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Articles

Liquid–Liquid Equilibria of Biphasic Systems Composed of Sodium Citrate + Polyethylene(glycol) 1500 or 4000 at Different Temperatures

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Phase diagrams of systems composed of polyethylene(glycol) (PEG) 1500 + sodium citrate + water and PEG 4000 + sodium citrate + water were determined at temperatures of (10, 35, and 45) °C and (5, 10, 25, 35, and 45) °C, respectively. The influence of temperature and PEG molar mass on the equilibrium data of the system was analyzed. Binodal curves shifted toward the origin with the increase in PEG molar mass.

Introduction

Extraction using aqueous two-phase systems (ATPS) is a useful technique for the separation and purification of biomaterials where the use of organic solvents would damage compounds with biological activities. These systems can be formed by adding either two incompatible polymers, such as polyethylene(glycol) (PEG) and dextran, or a polymer and a salt, such as PEG and $(NH_4)_2SO_4$, to water. The high water content of both phases provides high biocompatibility and low interfacial tension, minimizing the degradation of the biomolecules. These systems also present good resolution and yield, relatively high capacity, easy scale-up, relatively low material cost, and the possibility of polymer recycle.[1]

The most common polymer + polymer system is composed of PEG and dextran. However, a large-scale utilization of this system has a high cost. PEG + salt systems have some advantages when compared to those containing only polymers, because of their lower cost, lower viscosity, rapid phase disengagement, and easy scale-up. Poly(ethylene)glycol is one of the most useful constituents in ATPS since it is a low cost, nontoxic and noninflammable reagent. Several studies[1–7] have combined PEG with different types of salts, the majority with sulfate or phosphate salts, but to a lesser degree, citrate has been used as an alternative to substitute phosphate or sulfate in the ATPS formation. Citrate is biodegradable and nontoxic and can be discharged into biological wastewater treatment plants. Nevertheless, for PEG + citrate salts + water systems, a limited number of experimental data are reported in the literature.[8–14]

Vernau and Kula[8] have measured equilibrium data for the aqueous PEG 1550 + sodium citrate system at room temperature. Zafarani-Moattar et al.[9] and Perumalsamy et al.[10] determined the phase equilibrium compositions for the PEG 6000 + sodium citrate aqueous system at different temperatures. Murugesan and Perumalsamy[11] reported the binodal curve for PEG 2000 + sodium citrate at (25, 30, 35, 40, and 45) °C. Tubío et al.[12] determined the phase diagrams of systems comprised of PEG of molar masses (600, 1000, ∼1500, ∼4000, and 8000) $g \cdot mol^{-1}$ and sodium citrate at (22, 37, and 50) °C and pH values of (5.2, 8.2, and 9.2). Zafarani-Moattar and Hamidi[13] and Jayapal et al.[14] reported liquid–liquid equilibrium data of PEG 6000 + potassium citrate at (25, 30, and 45) °C and PEG 2000 + potassium citrate at (25, 35, and 45) °C, respectively.

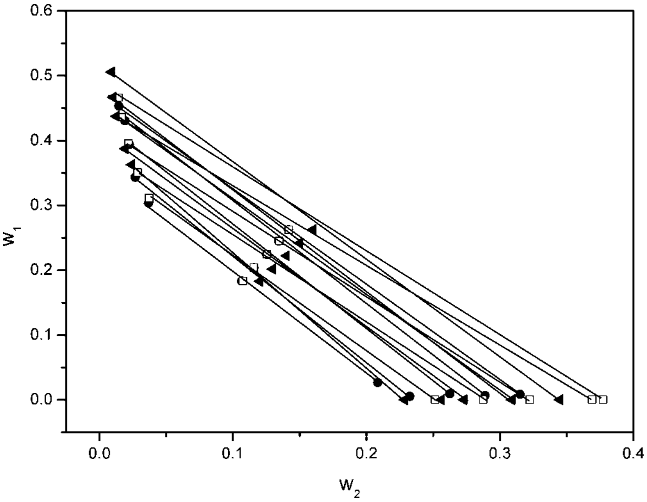

Figure 1. Temperature effect on the equilibrium phase compositions for the PEG 4000 (1) + sodium citrate + water (3) system at ●, 5 °C; □, 10 °C; "triangle left solid", 25 °C.

