiments.

The concentration of PVP in both phases was determined from refractive index measurements using a refractometer (Mettler Toledo, RE 50) with a precision of ± 0.0001.

Table 2. Binodal Data as Weight Fraction for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at Different Temperatures and pH 5

T/K = 298.15				T/K = 308.15				T/K = 318.15			
100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s
14.90	14.81	1.50	16.77	14.71	13.90	0.20	15.7	14.71	8.70	1.40	10.71
12.61	15.07	1.05	17.01	10.14	13.29	0.18	15.93	4.40	9.51	1.07	10.89
12.10	15.11	0.41	17.46	4.71	13.68	0.14	17.46	2.57	10.01	0.40	12.00
9.10	15.35	0.11	17.9	3.75	13.81	0.13	18.50	2.11	10.25	0.02	13.68
5.71	15.71	0.02	18.52	1.49	14.38	0.04	20.01	2.02	10.28	0.00	22.01
4.46	15.93	0.02	19.20	0.64	14.96	0.02	20.89				
3.00	16.21	0.00	22.00	0.49	15.07	0.01	22.05				
				0.36	15.25						

Table 3. Binodal Data as Weight Fraction for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at Different Temperatures and pH 7

T/K = 298.15				T/K = 308.15				T/K = 318.15			
100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s
1.99	12.71	0.14	16.04	2.01	9.67	0.10	12.38	2.01	8.17	0.43	9.25
1.50	12.8	0.03	21.95	1.60	9.73	0.07	12.7	1.62	8.22	0.39	9.35
1.02	13.03	0.02	22.30	1.42	9.74	0.06	13.03	1.10	8.41	0.33	9.44
0.60	13.35	0.01	22.51	0.70	10.05	0.05	13.35	1.02	8.52	0.21	10.01
0.51	13.59	0.01	22.85	0.35	10.63	0.01	17.84	0.89	8.60	0.10	10.63
0.27	14.51	0.00	23.03	0.20	11.25			0.85	8.73	0.07	11.25
0.20	15.51							0.80	8.79	0.04	12.38
								0.75	8.85	0.03	12.71
								0.75	8.89	0.00	15.58
								0.45	9.21		

Table 4. Experimental Phase Equilibrium Compositions for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at Different Temperatures and pH 5

T/K = 298.15				T/K = 308.15				T/K = 318.15			
top phase		bottom phase		top phase		bottom phase		top phase		bottom phase	
100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s
13.13	14.99	0.01	20.98	12.00	13.22	0.03	19.20	13.00	8.80	0.01	19.20
10.02	15.25	0.02	20.00	8.00	13.4	0.13	16.79	10.04	8.91	0.02	14.96
8.01	15.42	0.03	18.85	6.40	13.52	0.13	16.21	7.99	9.00	0.10	13.19

Table 5. Experimental Phase Equilibrium Compositions for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at Different Temperatures and pH 5

T/K = 298.15				T/K = 308.15				T/K = 318.15			
top phase		bottom phase		top phase		bottom phase		top phase		bottom phase	
100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s	100 w_p	100 w_s
1.70	12.75	0.04	21.4	1.80	9.70	0.02	15.96	1.70	8.19	0.01	15.00
1.31	12.92	0.07	18.98	1.20	9.75	0.02	14.50	1.55	8.25	0.01	13.54
0.75	13.25	0.09	18.02	1.00	9.80	0.04	13.58	1.40	8.30	0.02	13.02

Table 6. Values of the Parameters of Equation 2 for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at Different Temperatures and pH 5 and pH 7

pH	T/K	a	b	c	R[a]
5	298.15	−59.03	190.14	−4933.35	0.999
	308.15	94.17	−283.87	3030.48	0.997
	318.15	27.02	−102.00	1274.51	0.998
7	298.15	60.31	−191.40	1916.93	0.998
	308.15	70.30	−253.50	5065.59	0.965
	318.15	28.10	−117.69	2789.69	0.998

[a] R is the correlation coefficient.

Table 7. Values of the Parameters of Equation 3 for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at Different Temperatures and pH 5 and pH 7

pH	T/K	b	k	SD b[a]	SD k[b]	R[c]
5	298.15	−13.30	3.60	0.114	0.018	0.956
	308.15	−10.37	3.20	0.069	0.013	0.990
	318.15	−25.00	5.06	0.000	0.000	1.000
7	298.15	−14.30	3.79	0.034	0.005	0.994
	308.15	−22.16	4.70	0.014	0.002	0.998
	318.15	−27.49	5.21	0.186	0.022	0.991

[a] Standard deviation values of b. [b] Standard deviation values of and k. [c] R is the correlation coefficient.

Table 8. Values of the Parameters of Equation 4 for PVP (w_p) + K_2HPO_4/KH_2PO_4 (w_s) + H_2O at Different Temperatures and pH 5 and pH 7

pH	T/K	k	n	SD k[a]	SD n[b]	R[c]
5	298.15	0.026	4.100	0.011	0.317	0.997
	308.15	0.084	3.103	0.050	0.390	0.992
	318.15	0.619	1.537	0.000	0.000	1.000
7	298.15	0.000	0.000	0.000	0.000	1.000
	308.15	0.199	3.370	0.115	0.343	0.995
	318.15	9.200	1.050	0.560	0.330	0.955

[a] Standard deviation values of k. [b] Standard deviation values of n. [c] R is the correlation coefficient.

