Equilibrium Phase Behavior of Poly(ethylene glycol) + Potassium Phosphate + Water Two-Phase Systems at Various pH and Temperatures

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This work presents the effects of changes in temperature (4 °C, 25 °C, and 40 °C), pH (6, 7, and 9), and polymer molecular weight (1000 and 8000) on the equilibrium phase behavior of two-phase PEG + potassium phosphate + water systems. As the temperature and pH were increased, the binodal curve shifted to lower polyethylene and phosphate concentrations, resulting in a higher difference in polymer and salt concentrations between the top and bottom phases. This corresponds to an increase in the region of two-phase coexistence.

Introduction

Aqueous two-phase systems are formed by adding either two structurally different hydrophilic polymers, such as dextran and poly(ethylene glycol) (PEG), or a polymer and a salt, such as $K_2HPO_4 + KH_2PO_4$ and PEG, to water.

As first suggested by Albertsson (1960), the phases of these systems offer different physical and chemical environments and are gentle enough to be used for the purification of biological materials.

PEG + salt systems have been used in large scale protein separation due to larger droplet sizes, a higher density difference between the phases, and lower viscosity, leading to a much faster separation than PEG + dextran systems. Industrial applications of PEG + salt systems could be improved by the availability of commercial separators, which allow faster continuous protein separations (Coimbra et al., 1994, 1995; Hustedt et al., 1978).

Phase equilibrium data for the PEG + salt systems are mainly found in Albertsson's work. Phase diagrams for such systems have also been compiled by Zaslavsky (1995). However, these data are not yet complete, particularly regarding the behavior of such systems at different temperatures and pH.

The absence of experimental information on the simultaneous effects of pH, temperature, and polymer molecular weight on the phase equilibrium behavior of such systems hinders the development of theoretical treatments of two-phase aqueous partitioning.

Therefore, this work is aimed at studying the effect of temperature, pH, and polymer molecular weight changes on the binodal curve and tie lines of the phase equilibrium diagrams for PEG + potassium phosphate + water systems.

Experimental Section

Materials. All chemicals employed in the present work were analytical grade and used as received. The poly(ethylene glycol) (PEG) used in this work (PEG 1000 and PEG 8000) were purchased from Sigma. The molecular weight range for both polymers, reported by the supplier, is ±5%. Mono- and dibasic potassium phosphates, as well as KOH and HCl (37%), were purchased from Merck. The purities of PEG and potassium phosphate were above 99% and 99.8%, respectively. Distilled water was used in all experiments.

Apparatus and Procedures. The biphasic systems were prepared by mass with a precision of ±0.0001 g (analytical balance). The appropriate quantities of PEG, mono- and dibasic potassium phosphates, and water were fed into a liquid−liquid equilibrium cell (Figure 1). Typically, 60 g of a system was prepared. The solution was magnetically stirred for 30 min, after which the cell was tightly capped and then allowed to attain equilibrium for 5 h at the temperature and pH ranges studied, respectively (4, 25, and 40) °C and 6, 7, and 9. The temperature in the cell was controlled within ±0.05 deg in a water bath (TE084/TECNAL). The Henderson−Hasselbach equation was used to determine the ratio of mono- and dibasic salts necessary to bring the pH to the desired values.

After equilibrium was attained, samples of the top and bottom phases were collected, with the aid of a syringe. Potassium phosphate concentration was determined by titration, following the procedure described in detail by Greve (1989). The addition of HCl, until pH 4.0, transforms the phosphate salt into phosphoric acid, whose titration makes it possible to calculate phosphate concentrations in the samples. The potentiometric titration was performed with 0.1 M potassium hydroxide. The average relative error in the titration was about 0.24% (w/w), calculated by error propagation. The water concentration was determined to a precision of ±0.02% (w/w), following the procedure outlined by the AOAC (1970), method 29.001 (drying upon quartz sand), applicable to liquid and semiliquid products and viscous mixtures. Both analytical techniques are very simple and not yet applied to the determination of aqueous two-phase equilibria. Having determined the potassium phosphate and water concentrations, the PEG content is given by the difference. The average standard deviations of the phase concentrations (analyzed in triplicate) were ±0.024% for water, ±0.013% for salt, and ±0.008% for PEG. All concentrations are expressed as mass percentages.

