Liquid−Liquid Equilibria for Hydroxypropyl Starch + Poly(ethylene glycol) + Water at 25 °C

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The liquid−liquid equilibria for hydroxypropyl starch + poly(ethylene glycol) + water with various polymer molecular weights were measured at 25 °C. The binodal curves were compared with literature results.

Introduction

Aqueous two-phase systems have recently attracted considerable attention for the large-scale recovery and purification of bioproducts, such as plant and animal cells, proteins, enzymes, nucleic acids, antibiotics, and others (Albertsson, 1986; Walter et al., 1985; Guan et al., 1994). Generally, poly(ethylene glycol) (PEG) + dextran + water systems are more generally useful in the laboratory; however, the high cost of dextran limits extensive use in large-scale separations. Economic evaluations have shown that systems which minimize the use of dextran relative to PEG are more economical. This is primarily because the cost of PEG is only about 1−2% of that of the dextran. The choice of polymers for a two-phase partition was summarized by Carlson (1988). Several factors, including the phase-forming characteristics, the physical properties, the interaction of the polymer with product molecules, the cost of the polymer, and the end use of the product, should be taken into account. Other inexpensive PEG + salt + water systems have also been used, but the usefulness of these systems is restricted not only by the high salt concentration necessary for the formation of the phase but also by the high salt concentration in both phases which, in most cases, makes the use of phase-bound affinity ligands impossible. Moreover, the high salt concentration may destroy the sensitive biological structure. Attempts to exploit the research for suitable yet cheaper phase-forming polymers appear rather encouraging. Crude dextran, pullulan, polyampholytic acrylic copolymer, maltodextrins, and hydroxypropyl cellulose have been substituted for dextran.

Hydroxypropyl starch (HPS), a natural polymer, which is nontoxic, biodegradable, and biocompatible, was prepared by REPPE Glykos AB (Vaxjo, Sweden). It is particularly interesting for industrial downstream processing because of its comparably low cost. The PEG + HPS + water system has been used for separation and purification of enzymes, proteins, and whole cells (Tjerneld et al., 1986; Ortin et al., 1991). However, few experimental data for phase equilibria are available. The work presents the liquid−liquid equilibria for PEG + HPS + water in order to provide the potential application of such data for the laboratory extraction purpose and in the design of industrial-scale separation processes.

Experimental Section

1. Materials. Poly(ethylene glycol) (PEG) samples were purchased from Shanghai Chemical Reagent Factory (P. R. China), with three different average molecular weights (M_n's) of 2000 (1900−2200), 4000 (3000−4500), 6000 (5500−7500).

HPS100 (M_n 10 000) and HPS200 (M_n 20 000) were donated by REPPE Glykos AB (Vaxjo, Sweden).

Water was the common laboratory distilled product.

2. Experimental Procedure. The systems were prepared by mass from stock solutions, 30 mass % HPS and 40 mass % PEG. Known masses of these solutions and water were weighed in a test tube to have the desired initial overall compositions. After mixing in a closed test tube by inverting upside down 50 times, phase separation was sped up by centrifugation at 3000 rpm for 10 min. Then the tube was placed in a water bath at (25 ± 0.05) °C for 2 h, waiting for proper phase equilibration and separation, as indicated by the absence of turbidity in both top and bottom phases (Voros et al., 1993).

Samples of the top phase were carefully withdrawn first. And a layer of the solution at least 0.5 cm thick was left above the interface. Samples of the bottom phase were then withdrawn by using a plastic syringe with a long needle. A tiny bubble of air was retained on the needle tip and expelled once in the bottom phase to prevent contamination from the top phase material, with special attention given to avoid disturbing the equilibrium systems in the subsequent analysis.

3. Determination of Binodal and Tie Lines. The binodal curve represents the borderline between one and two phases. A detailed description of the determination has been given elsewhere (Albertsson, 1986). The tie line describes the compositions of the two phases when they are in equilibrium. After the polymer concentrations were determined, the tie line was constructed.