In the present work, we report liquid–liquid equilibrium data for the PEG 1500 + sodium citrate + water system at (10, 35, and 45) °C and a pH value of 8.24 and also for the PEG 4000 + sodium citrate + water system at (5, 10, 25, 35, and 45) °C aiming to study the influence of the temperature and PEG molar mass on the equilibrium data.

Experimental Section

Materials. The reagents of analytical grade used were PEG ($M = 1500$ $g \cdot mol^{-1}$ and 4000 $g \cdot mol^{-1}$, ISOFAR, Brazil) and

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Table 1. Mass Fraction of the PEG 4000 (1) + Sodium Citrate (2) + Water (3) System at Different Temperatures

	overall composition			upper phase			lower phase		
tie line	w_1	w_2	w_3	w_1	w_2	w_3	w_1	w_2	w_3
					5 °C				
1	0.1832	0.1067	0.7101	0.3037	0.0370	0.6593	0.0266	0.2086	0.7648
2	0.2037	0.1157	0.6806	0.3435	0.0269	0.6295	0.0050	0.2326	0.7624
3	0.2243	0.1256	0.6502	0.3939	0.0226	0.5835	0.0093	0.2625	0.7283
4	0.2450	0.1345	0.6205	0.4307	0.0192	0.5501	0.0065	0.2888	0.7046
5	0.2624	0.1417	0.5959	0.4534	0.0146	0.5321	0.0084	0.3152	0.6764
					10 °C				
1	0.1834	0.1076	0.7090	0.3114	0.0373	0.6513	0.0000	0.2514	0.7486
2	0.2039	0.1158	0.6803	0.3507	0.0287	0.6206	0.0000	00.2875	0.7125
3	0.2247	0.1254	0.6499	0.3957	0.0219	0.5824	0.0000	00.3224	0.6776
4	0.2451	0.1347	0.6202	0.4361	0.0169	0.5469	0.0000	0.3691	0.6309
5	0.2623	0.1418	0.5959	0.4665	0.0144	0.5191	0.0000	0.3773	0.6227
					25 °C				
1	0.1829	0.1202	0.6969	0.3628	0.0242	0.6130	0.0019	00.2286	0.7202
2	0.2020	0.1299	0.6682	0.3875	0.0192	0.5933	0.0000	0.2562	0.6885
3	0.2221	0.1401	0.6378	0.4377	0.0126	0.5497	0.0000	0.2731	0.6771
4	0.2416	0.1500	0.6084	0.4669	0.0103	0.5228	0.0000	0.3089	0.6317
5	0.2629	0.1602	0.5770	0.5059	0.0091	0.4850	0.0000	0.3448	0.5977
					35°C				
1	0.1831	0.1201	0.6968	0.3853	0.0225	0.5922	0.0000	00.2277	0.7723
2	0.2027	0.1299	0.6673	0.4249	0.0175	0.5577	0.0000	0.2568	0.7432
3	0.2220	0.1414	0.6367	0.4465	0.0106	0.5429	0.0000	0.2831	0.7169
4	0.2422	0.1501	0.6077	0.4833	0.0108	0.5059	0.0000	0.3119	0.6881
5	0.2632	0.1605	0.5762	0.5006	0.0090	0.4905	0.0000	0.3566	0.6434
					45 °C				
1	0.1831	0.1200	0.6968	0.3985	0.0145	0.5870	0.0000	0.2259	0.7741
2	0.2024	0.1300	0.6677	0.4336	0.0151	0.5513	0.0000	0.2395	0.7605
3	0.2225	0.1405	0.6370	0.4520	0.0116	0.5365	0.0000	0.2760	0.7240
4	0.2424	0.1496	0.6079	0.4998	0.0090	0.4912	0.0000	0.3053	0.6947
5	0.2633	0.1603	0.5764	0.5133	0.0070	0.4797	0.0000	0.3346	0.6654

tribasic sodium citrate (VETEC, Brazil), with a minimum purity of 99 %. The water (conductivity = 18.2 MΩ·cm) was deionized (Milli-Q, Millipore, USA). All reagents were used without further purification.