Results and Discussion

Complete phase separation was achieved within 5 h after stirring, resulting in two clear phases. Several equilibrium diagrams were built for the systems PEG 1000 + potassium

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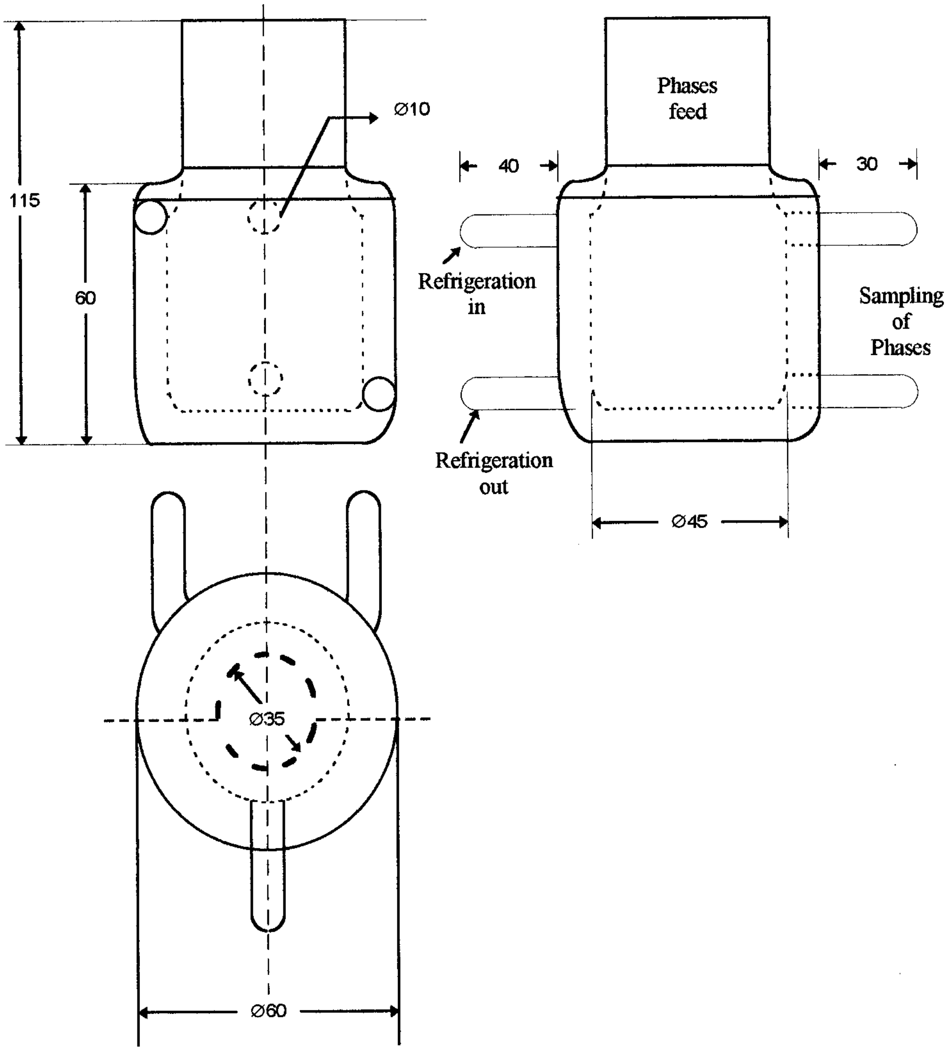

Figure 1. Scheme of the liquid–liquid equilibrium cell.