4. Analytical Procedure. The optical rotation is expressed by the angle α through which the plane of polarization is rotated when the incident polarized light passes through a layer of an optically active substance. The increase in optical rotation was proportional to the concentration of HPS; therefore, the concentration was determined by an automatic polarimeter capable of giving replicate readings within 0.005° (WZZ-2, ShengHua Ltd., P. R. China).

The optical rotation was determined at 25 °C, in a 1 dm thick layer, with the use of the D line of sodium. In addition, a blank determination was made in the same manner. It was necessary to dilute the sample with a known mass of water. The presence of PEG had no effect on the optical rotation of HPS. A calibration plot of optical rotation versus concentration of HPS is shown in Figure 1. There is a subtle difference between the two lines.

The concentration of water was determined by vacuum-drying samples in the presence of P_2O_5 for 24 h until the sample reached a constant mass.

The concentration of PEG was calculated by the difference of HPS and water concentrations.

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Table 1. Phase Compositions as Mass Fraction for PEG + Hydroxypropyl Starch + H_2O at 25 °C

tie line no.	(mass %) total system HPS	PEG	H_2O	bottom phase HPS	PEG	H_2O	top phase HPS	PEG	H_2O	STL
				PEG2000 + Hydroxypropyl Starch (M_n 10 000) + H_2O						
1	16.65	10.97	72.38	32.72	4.43	62.85	4.60	16.69	78.71	−0.436
2	16.65	9.99	73.36	30.49	4.65	64.86	5.48	15.40	79.12	−0.430
3	16.69	8.98	74.33	27.30	5.10	67.60	7.19	13.45	79.36	−0.415
4	16.50	8.40	75.10	23.50	5.70	70.80	8.00	12.00	80.00	−0.405
5	15.10	8.40	76.50	21.50	6.10	72.40	9.40	10.40	80.20	−0.355
										−0.408 ± 0.032 (av)
				PEG4000 + Hydroxypropyl Starch (M_n 10 000) + H_2O						
1	16.89	11.00	72.11	35.50	2.95	61.55	3.92	16.45	79.63	−0.427
2	16.69	10.00	73.31	32.40	3.17	64.43	4.55	15.09	80.36	−0.428
3	16.69	8.99	74.32	30.47	3.37	66.16	5.27	13.68	79.36	−0.409
4	16.68	8.00	75.32	27.98	3.48	68.54	6.52	12.23	81.05	−0.408
5	16.65	6.99	76.36	25.03	3.76	71.21	8.15	10.44	81.41	−0.395
										−0.413 ± 0.014 (av)
				PEG6000 + Hydroxypropyl Starch (M_n 10 000) + H_2O						
1	16.70	9.99	73.31	33.50	2.90	63.60	3.34	15.63	81.03	−0.422
2	16.68	9.00	74.32	30.89	2.85	66.26	3.88	14.24	81.88	−0.422
3	16.59	8.00	75.41	28.76	2.97	68.27	4.24	13.23	82.53	−0.418
4	16.67	7.01	76.32	26.30	3.19	70.51	5.12	11.50	83.38	−0.392
5	16.20	5.90	77.90	20.50	4.04	75.46	6.16	10.00	83.84	−0.416
										−0.414 ± 0.013 (av)
				PEG2000 + Hydroxypropyl Starch (M_n 20 000) + H_2O						
1	18.01	8.99	73.00	31.01	3.31	65.68	5.30	14.70	80.00	−0.443
2	18.00	8.00	74.00	28.12	3.59	68.29	6.57	12.53	80.90	−0.415
3	18.00	7.20	74.80	24.80	4.20	71.00	8.00	11.44	80.56	−0.431
4	18.00	6.76	75.24	21.18	5.31	73.51	10.72	9.37	79.91	−0.388
5	15.30	6.82	77.88	20.20	4.80	75.00	10.08	9.00	80.92	−0.415
										−0.418 ± 0.020 (av)
				PEG4000 + Hydroxypropyl Starch (M_n 20 000) + H_2O						
1	18.00	8.99	73.01	34.59	1.52	63.89	3.29	15.64	81.07	−0.451
2	17.99	8.00	74.01	32.68	1.52	65.80	3.29	14.70	82.01	−0.448
3	18.01	7.00	74.99	30.71	1.83	67.46	4.32	13.34	82.34	−0.436
4	18.00	5.98	76.02	28.21	1.51	70.28	5.00	11.41	83.59	−0.427
5	18.00	4.98	77.02	25.64	1.67	72.69	5.97	9.86	84.17	−0.416
										−0.436 ± 0.015 (av)
				PEG6000 + Hydroxypropyl Starch (M_n 20 000) + H_2O						
1	18.03	9.02	72.95	35.14	1.37	63.49	2.70	16.18	81.12	−0.456
2	18.01	8.00	73.99	33.06	1.58	65.36	2.98	14.71	82.31	−0.437
3	18.02	6.99	74.99	31.02	1.58	67.40	3.43	13.42	83.15	−0.429
4	18.06	5.99	75.95	28.76	1.65	69.59	3.88	11.75	84.37	−0.406
5	18.05	4.82	77.13	25.40	2.00	72.60	4.22	10.25	85.53	−0.390
										−0.424 ± 0.026 (av)