Experimental Procedure. Appropriate quantities of water, of aqueous solutions of PEG ($w = 0.50$), and of salt at a pH value of 8.24 were weighed with ± 0.1 mg precision (analytical balance Denver M-310, USA) aiming to prepare 40 g of the biphasic systems in 50 cm^3 centrifuge tubes. The mixture was vigorously stirred and then centrifuged (Eppendorf 5804, Germany) at 720*g* for 20 min. The tubes were transferred to a thermostatic bath (TECNAL, TE-184, Brazil) where the mixture was allowed to settle for 24 h at the desired temperature to ensure the phase separation and the equilibrium condition. The temperature was controlled at ± 0.1 °C. After equilibrium was achieved, each phase was collected using syringes and needles to determine its composition and density. The top phase was sampled first with care being taken to leave a layer of solution approximately 0.5 cm thick above the interface. The bottom phase was withdrawn using a syringe with a long needle.

Densities were measured using a 10 mL pycnometer and a balance reading at ± 0.1 mg (Denver M-310, USA).

The water was quantified by freeze-drying (Freezone 4.5, LABCONCO, USA).

The concentration of sodium citrate and of PEG in both phases was determined by flame photometry and by refractive index measurements at 30 °C (Analytic Jena AG Abbe refrac-

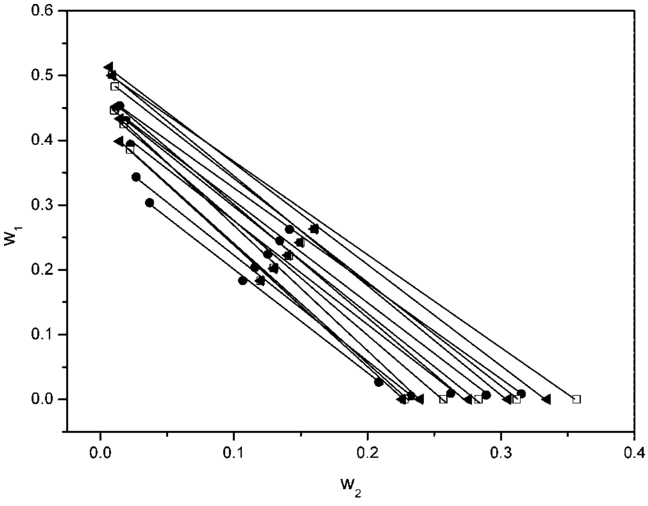

Figure 2. Temperature effect on the equilibrium phase compositions for the PEG 4000 (1) + sodium citrate + water (3) system at ●, 5 °C; □, 35 °C; "triangle left solid", 45 °C.

Table 2. Tie Line Slope for the PEG 4000 + Sodium Citrate + Water System at Different Temperatures

	temperature				
tie line	5 °C	10 °C	25 °C	35 °C	45 °C
1	1.61	1.45	1.77	1.88	1.89
2	1.65	1.36	1.64	1.78	1.93
3	1.60	1.32	1.68	1.64	1.71
4	1.57	1.24	1.56	1.61	1.69
5	1.48	1.29	1.51	1.44	1.57

Table 3. Tie Line Length for the PEG 4000 + Sodium Citrate + Water System at Different Temperatures

	temperature				
tie line	5 °C	10 °C	25 °C	35 °C	45 °C
1	32.59	37.79	41.47	43.65	45.11
2	39.61	43.59	45.42	48.77	48.82
3	45.32	49.68	50.93	52.31	52.36
4	50.26	56.06	55.42	56.94	58.10
5	53.70	59.10	60.71	60.94	60.89