Table 1. Compositions of Potassium Phosphate + PEG 1000 Systems

			total system			top phase			bottom phase		
tie line	pH	*t*/°C	PEG (w/w)	salt (w/w)	water (w/w)	PEG (w/w)	salt (w/w)	water (w/w)	PEG (w/w)	salt (w/w)	water (w/w)
B	7	4	17.90	10.50	71.60	26.09	4.08	68.93	7.46	19.47	71.60
C	7	4	19.99	11.39	68.62	32.00	3.25	64.75	6.86	22.74	69.38
D	7	4	21.99	12.29	65.72	36.01	2.03	60.96	4.99	25.42	67.59
B	7	25	17.38	10.19	72.43	25.14	4.71	70.15	4.65	19.30	76.06
C	7	25	19.97	11.38	68.64	32.78	3.31	63.91	3.32	22.10	74.58
D	7	25	22.00	12.30	65.70	37.37	3.00	59.63	4.91	24.18	70.91
B	7	40	17.90	10.50	70.34	27.70	4.01	66.22	3.50	18.92	77.58
C	7	40	19.98	10.50	68.62	31.77	3.23	65.00	2.75	21.86	75.34
D	7	40	22.00	12.30	65.70	38.41	3.06	58.83	3.26	24.83	71.91
B	9	4	17.89	10.49	71.62	26.86	4.84	68.30	5.19	19.49	75.32
C	9	4	20.00	11.40	68.60	32.73	3.30	63.97	3.76	22.28	73.96
D	9	4	22.00	12.30	65.70	37.10	2.90	59.91	2.91	24.65	72.44
B	9	25	17.89	10.50	71.61	29.19	5.02	65.79	3.62	18.29	78.09
C	9	25	20.00	11.40	68.60	35.68	3.47	60.85	2.27	21.67	76.06
D	9	25	22.00	12.30	65.70	39.70	3.13	57.17	2.33	22.92	75.75
B	9	40	17.89	10.50	71.61	30.86	5.16	63.98	2.96	18.75	78.29
C	9	40	19.99	11.40	68.61	36.73	3.54	59.73	6.47	23.55	69.97
D	9	40	21.99	12.29	65.72	40.66	3.19	56.15	0.91	23.67	75.42

[a] The average standard deviation of the phase concentrations (Tables 1 and 2) were ±0.024% for water, ±0.013% for salt, and ±0.008% for PEG. The standard deviation was determined by $S = \{[1/(N - 1)]\sum(x_i - \bar{x})^2\}^{1/2}$, $N = 3$.

phosphate and PEG 8000 + potassium phosphate for different values of temperature and pH. Four tie lines were determined for PEG 8000 and three for PEG 1000, as shown in Tables 1 and 2.

The complete phase diagram for the PEG 8000 + potassium phosphate + water system at 25 °C is shown in Figure 2. It can be seen that good linear fittings were obtained for the experimental data relative to both total and phase concentrations.

The tie lines are determined by linear regression of each corresponding set of total, bottom phase, and top phase concentrations. The binodal curve is drawn through the

Table 2. Compositions of Potassium Phosphate + PEG 8000 Systems

			total system			top phase			bottom phase		
tie line	pH	*t*/°C	PEG (w/w)	salt (w/w)	water (w/w)	PEG (w/w)	salt (w/w)	water (w/w)	PEG (w/w)	salt (w/w)	water (w/w)
G	6	25	16.03	9.39	74.58	26.84	3.31	68.16	2.82	16.60	80.58
H	6	25	17.92	10.52	71.56	31.66	2.42	63.84	2.83	18.43	78.74
J	6	25	20.00	11.60	68.40	35.47	1.97	60.53	2.88	20.56	76.56
G	6	40	16.09	9.99	73.92	29.89	3.42	66.69	2.56	16.50	80.94
H	6	40	17.89	10.81	71.30	34.75	2.92	62.33	2.96	17.83	79.21
J	6	40	20.00	11.60	68.41	37.92	3.02	59.06	3.62	18.87	77.51
F	7	4	13.89	7.70	78.41	20.87	3.68	75.45	4.43	12.89	82.68
G	7	4	16.09	9.99	73.92	29.57	2.22	68.21	2.92	16.63	80.45
H	7	4	17.88	10.79	71.33	33.06	2.01	64.93	4.10	18.84	77.06
J	7	4	21.89	12.29	65.82	38.28	1.78	59.94	4.83	24.36	70.81
F	7	25	13.87	7.68	78.45	25.97	3.98	70.05	1.88	12.45	85.67
G	7	25	16.07	10.13	73.80	35.68	2.65	61.67	2.31	16.47	81.22
H	7	25	17.88	10.79	71.33	38.59	2.22	59.19	1.44	18.04	80.52
J	7	25	21.85	12.27	65.88	44.02	1.59	54.39	0.98	22.35	76.67
F	7	40	13.90	7.70	78.40	26.70	3.38	69.92	1.50	12.41	86.09
G	7	40	16.10	9.99	73.91	35.34	2.64	62.02	1.42	16.25	82.33
H	7	40	17.80	10.74	71.46	38.62	2.22	59.16	1.08	17.93	80.99
J	7	40	21.89	12.30	65.81	45.93	1.64	52.43	1.62	22.93	75.45
F	9	4	13.90	7.70	78.40	20.92	3.71	75.37	2.74	12.61	84.65
G	9	4	16.10	9.99	73.91	31.06	2.45	66.49	2.23	16.45	81.32
H	9	4	17.87	10.78	71.35	33.58	2.02	64.40	2.48	18.35	79.17
J	9	4	21.89	12.30	65.81	41.22	1.50	57.28	3.49	23.77	72.74
F	9	25	13.89	7.69	78.42	22.45	3.77	73.78	1.61	12.67	85.72
G	9	25	16.10	9.99	73.91	32.04	2.49	65.47	1.58	16.30	82.12
H	9	25	17.89	10.79	71.32	37.76	2.19	60.05	1.62	18.43	79.95
J	9	25	21.89	12.30	65.81	44.46	1.60	53.94	2.12	23.00	74.88
F	9	40	15.12	8.38	76.50	28.65	3.43	67.92	3.87	13.13	83.00
G	9	40	16.10	10.00	73.90	37.07	2.42	60.51	3.17	16.19	80.64
H	9	40	17.89	10.79	71.31	41.92	2.02	56.06	3.34	18.61	78.05
J	9	40	21.89	12.30	65.81	46.79	1.50	51.71	2.05	22.56	75.39