The estimated uncertainties in the reported mass %, based on determinations with samples of known composition and duplications of five runs, are as follows: HPS100 and HPS200, 0.20%; water, 0.10%.

Results and Discussion

The experimental liquid−liquid equilibrium results for the aqueous two-phase systems, PEG2000 + HPS100, PEG4000 + HPS100, PEG6000 + HPS100, PEG2000 + HPS200, PEG4000 + HPS200, and PEG6000 + HPS200, are given in Table 1 and, as an example, in Figure 2, along with the binodal curve of the system PEG6000 + dextran D17 (M_n 23 000) for comparison. The phase diagram for the PEG6000 + HPS200 is similar to that for PEG6000 + dextran D17, but there is a parallel displacement between the two. A rough determination of the plait point compositions was obtained by drawing a straight line connecting the midpoints of all experimental tie lines and extrapolating it to the point of intersection with the binodal curve. The compositions of the plait points are listed in Table 2. The plait point of the PEG6000 + HPS200 system is higher than that of PEG6000 + dextran D17. This means that higher polymer concentrations are required to achieve phase separation with the starch derivative, which may be the direct result of lowering the molecular weight of the polymer in the bottom phase.

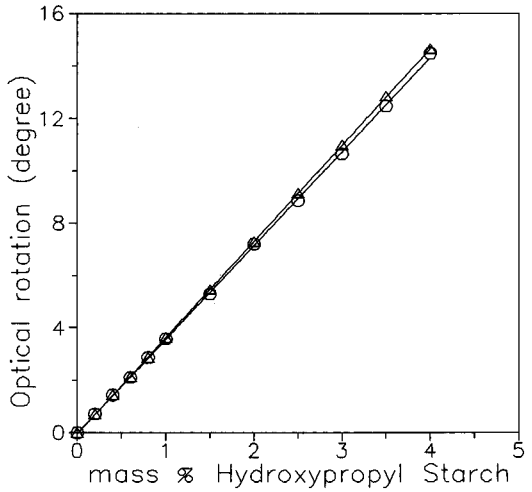

Figure 1. Optical rotation calibration curves for hydroxypropyl starch: (○) hydroxypropyl starch (M_n 10 000); (△) hydroxypropyl starch (M_n 20 000).

