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Equilibrium Data for PEG 4000 + Salt + Water Systems from (278.15 to 318.15) K

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Liquid−liquid equilibrium data of aqueous two-phase systems composed of PEG 4000 + sodium sulfate, PEG 4000 + lithium sulfate, and PEG 4000 + potassium phosphate were determined from (278.15 to 318.15) K. When the temperature was increased, the slope of the tie line (STL) tended to increase, reducing the quantity of salt necessary for phase splitting. From (308.15 to 318.15) K, for the aqueous systems PEG 4000 + lithium sulfate or potassium phosphate, a small decrease of the STL was verified. In spite of producing a phase diagram with a larger biphasic area, the sodium sulfate showed higher capability for inducing the phase splitting as compared to potassium phosphate and lithium sulfate.

Introduction

Aqueous two-phase systems (ATPS) are formed by mixing either aqueous solutions of different polymers or aqueous solutions of polymers and salts above certain critical thermodynamic conditions. Such systems are extensively applied to separate biocompounds since they present low interfacial tension, allied to high water content, which preserves the biological activity of labile constituents. Polymer + salt + water systems are particularly suitable for the partition of compounds due to their relatively low cost and low viscosity, which allows an efficient phase separation.[1,2]

Several studies have been carried out to find appropriate ATPS as well as proper operational conditions for the partition of different biocompounds.[3−9] The selective distribution of constituents of ATPS may be affected by different factors like the nature and size of the biocompound, molecular structure and size chain of the polymer, type of salt, pH, system initial composition, and temperature.[1,2,8,9] Therefore, a study of the behavior of ATPS under different operational conditions will expand and facilitate their application in the partitioning of biomaterials.

Liquid−liquid equilibrium (LLE) data are necessary for the development and scale-up of the extraction process using ATPS. Equilibrium data for aqueous systems with poly(ethylene glycol) (PEG) + potassium phosphate are reported in the literature.[1−3,10−12] However, for systems constituted by PEG + lithium sulfate and PEG + sodium sulfate, equilibrium data are relatively limited.[13−17]

The objective of the present work was to measure LLE data for PEG 4000 + sodium sulfate, PEG 4000 + lithium sulfate, and PEG 4000 + potassium phosphate ATPS from (278.15 to 318.15) K as well as to analyze the influence of the temperature and type of salt on the behavior of these systems.

Experimental Section

Materials. The analytical grade reagents used were PEG ($M = 4000\ \mathrm{g{\cdot}mol^{-1}}$, Isofar, Brazil), sodium sulfate, lithium sulfate, and potassium phosphate (Vetec, Brazil), with purities higher than 99 %.

Experimental Procedure. ATPS were prepared by adding appropriate quantities of water, salt, and stock solution of PEG (0.50 mass fraction) into a centrifuge tube to obtain 40 g of mixture. The water used was previously distilled and deionized (Milli-Q Device, Millipore, USA). The pH of the solution was monitored at ± 0.01 (Digimed DM-20 pH meter, Brazil). The pH of mixtures containing sodium sulfate and lithium sulfate were close to 7, needing no adjustment. The pH value of the mixtures with potassium phosphate was adjusted to 7 by adding mono and dibasic potassium phosphate in the proportion of 1:1.82, respectively.

Each system was further centrifuged at 2000 g for 20 min (Eppendörf AG-5804 Centrifuge, Germany) and transferred to an equilibrium cell, where the mixture was allowed to settle for 24 h at the operational temperature (278.15, 298.15, 308.15, and 318.15) K to obtain clear phase separation and to reach the equilibrium. The temperature was monitored with a precision of ± 0.01 K by a thermostatic water bath (Tecnal TE-184, Brazil). Each resultant phase was collected with a syringe, and its density was measured with a pycnometer (10 mL), previously calibrated with water at the operational temperature. The salt content was determined by flame photometry (Celm FC-180 Photometer, Brazil). The water was quantified by freeze drying (Heto VR-1 Speed Vacuum Concentration, Denmark) at 253.15 K for 24 h. The sample was previously weighed with an accuracy of ± 0.0001 g (Denver M-310 Analytical Balance, USA), and subsequently frozen. The amount of water was obtained from the variation of the sample weight before and after the drying process, corresponding to the mass of water evaporated. The quantity of PEG was determined from the difference between the mass of the dried sample and the amount of the salt, as shown by

