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Liquid−Liquid Equilibrium of Aqueous Two-Phase Systems Containing Poly(ethylene) Glycol 4000 and Zinc Sulfate at Different Temperatures

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Liquid–liquid equilibrium data of aqueous two-phase systems composed of polyethylene(glycol) (PEG) 4000 + zinc sulfate at (5, 10, 35, and 45) °C were measured. The effect of temperature in the systems was studied. The expansion of the two-phase area with the increase in temperature from (5 to 35) °C and also a small reduction of the biphasic region from (35 to 45) °C were observed. The slope of all tie lines increased with the temperature increase. The tie line length increased in the ranges of (5 to 25) °C and (35 to 45) °C and decreased from (25 to 35) °C.

Introduction

Aqueous two-phase systems (ATPS) are an important partition technique used for separation, purification, and recovery of biological materials. These systems are formed by adding two polymers soluble in water or a water-soluble polymer and a component of low molar mass, like inorganic salts.[1,2] The use of these systems presents several advantages when compared to other separation techniques. They show good resolution, high yield, and relatively low material costs. In addition, ATPS are easily scaled up and allow the recycle of the reagents utilized in the process. Their high water content implies high biocompatibility and low interfacial tension, minimizing the degradation of biomolecules.[3,4] Snyder et al.[5] have determined phase diagrams and their properties for polyethylene(glycol) (PEG) of molar masses of 1000, 3350, and 8000 + salt systems. Ananthapadmanabhan[6] conducted studies of aqueous two-phase formation of PEG molar masses of 1450, 3350, and 8000 and inorganic salts. The effect of temperature was investigated by Ho-Gutierrez et al.[7] for PEG (1000, 3350, and 8000) + Na_2SO_4 systems and by Zafarani-Moattar and Sadeghi[8] for PEG + Na_2HPO_4 and PEG + NaH_2PO_4 systems. The authors observed an expansion in the two-phase area with a temperature increase. The same effect was reported by Zafarani-Moattar and Gasemi[9] for PEG + $NH_4H_2PO_4$ systems and by Graber et al.[10] for PEG + $(NH_4)_2HPO_4$ systems.

Liquid–liquid equilibrium data for PEG + zinc sulfate aqueous systems are scarce. Zafarani-Moattar and Hamzehzadeh[11] presented binodal curves for PEG 6000 + $ZnSO_4$ at 25 °C, and Hey et al.[12] presented equilibrium data for the PEG 8000 + $ZnSO_4$ system at 25 °C. Therefore, this work was conducted aiming to determine equilibrium data of aqueous two-phase systems composed of PEG 4000 + $ZnSO_4$ at (5, 10, 35, and 45) °C as a function of temperature.

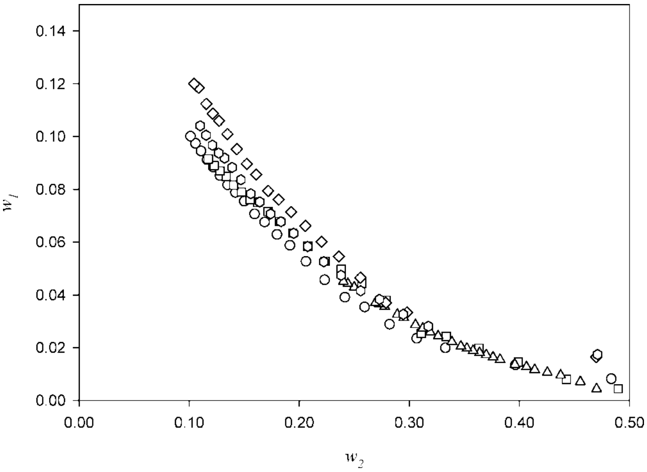

Figure 1. Binodal curves for the PEG 4000 (1) + $ZnSO_4$ (2) + water (3) system at ⬡, 5 °C; ◇, 10 °C; Δ, 25 °C; ○, 35 °C; □, 45 °C.