The tie lines for each mixture were constructed by plotting the best line that could fit the three points of initial compositions, of the top and the bottom phases. The linear correlation factor (r^2) in most cases is greater than 0.9970,

Table 2. Plait Point Compositions as Mass Fraction

system	HPS (mass %)	PEG (mass %)
PEG2000 + hydroxypropyl starch (M_n 10 000) + H_2O	15.5	7.6
PEG4000 + hydroxypropyl starch (M_n 10 000) + H_2O	14.9	6.0
PEG6000 + hydroxypropyl starch (M_n 10 000) + H_2O	12.8	5.7
PEG2000 + hydroxypropyl starch (M_n 20 000) + H_2O	14.8	6.6
PEG4000 + hydroxypropyl starch (M_n 20 000) + H_2O	12.8	4.1
PEG6000 + hydroxypropyl starch (M_n 20 000) + H_2O	11.9	4.0

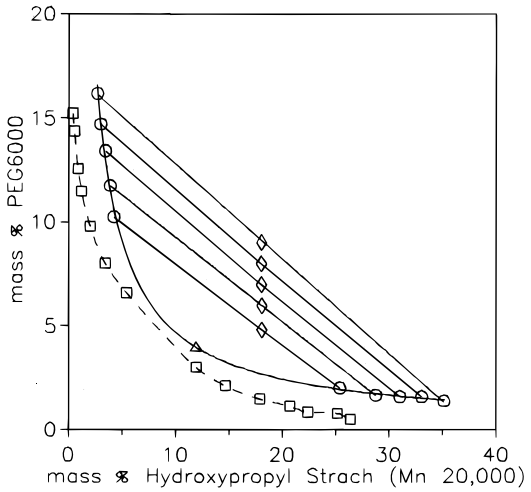

Figure 2. Phase diagrams for PEG6000 + hydroxypropyl starch (M_n 20 000) + H_2O: (◊) points obtained by mixing of polymer; (○) points obtained by analysis of separate phases; (△) plait point; (□) PEG6000 + dextran D17 + H_2O. The data for the system with dextran are taken from Albertsson.

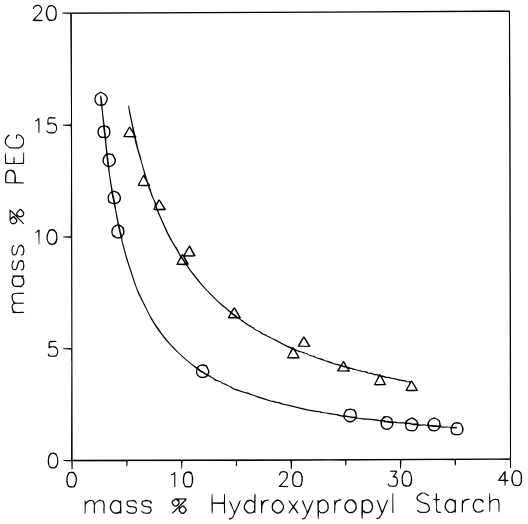

Figure 3. Influence of the polymer molecular weight on the binodals: (△) PEG2000 + hydroxypropyl starch (M_n 20 000) + H_2O; (○) PEG6000 + hydroxypropyl starch (M_n 20 000) + H_2O.

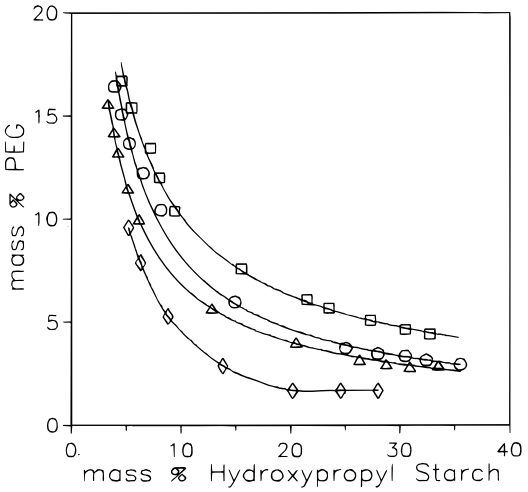

Figure 4. Comparison of binodals for various PEG + hydroxypropyl starch + H_2O: (□) PEG2000 + hydroxypropyl starch (M_n 10 000) + H_2O (this work); (○) PEG4000 + hydroxypropyl starch (M_n 10 000) + H_2O (this work); (△) PEG6000 + hydroxypropyl starch (M_n 10 000) + H_2O (this work); (◊) PEG8000 + hydroxypropyl starch (M_n 10 000) + H_2O (from manufacturer).

which indicates that the analytical determination of the constituents of the two equilibrium liquid phases was accurate and consistent.