Calibration plots of refractive index verses polymer concentration were prepared for different concentrations of salts at (298.15, 308.15, and 318.15) K. The relation between the refractive index (n) and the mass fractions of polymer (w_p) and salt (w_s) is given by

$$n = a_0 + a_1 w_p + a_2 w_s \tag{1}$$

The values of coefficients a_0, a_1, and a_2 at various temperatures are listed in Table 1. The precision of the mass fraction of PVP achieved using eq 1 is better than 0.002.

Results and Discussion

LLE data are given for the aqueous PVP + K_2HPO_4/KH_2PO_4 buffer system at different temperatures and at pH 5 and pH 7 in Tables 2 to 5. The binodal curves at different temperatures are given in Figures 1 and 2. These figures show that the two-phase area becomes larger over the whole concentration range for systems containing PVP + K_2HPO_4/KH_2PO_4 buffer at both pH 5 and pH 7, with increasing temperature.

The tie lines of our system at different temperatures are given in Figures 3 and 4. As these figures show, increasing the temperature of these systems will cause a decrease of the salt concentration at both pH values.

For correlating the binodal data of polyvinylpyrrolidone−K_2HPO_4/KH_2PO_4 buffer, we have used the following equation:[13]

$$\ln w_1 = a + b w_2^{0.5} + c w_2^{3} \tag{2}$$

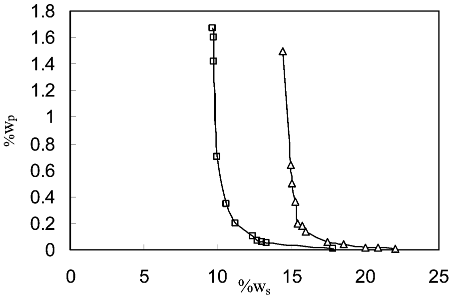

Figure 5. Effect of pH on the binodal curves at temperature 308.15 K: △, pH 5; □, pH 7.

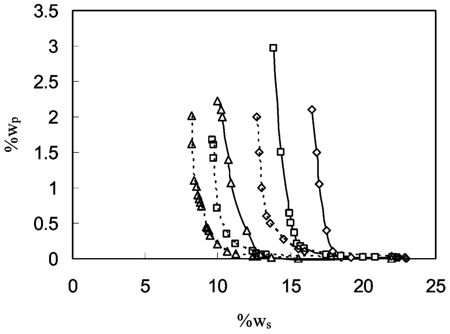

Figure 6. Effect of the temperature and pH on the binodal curves: ×, T = 298.15 K; ○, T = 308.15 K; and △, T = 318.15 K; —, pH 5; - - -, pH 7.

The coefficients of eq 2 along with the corresponding correlation coefficient (R) for PVP + K_2HPO_4/KH_2PO_4 are given in Table 6. As the obtained values of R show, eq 2 can be satisfactorily used to correlate the binodal curves of the PVP + K_2HPO_4/KH_2PO_4 system at different temperaures and at both pH 5 and pH 7.

The tie lines were described and correlated using eqs 3 and 4:

$$w_2^{t} = (r + k_1^{2}) w_1^{t} - \frac{\ln\left(\dfrac{w_2^{t}}{w_3^{b}}\right)}{k_1^{2}} \tag{3}$$

$$\left(\frac{1 - w_1^{t}}{w_1^{t}}\right) = k_2 \left(\frac{1 - w_2^{b}}{w_2^{b}}\right)^{n} \tag{4}$$

where k_1, k_2, r, and n represent fit parameters, and the superscripts t and b stand for the top and bottom phases, respectively. Recently, the Othmer−Tobias equation[14,15] (eq 4) has been used to correlate the tie line compositions of systems containing PEG.

The values of the parameters in eqs 3 and 4 for our system are given in Tables 7 and 8. Good agreement has been obtained in correlating the data of our system with these equations. Figure 5 shows the binodal curves for PVP + K_2HPO_4/KH_2PO_4 buffer at pH 5 and pH 7. This figure shows that the increase in pH causes an increase in the size of the two-phase region. The simultaneous effect of temperature and pH on the binodal curves is illustrated in Figure 6. It can be seen that the two-phase region is expanded with an increase in temperature and pH. In other words, the larger two-phase region occurs at pH 7 and 318.15 K.

Conclusions

For the PVP + K_2HPO_4/KH_2PO_4 buffer system, binodal and tie line data have been determined at (298.15, 308.15, and

318.15) K and pH 5 and pH 7. It was found that the two-phase area increases with increasing temperature and pH. The binodal curves for the investigated systems can be satisfactorily described by a three-parameter equation, and the tie lines were well described using the Othmer−Tobias equation together with a two-parameter equation.

Acknowledgment

The authors thank Azadeh Yeganeh for her useful assistance.

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Received for review October 14, 2006. Accepted February 3, 2007.

JE0604582