Experimental Section

Materials. The reagents of analytical grade were PEG (M = 4000 $g\cdot mol^{-1}$, ISOFAR, Brazil) and zinc sulfate (VETEC, Brazil), with a minimum purity of 99 %. The water (conductivity = 18.2 $M\Omega\cdot cm$) was deionized (Milli-Q, Millipore, USA). All reagents were used without further purification.

Experimental Procedure. The binodal curves were determined by the turbidimetric titration method. A PEG solution with known concentration was taken into the vessel and titrated with a zinc sulfate solution, also with known concentration. The amount of salt solution was recorded as the mixture became turbid. Subsequently, water was added until the solution became clear, and the mass of the mixture was recorded.

For the tie line determination, stock solutions of polymer (with mass fraction between 0.50 and 0.60) and salt (w = 0.30 and pH = 3.66) were initially prepared to make up the systems using an analytical balance (Denver Instrument, M-310, USA), with a precision of ± 0.1 mg. Appropriate amounts of stock solutions of PEG 4000 $g\cdot mol^{-1}$, zinc sulfate, and water were added into 50 cm^3 centrifuge tubes. The tubes were manually stirred for 5

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Table 1. Mass Fraction of the PEG 4000 (1) + $ZnSO_4$ (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	0.1821	0.1145	0.7034	0.2854	0.0205	0.6941	0.0534	0.2016	0.7450
2	0.1980	0.1187	0.6834	0.3255	0.0138	0.6607	0.0486	0.2276	0.7239
3	0.2223	0.1276	0.6501	0.3521	0.0081	0.6399	0.0522	00.2456	0.7022
4	0.2343	0.1318	0.6339	0.3749	0.0061	0.6190	0.0559	00.2637	0.6805
5	0.2535	0.1383	0.6082	0.4144	0.0043	0.5813	0.0678	00.2746	0.6576
10 °C									
1	0.2168	0.0949	0.6883	0.3255	0.0105	0.6640	0.0565	00.2185	0.7250
2	0.2316	0.0961	0.6723	0.3431	0.0084	0.6485	0.0611	00.2210	0.7179
3	0.2481	0.1025	0.6494	0.3776	0.0064	0.6160	0.0587	00.2488	0.6925
4	0.2584	0.1053	0.6363	0.3912	0.0051	0.6037	0.0631	00.2526	0.6843
5	0.2825	0.1131	0.6045	0.4226	0.0032	0.5742	0.0650	00.2751	0.6599
25 °C[a]									
1	0.1314	0.1482	0.7204	0.3335	0.0205	0.6460	0.0219	00.2167	0.7613
2	0.1376	0.1748	0.6876	0.3680	0.0131	0.6190	0.0191	00.2380	0.7429
3	0.1446	0.2015	0.6539	0.4112	0.0092	0.5796	0.0276	00.2548	0.7175
4	0.1495	0.2263	0.6242	0.4441	0.0047	0.5512	0.0376	00.2674	0.6950
5	0.1551	0.2455	0.5994	0.4692	0.0042	0.5266	0.0262	00.2881	0.6857
35 °C									
1	0.1816	0.0746	0.7438	0.2660	0.0187	0.7153	0.0380	00.1562	0.8058
2	0.2003	0.0870	0.7127	0.3180	0.0110	0.6709	0.0416	00.1811	0.7773
3	0.2098	0.0930	0.6971	0.3397	0.0108	0.6495	0.0462	00.1986	0.7552
4	0.2398	0.0975	0.6627	0.3786	0.0065	0.6149	0.0530	00.2225	0.7244
5	0.2510	0.1061	0.6429	0.3949	0.0056	0.5994	0.0498	00.2433	0.7069
45 °C									
1	0.1979	0.1074	0.6947	0.3219	0.0584	0.6197	0.0534	00.1697	0.7769
2	0.2241	0.1148	0.6610	0.3577	0.0558	0.5865	0.0519	00.1930	0.7551
3	0.2360	0.1202	0.6438	0.3701	0.0582	0.5717	0.0489	00.2270	0.7242
4	0.2569	0.1262	0.6168	0.4062	0.0585	0.5352	0.0541	00.2357	0.7102
5	0.2796	0.1324	0.5880	0.4315	0.0704	0.4981	0.0573	00.2232	0.7195