Table 4. Mass Fraction of the PEG 1500 (1) + Sodium Citrate (2) + Water (3) System at Different Temperatures

	overall composition			upper phase			lower phase		
tie line	w_1	w_2	w_3	w_1	w_2	w_3	w_1	w_2	w_3
					10 °C				
1	0.1843	0.1030	0.7127	0.3046	0.0303	0.6650	0.0176	0.2235	0.7588
2	0.2039	0.1157	0.6804	0.3483	0.0226	0.6291	0.0169	0.2562	0.7269
3	0.2244	0.1254	0.6503	0.3787	0.0245	0.5968	0.0123	0.2816	0.7062
4	0.2452	0.1345	0.6203	0.4171	0.0176	0.5652	0.0150	0.3014	0.6836
5	0.2625	0.1416	0.5959	0.4505	0.0204	0.5291	0.0215	0.3212	0.6573
					35 °C				
1	0.1831	0.1200	0.6969	0.3029	0.0337	0.6634	0.0304	0.2171	0.7525
2	0.2025	0.1299	0.6676	0.3489	0.0254	0.6257	0.0294	0.2403	0.7303
3	0.2226	0.1400	0.6374	0.3935	0.0216	0.5849	0.0206	0.2752	0.7042
4	0.2425	0.1497	0.6078	0.4320	0.0158	0.5522	0.0141	0.3010	0.6850
5	0.2635	0.1603	0.5762	0.4645	0.0121	0.5234	0.0143	0.3267	0.6590
					45 °C				
1	0.1834	0.1200	0.6966	0.3220	0.0354	0.6425	0.0363	0.2156	0.7481
2	0.2022	0.1298	0.6680	0.3552	0.0251	0.6197	0.0431	0.2330	0.7239
3	0.2226	0.1400	0.6374	0.3909	0.0206	0.5885	0.0343	0.2690	0.6966
4	0.2425	0.1496	0.6079	0.4450	0.0145	0.5405	0.0317	0.2811	0.6872
5	0.2631	0.1599	0.5770	0.4641	0.0114	0.5245	0.0351	0.3066	0.6583

tometer 09–2001, Germany), respectively. The relationship between the refractive index (η) and the mass fraction of salt (w_s) and PEG (w_p) is given by eq 1[9,11]

$$\eta = a_0 + a_1 w_s + a_2 w_p \qquad (1)$$

The values of the coefficients α_0, α_1, and α_2 for the present systems were 1.3307, $1.47 \cdot 10^{-3}$, and $1.86 \cdot 10^{-3}$, respectively, and the estimated errors are within ± 0.0012. The parameters α_1 and α_2 were essentially independent of the polymer molar mass. This behavior has also been reported for other PEG + salt systems.[7] The quantification of salt and PEG was made in triplicate, and that of water was made in duplicate.

Results and Discussion

Effect of Temperature. Table 1 presents data for the PEG 4000 + sodium citrate + water system. Five tie lines were determined at each temperature. The mean standard deviation in the quantification of the salt mass by flame photometry was equal to ± 0.0036, in the determination of polymer mass by refractive index was ± 0.0006, and in the water mass by freeze-drying was ± 0.0007 (expressed as mass fraction).

From Figures 1 and 2 for the PEG 4000 + citrate + water system, an expansion in the biphasic area can be seen with increasing temperature. This behavior is in agreement with literature data[4,8,9,11,13,18] for aqueous systems formed by citrate and PEG of different molar masses. It is possible therefore to conclude that an increase in temperature reduces the polymer quantities to phase splitting.[11–13]

Tables 2 and 3 show, respectively, the slope of the tie line (STL) and tie line length (TLL) for the ATPS containing PEG 4000 + sodium citrate at different temperatures. For each temperature analyzed, an increase in the TLL induced the tie lines to become further from the origin. The STL increased from (10 to 45) °C. Hence, the PEG + sodium citrate + water systems form two phases at high temperatures more easily, or in other words, a smaller mass fraction of polymer or salt is sufficient for phase separation. An exception was found from (5 to 10) °C. Similar results are reported in the literature.[12,16,17]

Table 4 presents equilibrium data for the system PEG 1500 + sodium citrate + water at (10, 35, and 45) °C. Five tie lines were determined for each temperature. Figure 3 shows the effect of temperature on the phase diagrams. The mean standard deviation in the salt mass by this method was equal to ± 0.0045, in the polymer mass was ± 0.0005, and in the water mass was ± 0.0007 (expressed as mass fraction).

