Liquid−Liquid Equilibrium Data for Aqueous Two-Phase Systems Composed of Ethylene Oxide Propylene Oxide Copolymers

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Phase equilibrium of aqueous two-phase systems (ATPS) containing UCON 50 HB-2000 + potassium phosphate and UCON 50 HB-2000 + sodium citrate were investigated in this work. The effect of temperature and type of salt were determined at (277.15, 298.15, and 313.15) K in pH 6.0, 7.0, and 8.0. Increasing the temperature of the phase system leads to an increase in the slope of the tie-line (STL) and in the tie-line length (TLL) causing a shift in the binodal position toward the origin. Sodium citrate showed a better capability to induce phase separation than potassium phosphate.

Introduction

Phase separation in solutions containing polymer mixtures is a very common phenomenon that was first reported in the literature in 1896,[1] when Beijerinck accidentally discovered that an aqueous solution of starch did not mix with an aqueous solution of gelatin, producing the first reported aqueous two-phase system.[2] However, the application of aqueous two phase systems (ATPS) for separation of biological materials was showed by Per-Åke Albertsson in the mid-1950s.[3] Comprehensive reviews of the early experimental phase equilibrium data of the ATPS containing two different kinds of polymers or a polymer and a salt have been reported by Albertsson[1] and Zaslavsky.[3]

In recent years, many research groups focused on the measurement of new phase equilibrium data for aqueous polymer + salt systems.[4−7] Partitioning of biomaterials in polymer ATPS is widely recognized today as a highly efficient separation technique.[4] The scale-up extraction in these systems has gained increasing attention as the separation method of choice in biotechnology.[5] ATPS are suitable for the separation of biomaterials, due to their low interfacial tension and elevated water content that preserve the natural activity of labile constituents. The polymer + salt ATPS are particularly useful, since they present relatively low cost and viscosity and provide an efficient phase separation.[6]

Prior to developing an ATPS-based process for the recovery of a biological product, it is essential to know the phase diagram for the system under study. The phase diagram delineates the potential working area for a particular two-phase system and is composed by a binodal curve intercepted by different tie-lines. The binodal separates the one-phase from the two-phase regions, and the tie-lines describe the composition of the two-phases in equilibrium. This data can be used to prepare top or bottom phases of the appropriate composition separately to use in extraction or multistep partitioning applications.[8] The phase diagram is thus an unique "fingerprint" of the ATPS under a certain set of conditions, including pH, temperature, and ionic strength.[7] One common problem faced by ATPS is the difficulty in separating target biomolecules from the polymer solution. Since separation between the phase-forming polymers and biomolecules after the primary extraction is an important step in laboratory and industrial-scale processes, expensive and time-consuming methods, such as ultrafiltration, electrophoresis, and chromatography, have been employed to separate bioproducts from polymer solutions. It has been demonstrated that the use of the temperature induced phase formation combined with a cheap aqueous two-phase system offers a simple solution to the problems of polymer removal and recycling.[6] Thermoseparating random copolymers of ethylene oxide (EO) and propylene oxide (PO) have been successfully used for the recovery of several biological products, including biopharmaceuticals such as antibodies and plasmid DNA.[9]

These polymers are thermoseparating exhibiting a decrease in solubility in water solutions upon a temperature increase. This phenomenon, has been explained by increase in polymer segment hydrophobicity with increasing temperature giving rise to a two-phase system after the system is heated above a critical temperature designated the cloud point temperature (CPT), with one polymer-rich bottom phase and a water-rich top phase, almost totally free of polymer.[10,11] This makes it possible to perform temperature induced phase separation whereby a target protein can be separated from the polymer and the polymer can be recycled.[12] Experimental liquid−liquid equilibrium (LLE) data for aqueous thermoseparating EOPO copolymers-salt mixtures are still relatively scarce.[13] Considering the potential application of such ATPS in the design of biological separation processes, in this work we have measured the phase diagrams data for EO 50% PO 50% (trade name UCON) 2000 Da systems at (277.15, 298.15, and 313.15) K, using two different salts, sodium citrate and potassium phosphate, which are not found in the literature.