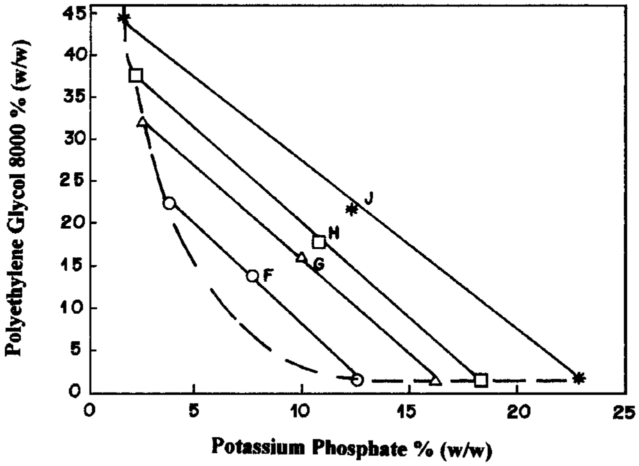

Figure 2. Binodal curve for the PEG 8000 + salt system at 25 °C and pH 7.

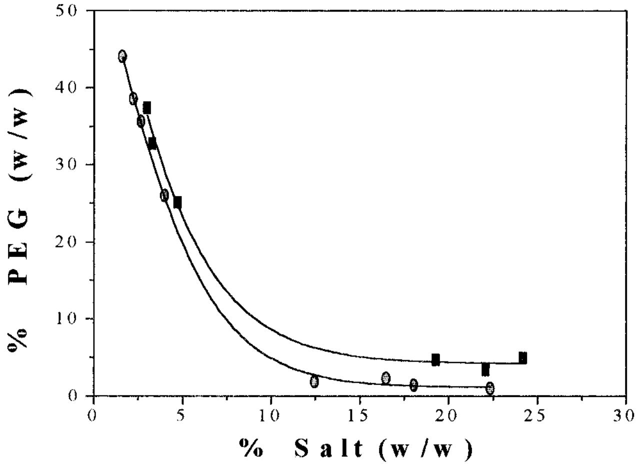

Figure 3. Binodal curves at 25 °C and pH 7 for both polymer molecular weights: (■) PEG 1000; (●) PEG 8000.

top and bottom phase points and is the location and trend of the top and bottom phase compositions. This curve was determined by fitting a sigmoidal (Boltzmann) equation.

The tie line compositions were confirmed, with an experimental error of 5%, by performing mass balances to determine if the amounts of potassium phosphate and PEG used to create the feed phase corresponded to the amounts in the top and bottom phases, based on the equilibrium compositions. These observations are in good agreement with results from the literature (Albertsson, 1960; Cheluget et al., 1994; Snyder et al., 1992).

Polymer Molecular Weight. Analysis of the binodal curves from the systems studied, revealed the effect of PEG molecular weight on phase separation. As the molecular weight is increased, the binodal curve shifts to lower PEG and phosphate concentrations, as seen in Figure 3. This trend is also in agreement with experimental results from the literature (Albertsson, 1960; Cheluget et al., 1994; Forciniti et al., 1991b; 1992; Lei et al., 1990; Vernau and Kula, 1990).

pH. Examination of the binodal curves in Figure 4 shows the effect of pH on phase separation at constant temperature. As the systems become more basic, the binodal curve is shifted toward lower PEG and salt concentrations, thus increasing the region of phase separation. This effect was more intense when the pH was changed from 6 to 7. The difference in position between the binodal curves begins to decrease at higher pH, with the binodals for pH 7 and 9 being very close. This trend is in agreement with the experimental results for other two-phase aqueous systems (Forciniti *et al.*, 1991b; Lei et al., 1990; Vernau and Kula, 1990).