Table 5. Tie Line Slope for the PEG 1500 + Sodium Citrate + Water System at Different Temperatures

	temperature		
tie lines	10 °C	35 °C	45 °C
1	1.49	1.49	1.59
2	1.42	1.49	1.50
3	1.43	1.47	1.44
4	1.42	1.47	1.55
5	1.43	1.43	1.45

Figure 3 shows the negligible effect of temperature on the displacement of the biphasic area. From (10 to 45) °C, a small increase in the monophasic region was verified. Results obtained by Carvalho[3] at (5 and 25) °C for the same type of system showed an increase in the biphasic area.

Tables 5 and 6 present the STL and the TLL for the PEG 1500 + sodium citrate system as a function of the temperature, respectively. It can be observed that the STL increases as the

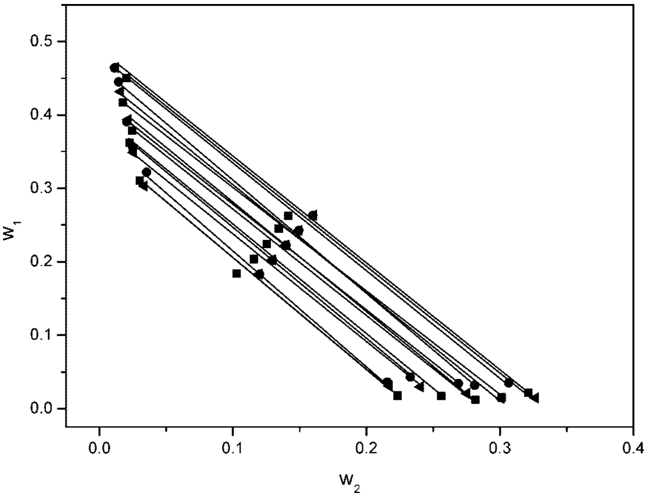

Figure 3. Temperature effect on the equilibrium phase compositions for the PEG 1500 (1) + sodium citrate + water (3) system at ■, 10 °C; "triangle left solid", 35 °C; ●, 45 °C.

Table 6. Tie Line Length for the PEG 1500 + Sodium Citrate + Water System at Different Temperatures

tie lines	temperature		
	10 °C	35 °C	45 °C
1	34.60	32.85	33.78
2	40.55	38.50	37.50
3	44.76	45.10	43.46
4	49.21	50.59	49.18
5	52.39	54.92	52.07

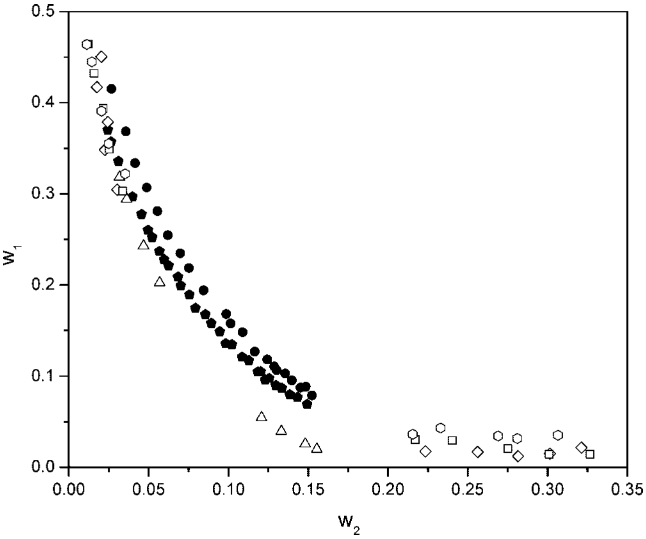

Figure 4. Binodal curves for the PEG 1500/2000 (1) + sodium citrate (2) + water (3) system at: "solid pentagon symbol", 35 °C; ●, 45 °C; Δ, 22 °C; ◇, 10 °C; □, 35 °C; and ○, 45 °C.