$$m_{\mathrm{PEG}}/\mathrm{g} = m_{\mathrm{DS}}/\mathrm{g} - m_{\mathrm{S}}/\mathrm{g} \qquad (1)$$

where m_{PEG}, m_{DS}, and m_{S} are the masses of PEG, dried sample, and salt, respectively. All measurements were made in triplicate.

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Table 1. Liquid−Liquid Equilibrium Data for PEG 4000 + Potassium Phosphate + Water System from (278.15 to 318.15) K

tie line	overall			top phase			bottom phase		
	w_{PEG}	w_S	w_W	w_{PEG}	w_S	w_W	w_{PEG}	w_S	w_W
278.15 K									
1	0.1731	0.0950	0.7319	0.2792	0.0409	0.6799	0.0120	0.2023	0.7857
2	0.1930	0.1036	0.7034	0.3114	0.0373	0.6513	0.0188	0.2292	0.7520
3	0.2147	0.1160	0.6693	0.3555	0.0298	0.6147	0.0393	0.2752	0.6855
4	0.2352	0.1281	0.6367	0.3963	0.0261	0.5776	0.0318	0.2910	0.6772
5	0.2521	0.1377	0.6102	0.4302	0.0232	0.5466	0.0042	0.3469	0.6489
298.15 K									
1	0.1756	0.0949	0.7295	0.2926	0.0377	0.6697	0.0018	0.1859	0.8123
2	0.2017	0.1067	0.6916	0.3583	0.0218	0.6199	0.0116	0.2220	0.7664
3	0.2189	0.1154	0.6657	0.3931	0.0145	0.5924	0.0121	0.2466	0.7413
4	0.2334	0.1249	0.6417	0.4181	0.0127	0.5692	0.0076	0.2738	0.7186
5	0.2552	0.1365	0.6083	0.4655	0.0055	0.5290	0.0232	0.3032	0.6736
308.15 K									
1	0.1727	0.0917	0.7356	0.3078	0.0255	0.6667	0.0018	0.1785	0.8197
2	0.1910	0.1007	0.7083	0.3496	0.0195	0.6309	0.0162	0.1928	0.7910
3	0.2110	0.1113	0.6777	0.3877	0.0183	0.5940	0.0206	0.2204	0.7590
4	0.2360	0.1221	0.6419	0.4287	0.0143	0.5570	0.0350	0.2405	0.7245
5	0.2525	0.1370	0.6105	0.4604	0.0156	0.5240	0.0426	0.2758	0.6816
318.15 K									
1	0.1666	0.0865	0.7469	0.3056	0.0293	0.6651	0.0331	0.1564	0.8105
2	0.1860	0.0984	0.7156	0.3571	0.0241	0.6188	0.0484	0.1821	0.7695
3	0.2054	0.1063	0.6883	0.3812	0.0204	0.5984	0.0396	0.2169	0.7435
4	0.2311	0.1201	0.6488	0.4332	0.0160	0.5508	0.0653	0.2392	0.6955
5	0.2450	0.1252	0.6298	0.4469	0.0141	0.5390	0.0354	0.2671	0.6975