The tie line slope (STL) is defined as the following ratio:

$$\mathrm{STL} = (\Delta\mathrm{PEG}/\Delta\mathrm{HPS})$$

where Δ is the difference between the concentrations of a given polymer in the two coexisting phases. Zaslavsky (1995) points out that the analysis of the phase diagrams reported in the literature implies that the STL value of an aqueous polymer system is usually constant in contrast to many nonaqueous polymer systems and solvent two-phase systems, but the mechanism of this phenomenon is not clearly understood. There has been no theoretical reason for constant slope yet. In our experiment, the expected uncertainty in the slopes is about 0.5% according to 0.2% uncertainty in composition measurements. However, the actual variation of the slopes with composition as given in Table 1 is from 3 to 8%. Thus, the slopes change slightly with composition. But in most cases, as shown in Figure 2, the STL value of the PEG6000 + HPS200 system is almost constant within experimental error, which indicates that the tie lines are almost parallel to each other. Averaging the STL values is probably the best way of obtaining reliable phase diagrams by checking and repeating the determination of the phase compositions away from the average STL value.

In Figure 3, the equilibrium phase compositions are strongly influenced by the molecular weight of the polymer. Lowering the molecular weight moves the binodal curve toward higher concentrations. The phase separation concentrations at the plait point vary with different polymer molecular weights: the larger the polymer molecular weight, the lower the plait point concentration.

The binodal curves for PEG + HPS systems have been studied, but no data are available for the tie lines. In Figure 4, our experimental results appear to be similar to those obtained from the manufacturer (REPPE Glykos AB). Although we have not measured the phase diagram for PEG8000 + HPS100(or 200) because PEG8000 was not available in China, the trend of the binodal curves for PEG + HPS systems with different molecular weights of PEG from this work is comparable with that of the manufacturer. However, the data provided by Tan et al. (1994) as shown in Figure 5 do not coincide with ours. There exist significant discrepancies. A possible explanation of this is that the molecular weight distributions of the polymer may not be the same, although the polymer has the same average molecular weight. The effect of the molecular weight distribution on liquid−liquid equilibrium is now

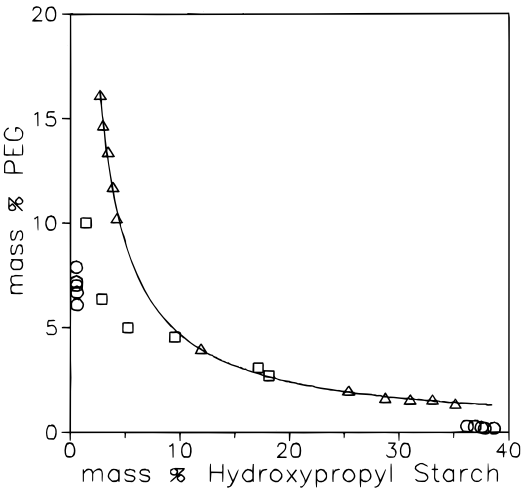

Figure 5. Comparison of liquid−liquid equilibrium data with Tan et al. (1994): (△) PEG6000 + hydroxypropyl starch (M_n 20 000) + H_2O (this work); (□) PEG6000 + hydroxypropyl starch (M_n 20 000) + H_2O (Tan et al.); (○) PEG20000 + hydroxypropyl starch (M_n 20 000) + H_2O (Tan et al.).

considered to be important (Forciniti et al., 1991). Moreover, the central part of the binodal curve of the system PEG20000 + HPS200 is somewhat arbitrary due to the lack of a composition of the plait point.

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