[a] Francisco, 2005.

min and then centrifuged (Centrifuge Eppendorf 5804, Germany) at 716*g* for 20 min, to accelerate the phase separation. The mixture was allowed to settle for 24 h at the desired temperature in a water bath (TECNAL, TE-184, Brazil) with a precision of ± 0.1 °C to ensure the phase separation and the equilibrium condition. Samples were taken in triplicate from the top and bottom phases to determine their composition, using syringes and needles. After that, the top phase was carefully sampled leaving, approximately, 0.5 cm of this phase above the interface. Syringes with long needles were used to withdraw samples from the bottom phase. The densities of both phases were determined using an analytical balance and a standard volumetric pycnometer of 10.0 mL previously calibrated with water.[13]

The concentrations of zinc sulfate in the top and bottom phases were quantified by zinc analysis using atomic absorption spectroscopy[10,12] (Varian Spectr AA-200, Australia). The content of PEG was obtained from refractive index measurements at 30 °C using a refractometer (Analytic Jena AG Abbe refractometer 09-2001, Germany). Since the refractive index of the phase samples depends on the polymer and salt concentrations, the polymer content was determined by the difference between the salt concentration measured by spectrophotometry and the total solution composition.[14,15] The water concentration was quantified by freeze-drying (Freezone 4.5 - LABCONCO, USA). Salt and PEG content measurements were performed in triplicate, and water content quantification was performed in duplicate.

Results and Discussion

The mean standard deviations in the quantification of the salt, polymer, and water were, respectively, equal to ± 0.0024, ± 0.0006, and ± 0.0006 (expressed as mass fraction).

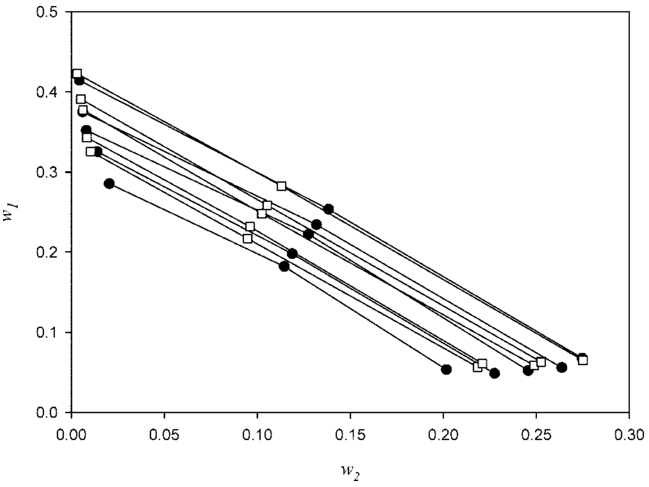

Figure 2. Temperature effect on the equilibrium phase compositions for the PEG 4000 (1) + $ZnSO_4$ (2) + water (3) system at ●, 5 °C; □, 10 °C.

The effect of temperature on the binodal curves from (5 to 45) °C, obtained from turbidimetric titrations, is shown in Figure 1. An increase in the biphasic region can be observed, from (5 to 35) °C.

Table 1 presents the equilibrium compositions of the aqueous two-phase system PEG + $ZnSO_4$ determined at (5, 10, 35, and 45) °C and the data at 25 °C which were previously determined by Francisco.[16] The tie lines obtained by connecting the experimental equilibrium phase composition results are illustrated in Figures 2 to 5.