Materials and Methods

Materials. UCON 50 HB-2000, a random copolymer composed of 50% ethylene oxide and 50% propylene oxide with an average molecular weight of 2000 Da was a kind offer of Dow Chemical (Midland, MI, U.S.A.). Trisodium citrate dihy-

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Table 1. Liquid−Liquid Equilibrium Data for UCON 2000 + Sodium Citrate + Water Systems from (278.15 to 318.15) K and pH (6.0 to 8.0)

	total composition		top phase		bottom phase	
	w_S	w_{UCON}	w_S	w_{UCON}	w_S	w_{UCON}
			277.15 K			
pH 6.0	0.0603 ± 0.3380	0.1499 ± 0.3321	0.0232 ± 0.1474	0.2924 ± 0.5609	0.0957 ± 0.4949	0.0001 ± 0.3551
	0.0719 ± 0.3131	0.1803 ± 0.4110	0.0142 ± 0.0246	0.4058 ± 0.3696	0.1187 ± 0.3271	0.0061 ± 0.3017
	0.0862 ± 0.1746	0.2153 ± 0.2791	0.0090 ± 0.0387	0.4992 ± 0.3375	0.1534 ± 0.4304	0.0057 ± 0.1234
	0.1038 ± 0.5317	0.2592 ± 0.0361	0.0103 ± 0.0375	0.5683 ± 0.3594	0.1889 ± 0.3185	0.0105 ± 0.5340
pH 8.0	0.0601 ± 0.0306	0.1501 ± 0.0204	0.0236 ± 0.0342	0.2629 ± 0.2527	0.0827 ± 0.0647	0.0439 ± 0.5365
	0.0719 ± 0.4117	0.1796 ± 0.1259	0.0161 ± 0.1908	0.3172 ± 0.2675	0.1147 ± 0.3372	0.0377 ± 0.2259
	0.0862 ± 0.2590	0.2158 ± 0.4816	0.0107 ± 0.3762	0.3967 ± 0.2139	0.1538 ± 0.1486	0.0203 ± 0.1970
	0.1038 ± 0.4284	0.2591 ± 0.2087	0.0064 ± 0.2438	0.4032 ± 0.3392	0.2002 ± 0.1771	0.0346 ± 0.1278
			298.15 K			
pH 7.0	0.0597 ± 0.1525	0.1504 ± 0.3196	0.0231 ± 0.2885	0.2865 ± 0.2112	0.0918 ± 0.2768	0.0180 ± 0.2041
	0.0717 ± 0.3257	0.1814 ± 0.3335	0.0160 ± 0.1035	0.3763 ± 0.3731	0.1150 ± 0.1174	0.0086 ± 0.1270
	0.0864 ± 0.1939	0.2162 ± 0.0531	0.0109 ± 0.1112	0.4540 ± 0.4588	0.1510 ± 0.1636	0.0068 ± 0.0218
	0.1043 ± 0.2121	0.2587 ± 0.2397	0.0078 ± 0.1387	0.5333 ± 0.2487	0.1844 ± 0.4214	0.0153 ± 0.3048
pH 8.0	0.0603 ± 0.1005	0.1499 ± 0.3344	0.0158 ± 0.0623	0.3034 ± 0.4727	0.0767 ± 0.1691	0.0005 ± 0.1269
	0.0719 ± 0.4069	0.1800 ± 0.1005	0.0101 ± 0.0641	0.3977 ± 0.2928	0.0978 ± 0.4023	0.0066 ± 0.0521
	0.0862 ± 0.1267	0.2162 ± 0.1391	0.0061 ± 0.2662	0.5034 ± 0.1269	0.1279 ± 0.2605	0.0111 ± 0.0992
	0.1038 ± 0.2082	0.2590 ± 0.2782	0.0025 ± 0.0266	0.5944 ± 0.2951	0.1544 ± 0.3728	0.0194 ± 0.3531

drated ($Na_3C_6H_5O_7.2H_2O$) was obtained from Merk (Darmstadt, Germany), citric acid monohydrated ($C_6H_8O_7.H_2O$) was obtained from Merk (Darmstadt, Germany), and potassium phosphate monobasic (KH_2PO_4) and dibasic (K_2HPO_4) were obtained from Panreac Quimica Sau (Barcelona, Spain). All chemicals employed in the present work were of analytical grade and used as received. Milli-Q water was used for the preparation of all aqueous solutions.