Table 2. Liquid−Liquid Equilibrium Data for PEG 4000 + Sodium Sulfate + Water System from (278.15 to 318.15) K

tie line	overall			top phase			bottom phase		
	w_{PEG}	w_S	w_W	w_{PEG}	w_S	w_W	w_{PEG}	w_S	w_W
278.15 K									
1	0.0860	0.0802	0.8338	0.1806	0.0496	0.7698	0.0176	0.1233	0.8591
2	0.1127	0.0902	0.7971	0.2633	0.0320	0.7047	0.0407	0.1514	0.8079
3	0.1238	0.0999	0.7763	0.3050	0.0226	0.6724	0.0375	0.1742	0.7883
4	0.1486	0.1095	0.7419	0.3657	0.0176	0.6167	0.0165	0.1935	0.7900
5	0.1717	0.1181	0.7102	0.3805	0.0150	0.6045	0.0205	0.2230	0.7565
278.15 K									
1	0.0873	0.0765	0.8362	0.1650	0.0496	0.7854	0.0161	0.1033	0.8806
2	0.1113	0.0912	0.7975	0.2873	0.0296	0.6831	0.0064	0.1416	0.8520
3	0.1279	0.1005	0.7716	0.3331	0.0204	0.6465	0.0013	0.1515	0.8472
4	0.1498	0.1040	0.7462	0.3682	0.0155	0.6163	0.0085	0.1748	0.8167
5	0.1723	0.1108	0.7169	0.4159	0.0100	0.5741	0.0056	0.1904	0.8040
308.15 K									
1	0.0864	0.0795	0.8341	0.2170	0.0395	0.7435	0.0172	0.1019	0.8809
2	0.1119	0.0901	0.7980	0.3032	0.0257	0.6711	0.0010	0.1316	0.8674
3	0.1281	0.0997	0.7722	0.3500	0.0190	0.6310	0.0052	0.1463	0.8485
4	0.1520	0.1068	0.7412	0.3933	0.0154	0.5913	0.0032	0.1675	0.8293
5	0.1690	0.1080	0.7230	0.4046	0.0145	0.5809	0.0036	0.1770	0.8194
318.15 K									
1	0.1928	0.0660	0.7412	0.2925	0.0289	0.6786	0.0083	0.1302	0.8615
2	0.2242	0.0733	0.7025	0.3500	0.0209	0.6291	0.0024	0.1422	0.8554
3	0.2490	0.0787	0.6723	0.3934	0.0192	0.5874	0.0054	0.1648	0.8298
4	0.2814	0.0858	0.6328	0.4309	0.0135	0.5556	0.0002	0.1857	0.8141
5	0.3331	0.0989	0.5680	0.5008	0.0095	0.4897	0.0013	0.2265	0.7722

Results and Discussion

Tables 1 to 3 and Figures 1 to 3 present LLE data for PEG 4000 + sodium sulfate, PEG 4000 + lithium sulfate, and PEG 4000 + potassium phosphate ATPS from (278.15 to 318.15) K. Five tie lines were found for each temperature studied. PEG, salt, and water quantities are expressed as mass fractions (*w*). Measurement uncertainties were statistically analyzed by estimating the corresponding mean standard deviation, with values equal to ± 0.0020 and ± 0.0044 for salt and water measurements, respectively. The amount of PEG 4000 was obtained according to eq 1.

The influence of the temperature on the phase equilibrium was analyzed by applying the slope of the tie line (STL) concept. The STL explains the effect of the operational conditions on system composition. This value is computed as the ratio between the variation of the polymer and salt concentration in each phase of the system (eq 2):

$$\mathrm{STL} = \frac{C_P^T/\mathrm{g \cdot cm^{-3}} - C_P^B/\mathrm{g \cdot cm^{-3}}}{C_S^T/\mathrm{g \cdot cm^{-3}} - C_S^B/\mathrm{g \cdot cm^{-3}}} \qquad (2)$$

where C_P and C_S are the polymer and salt concentrations, and the superscripts T and B refer to the top and bottom phases, respectively. Table 4 shows the STL values for PEG 4000 + salt aqueous systems.