Temperature. The effect of temperature on the binodal curve of the PEG 1000 + potassium phosphate + water system is illustrated in Figure 5. The temperature effects on the equilibrium behavior of the systems studied were

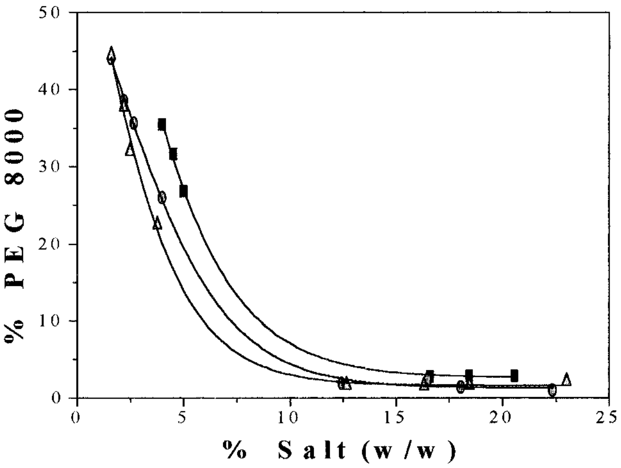

Figure 4. Binodal curves for the PEG 8000 + potassium phosphate + water system over the pH range of 6−9 at 25 °C. (■) pH = 6; (●) pH = 7; (▲) pH = 9.

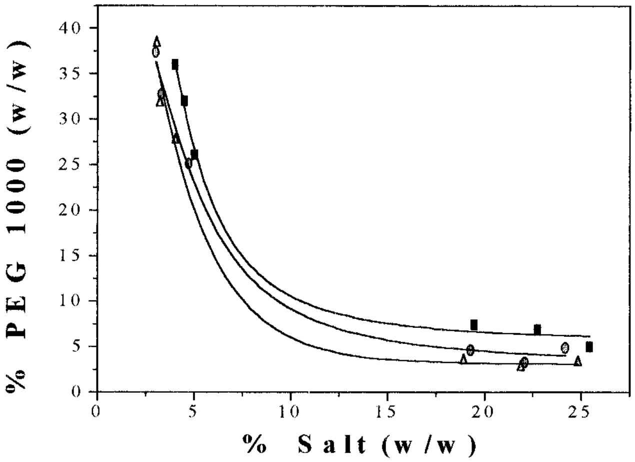

Figure 5. . Temperature effects on the binodal curve for the PEG 1000 + potassium phosphate + water system at pH 7: (■) *T* = 4 °C; (●) *T* = 25 °C; (▲) *T* = 40 °C.

similar to those observed for the changes in pH. For both polymer and salt fractions, as the temperature is increased, there was a decrease in miscibility, therefore increasing the area of two-phase coexistence, which is a behavior opposite to that exhibited in many traditional liquid−liquid systems. However, the temperature influence on the behavior of the systems studied was found to be higher in the (4−25) °C region, relative to the (25−40) °C range (Cheluget et al., 1994; Forciniti et al., 1991a,b; Mishima et al., 1995).

It was also observed that temperature increases enhance phase dispersion and accelerate coalescence once agitation is stopped, two important effects for liquid−liquid extraction. At 4 °C and pH 6, for the two polymer molecular weights and all tie lines, the formation of a third solid phase was observed (Cheluget et al., 1994).

Conclusions

The present work studied the effects of pH, temperature, and polymer molecular weight on the equilibrium phase behavior of polymer + salt systems.

The observed effect of PEG molecular weight on phase separation was in close agreement with the literature. As the molecular weight was increased, the binodal curve shifted to lower phase concentrations.

Temperature affects phase equilibrium by changing the gradient of tie lines and shifting the binodal curve, thus affecting the separation region.

The temperature influence on the equilibrium behavior of the systems studied was found to be higher in the (4−25) °C region, relative to the (25−40) °C range.

Increases in pH shifted the binodal curve toward lower PEG and salt concentrations, thus increasing the region of phase separation, but little is known or reported in the literature about this subject.

At lower temperature and pH values, a third solid phase was observed. Increases in temperature reduced phase miscibility, therefore increasing the area of two-phase coexistence.

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