Phase Diagram Determination. For the determination of the tie lines, different aqueous two-phase systems were prepared by weighting the appropriate amounts of components from stock solutions of mass fraction of 0.50 UCON 50-HB-2000, 0.30 potassium phosphate buffer or 0.35 sodium citrate buffer, to a final weight of 10 g in 15 mL graduated centrifuge tubes (uncertainty ± 0.1 mL). The pH value of the mixtures with potassium phosphate was adjusted to 6.0, 7.0, and 8.0 by adding monobasic and dibasic potassium phosphate in the proportion of 1:0.7, 1:1.91, and 1:12.04, respectively. The pH value of the citrate stock solution was adjusted to 6.0, 7.0, and 8.0 by the addition of the citric acid mass fraction 0.35 in a pH meter (744 Metrohm, Switzerland) (uncertainty ± 0.01). All system components were thoroughly mixed in a vortex (Ika, Staufen, Germany) and phases were left to separate at the temperature under study, namely (277.15, 298.15, and 313.15) K, in a thermostatic bath for 12 h (overnight). To ensure complete phase separation, the systems were centrifuged at 1400 × *g* for 10 min at the respective temperature (Eppendorf, Hamburg, Germany). After reaching the phase equilibrium, visual estimates of top and bottom volumes were made. Phases were then separated and characterize in terms of polymer and salt concentration. All studies at 277.15 K were performed in a cold chamber.

Salt concentration (sodium citrate or potassium phosphate) was determined by conductivity measurements using a condutivimeter (Waterproof EC Testr Low, Oaklon Instruments). UCON concentration was determined by refractive index measurements at 298.15 K, using a refractometer (30PX, Mettler Toledo, Greifensee, Switzerland), after subtracting the salt contribution to the refractive index. Standard curves for the refraction index of pure UCON and salts were prepared in water in the range 0.5 to 30.0 % (w/w) and for conductivity of salts in the range 0.0 to 0.03 % (w/w). All analytical measurements were performed in triplicate. Slope of the tie-lines and tie-line length were determined according to eqs 1 and 2, respectively

$$\mathrm{STL} = \frac{[\mathrm{P}]_{top} - [\mathrm{P}]_{bottom}}{[\mathrm{S}]_{top} - [\mathrm{S}]_{bottom}} \quad (1)$$

$$\mathrm{TLL} = \sqrt{([\mathrm{P}]_{top} - [\mathrm{P}]_{bottom})^2 + ([\mathrm{S}]_{top} - [\mathrm{S}]_{bottom})^2} \quad (2)$$

where [P] and [S] are the polymer and salt concentrations determined in each phase.

Results and Discussion

The equilibrium compositions and tie-line lengths have been measured for two different ATPS, namely UCON 2000-potassium phosphate and UCON 2000-sodium citrate, and are given in Tables 1 and 2. For each copolymer/salt system, four distinct total compositions were selected. The tie-lines were obtained by linear regression of the corresponding set of total, bottom phase, and top phase concentrations determined at (277.15, 298.15, and 313.15) K at pH (6.0, 7.0 and 8.0).

Effect of the Temperature on the Phase Equilibrium. The effect of temperature on the phase diagrams of the investigated aqueous UCON−salt systems is illustrated, as an example, in Figure 1 for the UCON 2000−potassium phosphate system at pH 7.0. Figure 1 shows that an increase in the temperature induces an increase in the two-phase area, which is more pronounced at the higher temperature (313.15 K). The effect of temperature on the phase diagrams at pH of 6.0 and 8.0 presents a similar behavior as the one observed in Figure 1 for pH 7.0. Similar trends were also observed for the UCON−citrate systems.

Tables 3 and 4 show the values of tie line slope (STL) and tie-line length (TLL) for all of the different pH values and temperatures evaluated. Accordingly, for each system under study, the slope and length of the tie lines increases with an increase in the temperature for each total composition evaluated, as has been observed for other PEG−salt systems.[3,14] An increase in the system temperature will result in an increasing polymer concentration at the polymer-rich phase, whereas in the salt-rich phase the salt concentration decreases due to the transfer of the water from the polymer-rich to the salt-rich phase.