According to Silva and Loh,[16] the interaction between polymer and salt is an endothermic event, and the temperature increment should be favorable to this reaction, leading to an

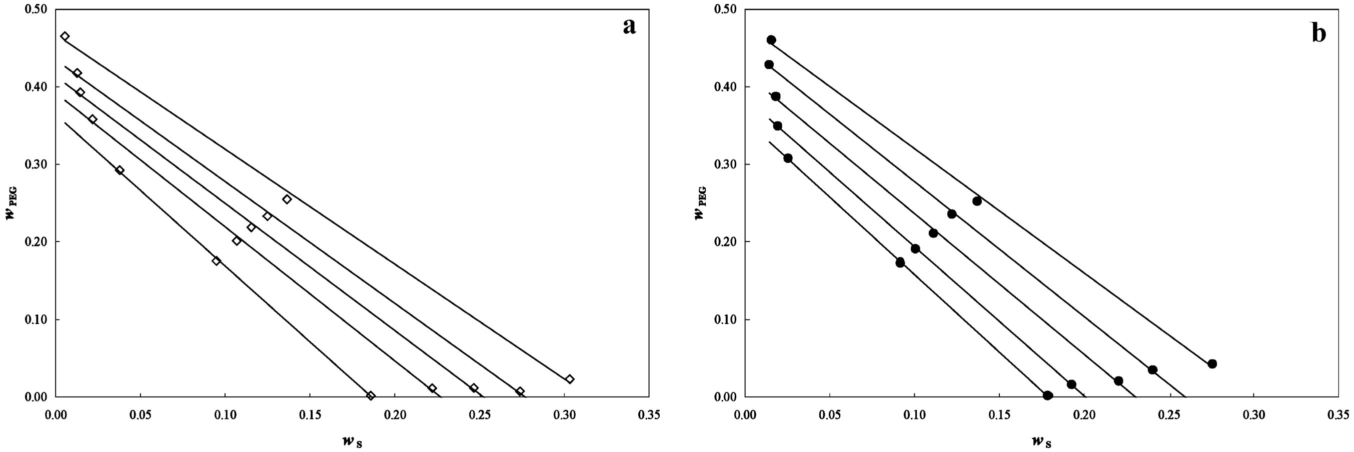

Figure 1. Liquid−liquid equilibrium data for PEG 4000 + potassium phosphate + water system from (a) 298.15 K to (b) 308.15 K: ◇, 298.15 K; ●, 308.15 K; −, tie line.