Figure 2 shows a reduction of the biphasic area in all ranges of concentrations analyzed when the temperature increases from

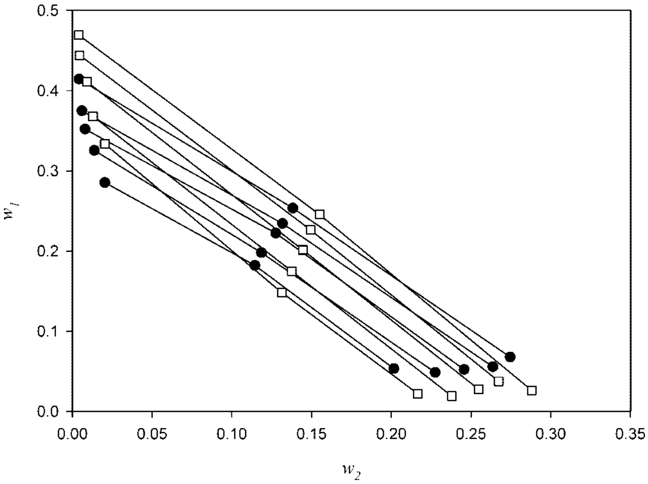

Figure 3. Temperature effect on the equilibrium phase compositions for the PEG 4000 (1) + $ZnSO_4$ (2) + water (3) system at ●, 5 °C; □, 25 °C.

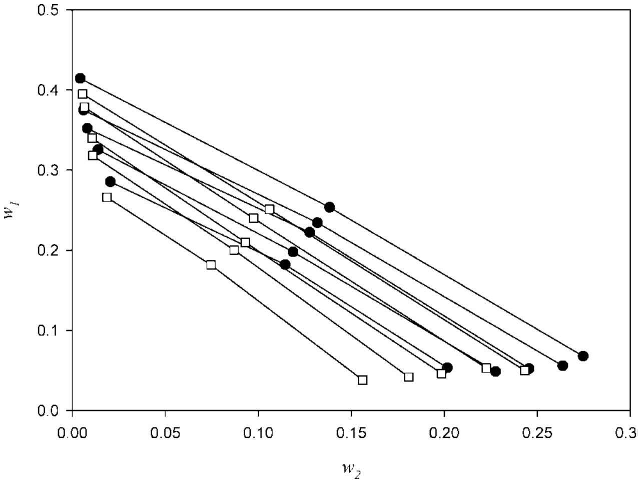

Figure 4. Temperature effect on the equilibrium phase compositions for the PEG 4000 (1) + $ZnSO_4$ (2) + water (3) system at ●, 5 °C; □, 35 °C.

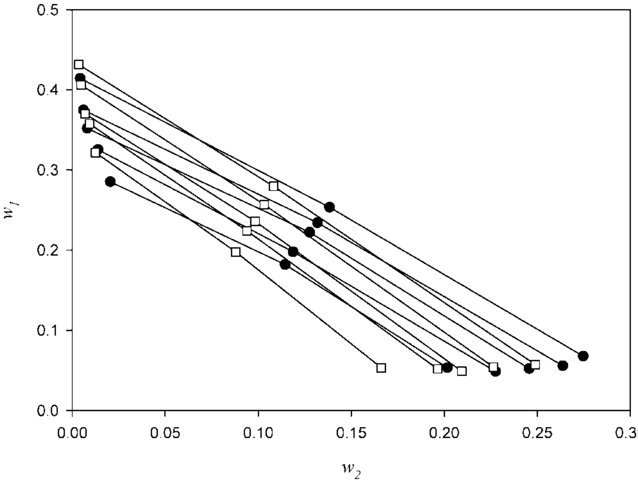

Figure 5. Temperature effect on the equilibrium phase compositions for the PEG 4000 (1) + $ZnSO_4$ (2) + water (3) system at ●, 5 °C; □, 45 °C.