Table 2. Liquid−Liquid Equilibrium Data for UCON 2000 + Potassium Phosphate + Water Systems from (278.15 to 318.15) K and pH (6.0 to 8.0)

	total compositions		top phase		bottom phase	
	w_S	w_{UCON}	w_S	w_{UCON}	w_S	w_{UCON}
			pH 6.0			
			277.15 K			
1	0.0675 ± 0.0032	0.1601 ± 0.0112	0.0251 ± 0.1739	0.2895 ± 0.2905	0.0971 ± 0.2494	0.0402 ± 0.2568
2	0.0721 ± 0.0042	0.1800 ± 0.0047	0.0241 ± 0.0953	0.3028 ± 0.1214	0.1125 ± 0.2913	0.0523 ± 0.1128
3	0.0865 ± 0.0048	0.2158 ± 0.0114	0.0160 ± 0.0324	0.3651 ± 0.2322	0.1552 ± 0.0726	0.0237 ± 0.1785
4	0.1050 ± 0.0852	0.2573 ± 0.1140	0.0118 ± 0.1086	0.4373 ± 0.0796	0.2007 ± 0.1904	0.0469 ± 0.3617
			298.15 K			
1	0.0600 ± 0.0012	0.1499 ± 0.0100	0.0230 ± 0.0450	0.3038 ± 0.0976	0.0712 ± 0.1602	0.0522 ± 0.4302
2	0.0720 ± 0.0006	0.1801 ± 0.0137	0.0167 ± 0.0214	0.3781 ± 0.2656	0.1027 ± 0.4437	0.0211 ± 0.1408
3	0.8650 ± 0.0076	0.2158 ± 0.0066	0.0115 ± 0.0329	0.4649 ± 0.1268	0.1286 ± 0.1965	0.0364 ± 0.0067
4	0.1050 ± 0.0790	0.2573 ± 0.1050	0.0049 ± 0.2795	0.5530 ± 0.2771	0.1608 ± 0.2770	0.0454 ± 0.1055
			313.15 K			
1	0.0598 ± 0.0137	0.1495 ± 0.0455	0.0101 ± 0.3795	0.4663 ± 0.2935	0.0712 ± 0.3203	0.0154 ± 0.2613
2	0.0720 ± 0.0006	0.1798 ± 0.0107	0.0080 ± 0.2198	0.4819 ± 0.1716	0.0915 ± 0.3293	0.0322 ± 0.2444
3	0.0865 ± 0.0015	0.2159 ± 0.0017	0.0076 ± 0.3010	0.5503 ± 0.1380	0.1153 ± 0.1256	0.0223 ± 0.3620
4	0.1051 ± 0.0941	0.2573 ± 0.1094	0.0022 ± 0.0794	0.6509 ± 0.2824	0.1475 ± 0.0794	0.0177 ± 0.1707
			pH 7.0			
			277.15 K			
1	0.0675 ± 0.4349	0.1600 ± 0.0094	0.0237 ± 0.1081	0.2895 ± 0.2812	0.1118 ± 0.0962	0.0393 ± 0.3167
2	0.0720 ± 0.0135	0.1800 ± 0.0128	0.0209 ± 0.0463	0.3059 ± 0.1230	0.1328 ± 0.0409	0.0322 ± 0.2260
3	0.0864 ± 0.0058	0.2159 ± 0.0080	0.0174 ± 0.3262	0.3502 ± 0.0743	0.1769 ± 0.3260	0.0431 ± 0.0445
4	0.1049 ± 0.0629	0.2574 ± 0.0914	0.0122 ± 0.1889	0.4370 ± 0.2670	0.2434 ± 0.2176	0.0456 ± 0.1998
			298.15 K			
1	0.0601 ± 0.0047	0.1500 ± 0.0043	0.0202 ± 0.2430	0.3066 ± 0.2929	0.0894 ± 0.2875	0.0342 ± 0.1321
2	0.0720 ± 0.0053	0.1799 ± 0.0008	0.0153 ± 0.0776	0.3794 ± 0.3632	0.1195 ± 0.0371	0.0181 ± 0.3565
3	0.0863 ± 0.0070	0.2154 ± 0.0278	0.0108 ± 0.0824	0.4656 ± 0.0779	0.1482 ± 0.2248	0.0170 ± 0.0538
4	0.1039 ± 0.0026	0.2588 ± 0.0006	0.0042 ± 0.3280	0.5537 ± 0.3249	0.1965 ± 0.0270	0.0102 ± 0.2328
			313.15 K			
1	0.0600 ± 0.0020	0.1500 ± 0.0086	0.0080 ± 0.1791	0.4819 ± 0.2456	0.0852 ± 0.1789	0.0016 ± 0.1636
2	0.0718 ± 0.0151	0.1791 ± 0.0594	0.0076 ± 0.1950	0.5231 ± 0.2537	0.1076 ± 0.0313	0.0299 ± 0.4115
3	0.0866 ± 0.0110	0.2157 ± 0.0126	0.0059 ± 0.1773	0.6065 ± 0.1982	0.1370 ± 0.2459	0.0281 ± 0.0655
4	0.1037 ± 0.0053	0.2587 ± 0.0114	0.0059 ± 0.1802	0.6337 ± 0.2984	0.1804 ± 0.1257	0.0261 ± 0.2278
			pH 8.0			
			277.15 K			
1	0.0676 ± 0.3165	0.1600 ± 0.3870	0.0262 ± 0.1777	0.3007 ± 0.4150	0.1342 ± 0.2828	0.0172 ± 0.0682
2	0.0720 ± 0.0003	0.1796 ± 0.0215	0.0227 ± 0.0697	0.3042 ± 0.1709	0.1587 ± 0.3763	0.0067 ± 0.1756
3	0.0866 ± 0.0254	0.2158 ± 0.0307	0.0167 ± 0.0214	0.3781 ± 0.2656	0.2119 ± 0.2413	0.0086 ± 0.3742
4	0.1038 ± 0.0055	0.2590 ± 0.0148	0.0108 ± 0.2224	0.4792 ± 0.1770	0.2707 ± 0.1373	0.0025 ± 0.1206
			298.15 K			
1	0.0664 ± 0.0756	0.1458 ± 0.1144	0.0178 ± 0.1874	0.3498 ± 0.1580	0.1146 ± 0.1768	0.0094 ± 0.4025
2	0.0722 ± 0.0101	0.1798 ± 0.4054	0.0136 ± 0.0354	0.4356 ± 0.2028	0.1370 ± 0.3889	0.0281 ± 0.0241
3	0.0866 ± 0.0050	0.2158 ± 0.0421	0.0097 ± 0.4455	0.4802 ± 0.4436	0.1734 ± 0.1273	0.0058 ± 0.1107
4	0.1038 ± 0.0872	0.2588 ± 0.3536	0.0033 ± 0.0309	0.5954 ± 0.1462	0.2133 ± 0.1839	0.0072 ± 0.0572