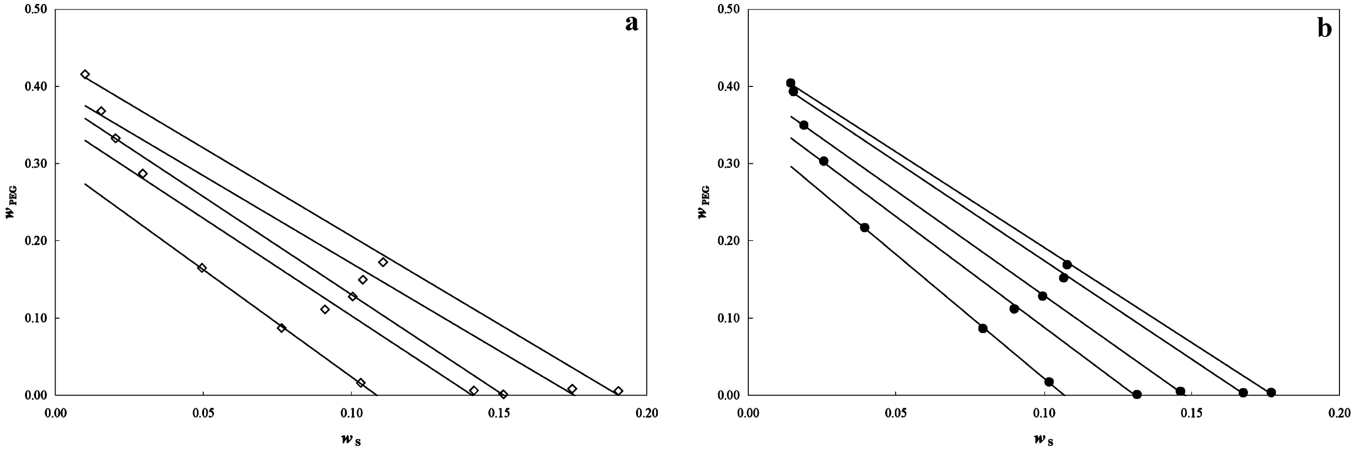

Figure 2. Liquid−liquid equilibrium data for PEG 4000 + sodium sulfate + water system from (a) 298.15 K to (b) 308.15 K: ◇, 298.15 K; ●, 308.15 K; −, tie line.

Table 3. Liquid−Liquid Equilibrium Data for PEG 4000 + Lithium Sulfate + Water System from (278.15 to 318.15) K

tie line	overall			top phase			bottom phase		
	w_{PEG}	w_S	w_W	w_{PEG}	w_S	w_W	w_{PEG}	w_S	w_W
				278.15 K					
1	0.2373	0.0745	0.6882	0.3282	0.0384	0.6334	0.0354	0.1447	0.8199
2	0.2485	0.0825	0.6690	0.3705	0.0351	0.5944	0.0381	0.1522	0.8097
3	0.2729	0.0921	0.6350	0.4427	0.0205	0.5368	0.0345	0.1929	0.7726
4	0.2957	0.1002	0.6041	0.4954	0.0142	0.4904	0.0324	0.1991	0.7685
5	0.3130	0.1112	0.5758	0.5754	0.0089	0.4157	0.0359	0.2278	0.7363
				278.15 K					
1	0.2426	0.0736	0.6838	0.3596	0.0352	0.6052	0.0263	0.1469	0.8268
2	0.2598	0.0815	0.6587	0.4102	0.0278	0.5620	0.0270	0.1667	0.8063
3	0.2846	0.0900	0.6254	0.4548	0.0225	0.5227	0.0324	0.1822	0.7854
4	0.3048	0.0992	0.5960	0.5042	0.0179	0.4779	0.0435	0.1922	0.7643
5	0.3433	0.1071	0.5496	0.5603	0.0120	0.4277	0.0434	0.2223	0.7343
				308.15 K					
1	0.2279	0.0687	0.7034	0.3290	0.0341	0.6369	0.0529	0.1072	0.8399
2	0.2553	0.0774	0.6673	0.4016	0.0240	0.5744	0.0268	0.1537	0.8195
3	0.2753	0.0868	0.6379	0.4547	0.0190	0.5263	0.0252	0.1658	0.8090
4	0.2950	0.0946	0.6104	0.4996	0.0136	0.4868	0.0384	0.1850	0.7766
5	0.3190	0.1045	0.5765	0.5470	0.0114	0.4416	0.0411	0.2074	0.7515
				318.15 K					
1	0.2281	0.0698	0.7021	0.3801	0.0229	0.5970	0.0017	0.1357	0.8626
2	0.2420	0.0730	0.6850	0.4088	0.0193	0.5719	0.0084	0.1537	0.8379
3	0.2590	0.0824	0.6586	0.4517	0.0175	0.5308	0.0021	0.1809	0.8170
4	0.2745	0.0906	0.6349	0.4970	0.0127	0.4903	0.0049	0.1996	0.7955
5	0.2927	0.0964	0.6109	0.5317	0.0106	0.4577	0.0139	0.2088	0.7773

increase in the amount of salt necessary for phase splitting. Nevertheless, the polymer chain rolls up with the rise of temperature, increasing its conformational entropy. As a result, the solubility of the polymer decreases and a transfer of water from the top to the bottom phase of the system occurs, reducing the concentration of salt in the bottom phase. Thus, the entropic