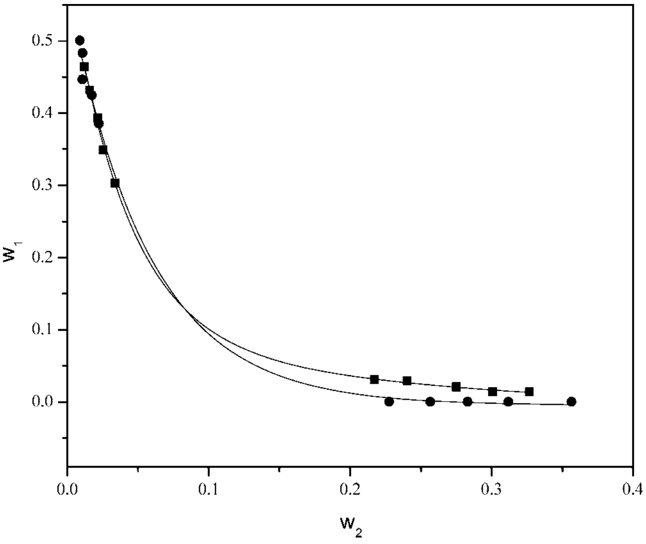

Figure 5. Effect of PEG molar mass for systems PEG (1) + sodium citrate (2) + water (3) at 30 °C: ●, PEG 1500; and ■, PEG 4000.

temperature increases. Similar results were obtained by other authors for PEG−citrate systems.[8,11,16,17] For temperature values between (10 and 45) °C, a TLL reduction on the temperature increment is observed.

In Figure 4, the binodal curves for PEG 2000 + sodium citrate[11] at (35 and 45) °C, PEG 1500 + sodium citrate[12] at 22 °C and pH 8.2, and data obtained in this work, PEG 1500 + sodium citrate at (10, 35, and 45) °C and pH 8.24, are shown. From the figure, no difference between the data from Tubío et al.[12] and ours was observed in the temperature and pH range studied. From the full symbols (Murugesan and Perumalsamy[11]), an increase in the biphasic area as temperature increases was verified.

Effect of PEG Molar Mass. The exclusion of the PEG from the salt-rich phase with an exception at 5 °C, where very small quantities of PEG were found in the bottom phase, can be observed from Table 1. In the polymer-rich phase, the opposite behavior is observed with the salt concentration being very small in the top phase. Similar results were reported in the literature for systems composed of PEG (600, 1000, 1450, 3350, and 8000) + sodium citrate aqueous systems[12] and the PEG + potassium phosphate + water system,[15] although for PEG 1500 this behavior was not verified in our work (Table 4). Figure 5 shows the binodal curves for PEG + sodium citrate ATPS, at 35 °C, as a function of PEG molar mass. It can be seen that the binodal curves shift to lower PEG and salt concentrations as the PEG molar mass increases. A similar behavior was also reported by Tubío et al.[12] for PEG 2000 + sodium citrate systems, and it was attributed to the increase in the incompatibility between the system components, since the PEG with a higher molar mass has a more hydrophobic character, when compared to the polymer presenting a smaller molar mass.

Conclusions

Equilibrium data for water + sodium citrate + polyethylene(glycol) of (1500 and 4000) g·mol^{-1} determined at different temperatures showed distinct behavior relative to the biphasic area. In the first, the temperature increase led to a biphasic area increase, and for PEG 1500, the biphasic area decreased as the temperature increased. The effect of polymer molar mass on the binodal curves promoted an enlargement of the biphasic area as the PEG molar mass increased.

Acknowledgment

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