Systems with total composition closer to the critical point seem to be more sensitive to temperature variations, whereas at low temperatures, this effect does not occur because the copolymer is strongly hydrated.[3] The effect of the temperature caused accentuated changes in both the slope of tie lines and the binodal position (Figure 2). The results for the experiments carried out

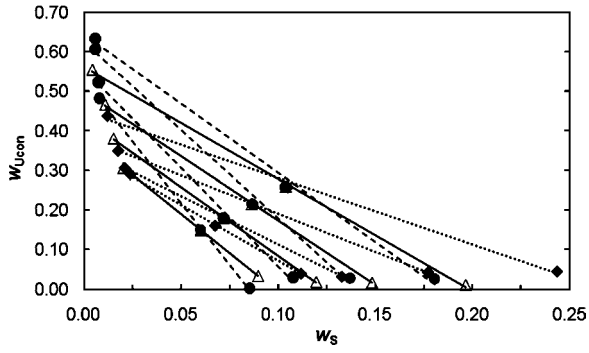

Figure 1. Effect of temperature on the liquid−liquid phase diagram for UCON 2000 (w_{UCON}) + potassium phosphate (w_S) at 277.15 K (♦; dotted line), 298.15 K (Δ; full line), and 313.15 K (●; dashed line), pH 7.0.