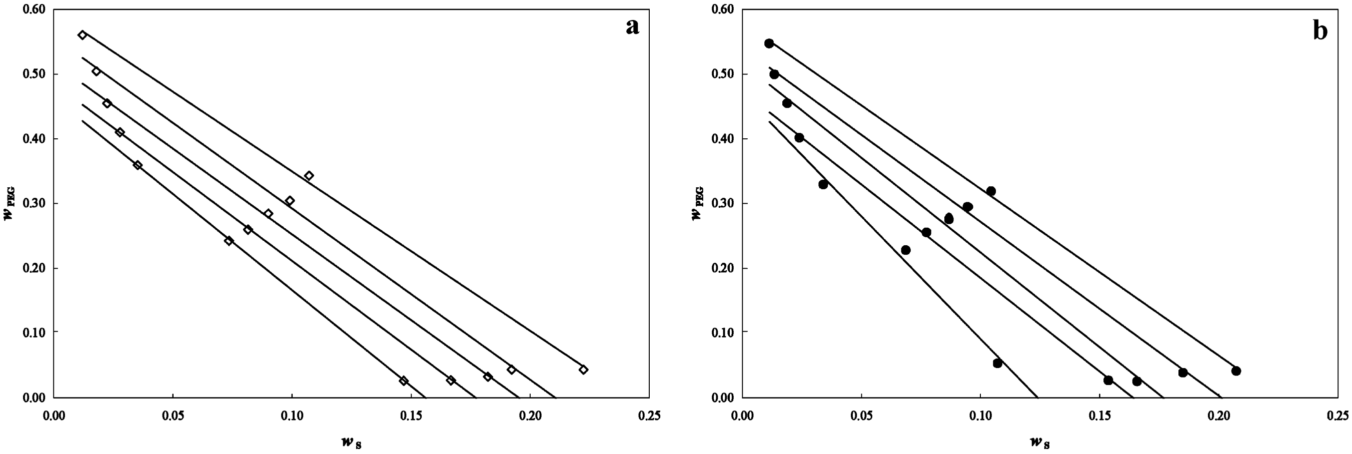

Figure 3. Liquid−liquid equilibrium data for PEG 4000 + lithium sulfate + water system from (a) 298.15 K to (b) 308.15 K: ◇, 298.15 K; ●, 308.15 K; —, tie line.

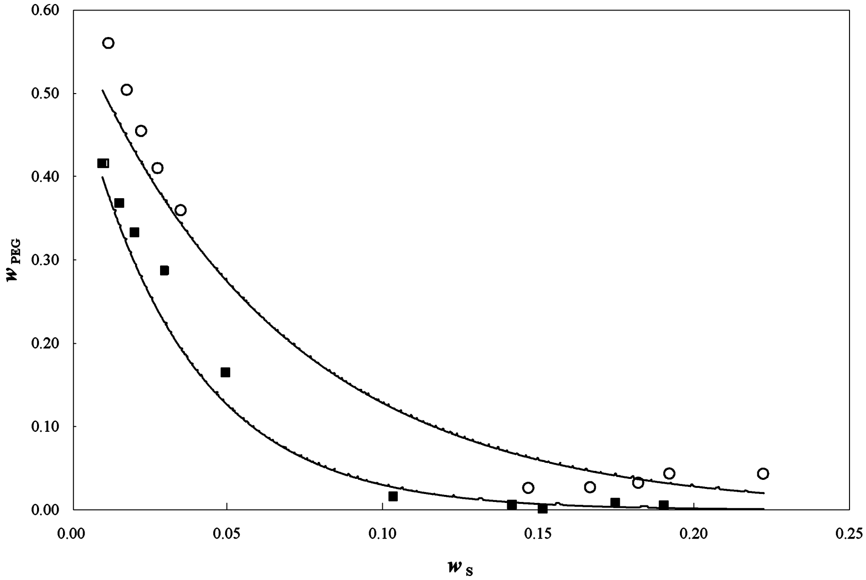

Figure 4. Influence of cationic species on the behavior of (■) PEG 4000 + sodium sulfate and (○) PEG 4000 + lithium sulfate aqueous systems at 298.15 K. —, binodal curve).