(5 to 10) °C. The same effect was observed for the PEG 1000 + $NaHSO_4$ system studied by Zafarani-Moattar and Sadeghi[8] and for the system PEG + $(NH_4)_2SO_4$ examined by Voros et al.[17]

Table 2. Tie Line Slope for the PEG 4000 + $ZnSO_4$ + Water System at Different Temperatures

	temperature				
tie line	5 °C	10 °C	25 °C[a]	35 °C	45 °C
1	1.28	1.29	1.59	1.66	2.41
2	1.30	1.33	1.55	1.63	2.23
3	1.26	1.32	1.56	1.56	1.90
4	1.24	1.33	1.55	1.51	1.99
5	1.28	1.32	1.56	1.45	2.45

[a] Francisco, 2005.

Table 3. Tie Line Length for the PEG 4000 + $ZnSO_4$ + Water System at Different Temperatures

	temperature				
tie line	5 °C	10 °C	25 °C[a]	35 °C	45 °C
1	29.44	34.01	36.83	26.62	29.06
2	34.98	35.32	41.50	32.45	33.52
3	38.26	40.06	45.55	34.84	36.29
4	41.01	41.10	48.40	39.07	39.42
5	43.93	44.92	52.62	41.90	40.42

[a] Francisco, 2005.

Figure 3 shows, for the temperature rise of (5 to 25) °C, an increase in the PEG concentration in the top phase and a reduction in the salt concentration in the bottom phase. In Figure 4, from (5 to 35) °C, it can be seen that the biphasic area expands with a temperature increase. This PEG + $ZnSO_4$ system, at 35 °C, presented the largest biphasic area compared to all other temperatures. Finally, at 45 °C (Figure 5), a small reduction of the biphasic area is observed. Similar behavior was also verified by Zafarani-Moattar and Sadeghi[8] for PEG 6000 + Na_2HPO_4 or NaH_2PO_4, by Mishima et al.[18] for PEG 6000 + K_2HPO_4, and by Voros et al.[17] for PEG 1000 + Na_2CO_3 and PEG 2000 + Na_2CO_3 aqueous systems.

The slope of the tie line (STL) is given by the ratio of the difference of the polymer and the salt concentration, (C(P)) and (C(S)), respectively, in the top and bottom phases, as presented by eq 1

$$\mathrm{STL} = \{[C(\mathrm{P})^{\mathrm{top}} - C(\mathrm{P})^{\mathrm{bottom}}]/[C(\mathrm{S})^{\mathrm{top}} - C(\mathrm{S})^{\mathrm{bottom}}]\} \quad (1)$$

Table 2 shows the increase in the STL with the temperature rise. Similar results were reported in other aqueous two-phase systems.[8,9,14] An increase of the polymer concentration in the upper phase and an increase of the salt concentration in the lower phase was observed when the temperature increased.

Tie-line lengths (TLL) of the systems are presented in Table 3. The tie-line length is an empirical measure of the compositions of the two phases and can be calculated through eq 2

$$\mathrm{TLL} = \{[C(\mathrm{P})^{\mathrm{top}} - C(\mathrm{P})^{\mathrm{bottom}}]^2 + [C(\mathrm{S})^{\mathrm{top}} - C(\mathrm{S})^{\mathrm{bottom}}]^2\}^{0.5} \quad (2)$$

where $C(\mathrm{P})^{\mathrm{top}}$ and $C(\mathrm{P})^{\mathrm{bottom}}$ are the polymer concentrations in the top and bottom phases and $C(\mathrm{S})^{\mathrm{top}}$ and $C(\mathrm{S})^{\mathrm{bottom}}$ are the salt concentrations in the top and bottom phases. It is observed in Table 3 that the TLL increases with a temperature elevation from (5 to 25) °C and from (35 to 45) °C, and the TLL reduces from (25 to 35) °C.

Conclusions

Liquid–liquid equilibrium data for the system PEG 4000 + zinc sulfate + water at (5, 10, 35, and 45) °C were obtained. It was observed that an expansion in the biphasic area occurred with an increment in temperature and a small reduction from (35 to 45) °C. The slope of tie lines increased with increasing temperature.

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