Table 3. STL and TLL Values for UCON 2000 + Potassium Phosphate ATPS

	277.15 K		298.15 K		313.15 K	
tie-line	STL	TLL	STL	TLL	STL	TLL
			pH 6.0			
1	−3.44	25.82	−5.22	25.62	−7.37	45.49
2	−2.83	26.57	−4.15	36.72	−5.38	45.75
3	−2.45	36.87	−3.66	44.42	−4.90	53.89
4	−2.07	43.37	−3.25	53.10	−4.36	64.96
			pH 7.0			
1	−2.84	26.53	−3.93	28.10	−6.22	48.65
2	−2.45	29.57	−3.47	37.60	−4.93	50.33
3	−1.92	34.60	−3.26	46.91	−4.41	59.31
4	−1.69	45.46	−2.83	57.65	−3.48	63.22
			pH 8.0			
1	−2.62	30.34	−3.51	35.40		
2	−2.19	32.71	−3.30	42.58		
3	−1.89	41.78	−2.90	50.19		
4	−1.88	55.27	−2.80	62.46		

at pH 8.0 and 313.15 K are not shown, as the polymer-rich phase was always turbid. This probably occurred because at those conditions the polymer had already changed its configuration from the linear, strongly hydrated, and soluble form to

Table 4. STL and TLL Values for UCON 2000 + Sodium Citrate ATPS at Different Temperatures and pH Values

	277.15 K		298.15 K	
tie-line	STL	TLL	STL	TLL
		pH 6.0		
1	−4.03	30.12		
2	−3.82	41.32		
3	−3.42	51.42		
4	−3.13	58.57		
		pH 7.0		
1			−3.91	27.71
2			−3.72	38.08
3			−3.19	46.87
4			−5.51	54.72
		pH 8.0		
1	−3.70	22.68	−4.97	30.90
2	−2.84	29.64	−4.46	40.08
3	−2.63	40.27	−4.04	50.72
4	−1.90	41.64	−3.79	59.47

the more compact and insoluble form; that is, the CPT had been reached at pH 8 and the polymer had precipitated.

Effect of pH. The effect of pH on phase separation was evaluated in both UCON−phosphate and UCON−citrate and is illustrated in Figures 3 and 4, respectively. For the UCON−phosphate ATPS (Figure 3), the tie-line slope was not significantly modified by a variation in pH, although a discrete change in the composition of the phases was observed. According to the phase diagram, an increase in the pH leads to a decrease in the polymer concentration and to an increase in the salt concentration in the bottom phase, which is probably related to the lower partitioning coefficient of the basic salt form (HPO_4^{2-}), compared to the acid form ($H_2PO_4^-$). Figure 5 shows

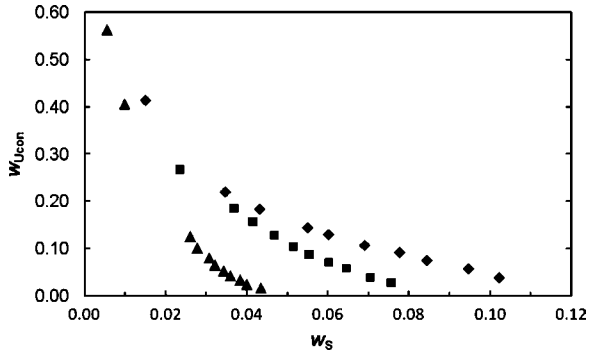

Figure 2. Binodal curves for the systems UCON 2000 (w_{UCON}) + potassium phosphate (w_S) ATPS for different temperatures: (♦) 277.15 K, (■) 298.15 K, and (▲) 313.15 K, at pH 7.0.

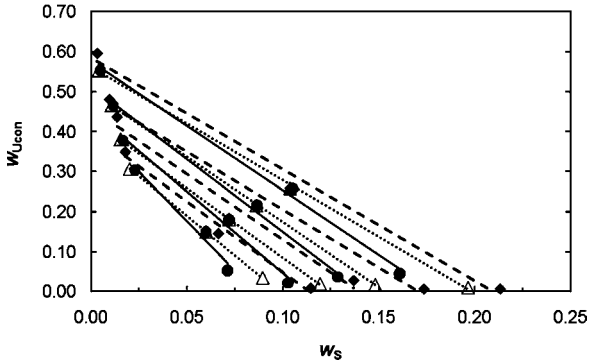

Figure 3. Effect of pH on the liquid−liquid phase diagram for UCON 2000 (w_{UCON}) + potassium phosphate (w_S) ATPS at 298.15 K: (●; full line) pH 6.0, (Δ; dotted line) pH 7.0, and (♦; dashed line) pH 8.0.