Table 4. STL Values for PEG 4000 + Salt + Water Systems

	T/K			
tie line	278.15	298.15	308.15	318.15
	PEG 4000 + Potassium Sulfate + Water			
1	0.0175	0.0204	0.0206	0.0235
2	0.0165	0.0185	0.0202	0.0225
3	0.0148	0.0174	0.0194	0.0199
4	0.0152	0.0161	0.0187	0.0190
5	0.0134	0.0157	0.0178	0.0185
	PEG 4000 + Sodium Sulfate + Water			
1	0.0219	0.0284	0.0308	0.0282
2	0.0207	0.0247	0.0276	0.0291
3	0.0192	0.0247	0.0269	0.0261
4	0.0191	0.0217	0.0251	0.0251
5	0.0179	0.0224	0.0233	0.0228
	PEG 4000 + Lithium Sulfate + Water			
1	0.0275	0.0298	0.0378	0.0336
2	0.0284	0.0276	0.0289	0.0298
3	0.0237	0.0265	0.0293	0.0275
4	0.0250	0.0264	0.0269	0.0263
5	0.0247	0.0246	0.0258	0.0261

contribution appears to prevail over the enthalpic contribution in the behavior of these systems.

For PEG 4000 + potassium phosphate ATPS, the STL increase became less pronounced with the rise of temperature. Between (278.15 and 298.15) K the STL was 14.0 %, while from (298.15 to 308.15) K and from (308.15 to 318.15) K the STL values were 10.0 % and 6.8 %, respectively.

For aqueous systems composed of PEG 4000 + sodium sulfate and PEG 4000 + lithium sulfate, a STL increase of 15.8 % and 9.4 % from (278.15 to 298.15) K and from (298.15 to 308.15) K, respectively, was observed. However, the STL showed a decrease of 3.6 % from (308.15 to 318.15) K. Such a value may be considered relatively small as compared to the STL values found for the other intervals of temperature analyzed.

Figure 4 exemplifies the influence of cationic species on the behavior of PEG 4000 + sodium sulfate and PEG 4000 + lithium sulfate aqueous systems. Sodium sulfate generally presented higher capability to induce phase separation for each temperature studied. Such a behavior may be explained by the difference between the size of the lithium and sodium radius. Lithium has a smaller radius, and consequently greater charge density, resulting in a stronger interaction between the polymer and the salt. As a result, more salt will be necessary for saturating the polymer chain and to induce the phase splitting.

The aqueous system composed by PEG 4000 + lithium sulfate presented a smaller biphasic area as compared to the system containing PEG 4000 + sodium sulfate. However, the