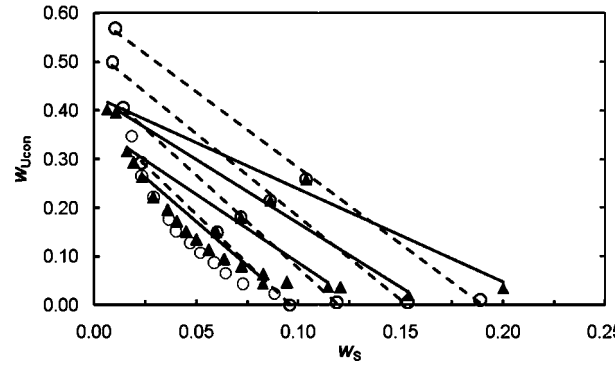

Figure 4. Effect of pH on the liquid−liquid phase diagram for UCON 2000 (w_{UCON}) + sodium citrate (w_S) at 4 °C: (○; dashed line) pH 6.0 e (▲; full line) pH 8.0. Representation of tielines and binodals.

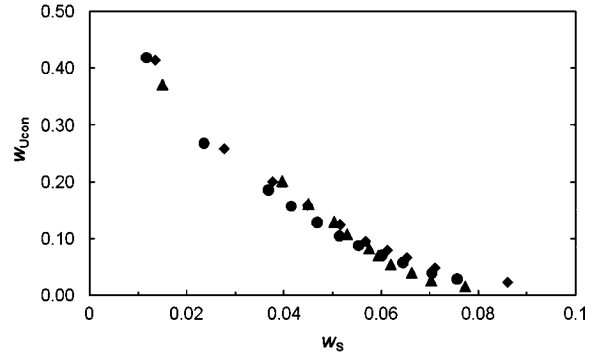

Figure 5. Binodal curves for the systems UCON 2000 (w_{UCON}) + potassium phosphate (w_S) ATPS for different pHs: (♦) pH 6.0, (■) pH 7.0, and (▲) pH 8.0 at 298.15 K.

that the pH has little effect on the position of binodal curves in the studied range.

For the UCON−sodium citrate systems, the effect of pH in the STL is more significant at 277.15 K. As can be seen in Figure 4, at 277.15 K the phase diagram at pH 6.0 shows a slighly larger two phase area than the diagram at pH 8.0.

Effect of Salt Type. The salts used for the generation of the phases diagram contain ions with different hydrophobicities. The salting-out efficacy of an electrolyte, described by the Hofmeister series, depends upon the tendencies of its ions to become hydrated.[15] The effectiveness is much stronger for anions, where this series is given by in decreasing order: $citrate^{3-} > SO_4^{2-} > HPO_4^{2-} > CH_3COO^- > Cl^- > Br^- > I^- > SCN^-$ and that for cations is given by $Li^+ > Na^+ \sim K^+ > NH_4^+ > Mg^{2+}$.[16] Thus, for the ions studied in this work, the salting out effectiveness is given by the following order: citrate > phosphate considering that cation exerts the same influence. Citrate has shown a better capability to induce phase separation, when compared to phosphate, for all systems studied as expected from the Hofmeister series. The citrate ion is preferentially hydrated and is able to remove water molecules from the copolymer hydration layer, thus reducing its solubility and promoting phase separation.

Conclusion

In this work, phase equilibrium data for UCON 50 HB-2000−potassium phosphate and UCON 50 HB-2000−sodium citrate, at three temperatures and three different pH values were measured. The effect of the type of salt and temperature on the behavior of the ATPS was also analyzed. The effect of temperature on the equilibrium data of the aqueous two-phase systems was rather significant in the displacement of the position

of the binodals and tie-lines. On the other hand, pH had a much more discrete effect on the phase diagram. Sodium citrate exhibited a higher capacity to induce phase separation than potassium phosphate due to the strongly interaction with the water molecules.

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