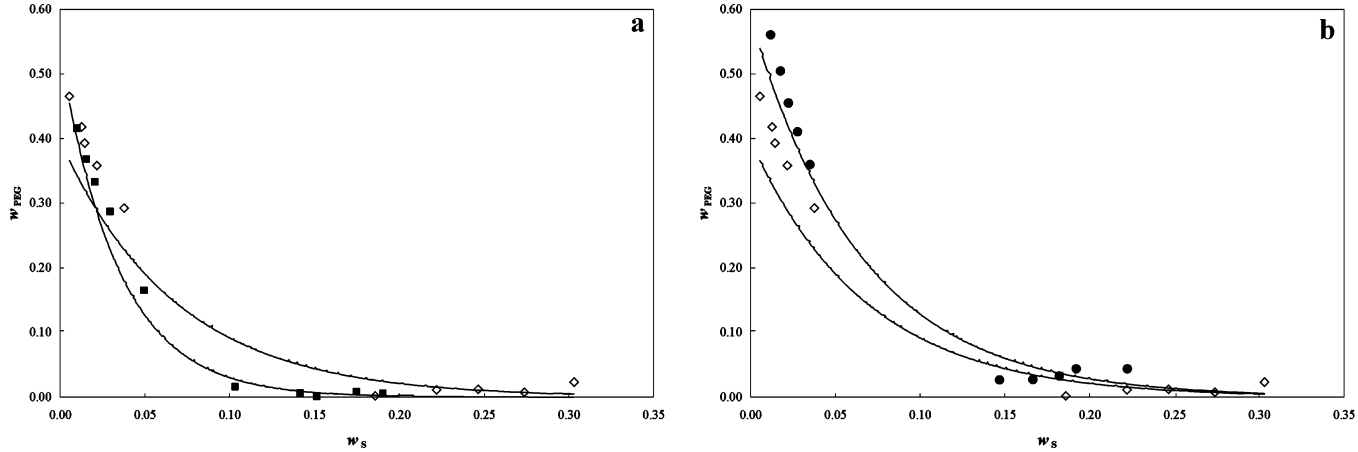

Figure 5. Influence of ionic species on the behavior of (a) (■) PEG 4000 + sodium sulfate and (◇) PEG 4000 + potassium phosphate; (b) (●) PEG 4000 + lithium sulfate and (◇) PEG 4000 + potassium phosphate aqueous systems at 298.15 K. —, binodal curve.

STL values determined for the system with lithium sulfate were larger than those found for the system containing sodium sulfate. The reduction of the ionic radius increased the STL and decreased the biphasic area. It shows that the increase of the concentration of polymer in the top phase was greater than the increase of the concentration of salt in the bottom phase, which may be explained by the transfer of water from the top to the bottom phase, increasing the STL.

The phase diagram for PEG 4000 + potassium phosphate aqueous system presented a biphasic area smaller than the system containing sodium sulfate and larger than the system with lithium sulfate (Figure 5). Such a behavior was also observed for potassium sulfate, as described by Ananthapadmanabhan and Goddard.[13] Phosphate shows higher capability to induce phase splitting than sulfate, as well as sodium presents higher capability than potassium. Consequently, the sodium (from sodium sulfate) prevails over the phosphate (from potassium phosphate) in the formation of ATPS.

Cationic and anionic species have a similar influence on the behavior of ATPS. Ionic species with smaller radii show greater charge density, facilitating their interaction with the polymer chain, and consequently increasing the amount of salt required for inducing the phase separation.

Sé and Aznar,[10] Ananthapadmanabhan and Goddard,[13] Hammer et al.,[14] and Zafarani-Moattar and Gasemi[18] also analyzed the influence of the temperature and ionic species on the behavior of ATPS composed of PEG and salt. The systems studied by these authors presented similar behavior to those analyzed in this work. Sé and Aznar[10] studied the phase equilibrium of PEG 4000 + potassium phosphate aqueous systems at (283.15, 293.15, and 303.15) K. Ananthapadmanabhan and Goddard[13] analyzed aqueous systems constituted by PEG 3350 + magnesium sulfate, PEG 3350 + sodium sulfate, and PEG 3350 + sodium phosphate. Hammer et al.[14] studied aqueous systems containing PEG 1550 + sodium sulfate, PEG 3000 + sodium sulfate, and PEG 6000 + sodium sulfate at (293.15, 303.15, and 313.15) K. Zafarani-Moattar and Gasemi[18] measured equilibrium data for PEG 6000 + ammonium dihydrogen phosphate and PEG 6000 + diammonium hydrogen phosphate aqueous systems at (298.15, 308.15, and 318.15) K.

Conclusions

The present work analyzed the influence of the temperature and type of salt on the LLE of ATPS composed of PEG 4000 + sodium sulfate, PEG 4000 + lithium sulfate, and PEG 4000 + potassium phosphate from (278.15 to 318.15) K. The results agreed well with the literature. The increase of the temperature generally led to an increase of the STL, reducing the quantity of salt necessary for phase splitting, which was shown by the enlargement of the biphasic area and the increase of the STL. The salt influence is related to the radius size of the ionic species. In spite of producing a phase diagram with a larger biphasic area, the potassium sulfate showed higher capability for inducing phase splitting as compared to potassium phosphate and lithium sulfate.

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