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Effect of Aqueous Two-Phase System Constituents in Different Poly(ethylene glycol)–Salt Phase Diagrams

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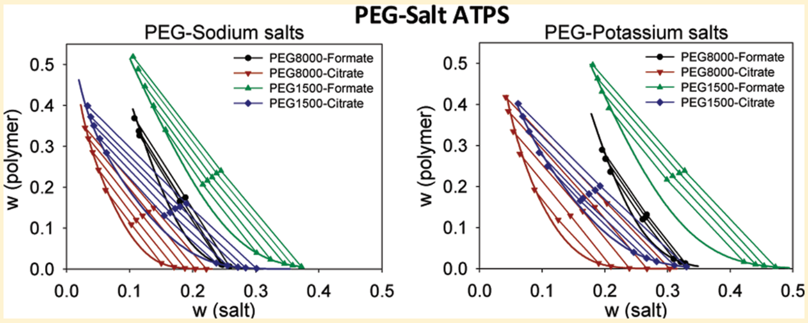

ABSTRACT: Eight different poly(ethylene glycol) (PEG)/salt aqueous two-phase systems (ATPS's) were characterized at 23 °C. PEGs with different molecular weights (1500 and 8000) were combined with different salts (sodium or potassium citrates and formates) to study the effect of each ATPS constituent in the phase diagram. The binodal curve and several tie-lines were determined for each system using common analytical techniques described in the literature for ATPS's (cloud point method, conductivity, and gravimetric analysis after lyophilization). According to the results, it was found that each ATPS constituent produced a particular effect in the phase diagram. For the salts, changes in the anion produced larger variations in the heterogeneous region size than changes of the cation. Thus, the effect of salt type seems to be largely dependent on the anion, which makes the dominant contribution. The ability to form ATPS's is connected with the Gibbs energy of hydration, ΔG_{hyd}, of the anions: more negative ΔG_{hyd} values facilitate ATPS formation. Changes in the PEG molecular weight affected also the phase diagram: the size of the heterogeneous region increases with the increase in the polymer molecular weight.

■ INTRODUCTION

Aqueous two-phase systems (ATPS's) are biphasic mixtures where both phases are composed mainly (70 to 90 %) by water. They can be obtained by mixing in water two water-soluble polymers (polymer–polymer ATPS) or a polymer and an inorganic salt (polymer–salt ATPS).[1−3] Other ATPS's have been obtained using surfactants or, recently, ionic liquids.[4,5] As biphasic systems, they can find applications for liquid extraction processes. Indeed, ATPS's are well-known in biotechnology for the recovery of biomolecules (proteins, enzymes, antibodies, DNA, and RNA) or even whole cells and viruses.[6−9] The reasons for this lay on the aqueous nature of both phases, which provides a benign environment for biomolecules or even living organisms. Other applications of ATPS's include the separation of metal ions or pollutants.[10,11]

The choice among polymer–polymer or polymer–salt ATPS's is mainly driven by the recovery yield and selectivity for the target solute and price of the ATPS constituents. As inorganic salts are, in general, cheaper than polymers, polymer–salt ATPS's are favored in the economic aspect. Poly(ethylene glycol) (PEG) is a hydrophilic polymer largely used to form ATPS's due to its low price and biodegradability. This polymer can be combined both with other polymers or salts to obtain biphasic systems. PEGs (phosphate or sulfate salts) are the most common polymer–salt ATPS's found in the literature.[2,12,13] However, the use of these salts in large scale can cause some environmental problems. For this reason, Vernau and Kula[14] proposed citrate salts as benign substitutes for phosphates and sulfates. Citrates and formates are organic anions that can be used to produce ATPS's with better performance regarding environmental and toxicity issues.

In this work, the phase diagrams for eight PEG–salt ATPS's were studied at 23 °C. The ATPS's were obtained combining PEG of two different molecular weights (M_w = 1500 and 8000) with four salts: sodium and potassium citrates and formates. The binodal curves and several tie-lines are reported. Besides, results allow the comparison of the effect of polymer molecular weight and the effect of both anions and cations in the size of the heterogeneous region and in the slope of the tie-lines of ATPS's.

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Table 1. Number of Solubility Points Used to Determine Each Binodal Curve (*N*), together with the Adjustable Parameters (*a*, *b*, and *c*) Obtained from Merchuk Equation (Equation 1) and Respective Coefficients of Determination (r^2)

system	*N*	*a*	*b*	*c*	r^2
PEG8000−sodium citrate	25	0.955 ± 0.028	−5.73 ± 0.17	581 ± 29	0.9993
PEG8000−sodium formate	25	1.24 ± 0.33	−2.76 ± 0.85	226 ± 18	0.9976
PEG8000−potassium citrate	25	1.73 ± 0.12	−6.69 ± 0.30	286 ± 21	0.9985
PEG8000−potassium formate	20	1.66 ± 1.42	−2.13 ± 2.20	103 ± 16	0.9956
PEG1500−sodium citrate	43	0.826 ± 0.011	−4.147 ± 0.066	151 ± 6	0.9994
PEG1500−sodium formate	47	1.338 ± 0.090	−2.79 ± 0.20	73 ± 3	0.9986
PEG1500−potassium citrate	42	1.111 ± 0.049	−4.10 ± 0.16	87 ± 4	0.9987
PEG1500−potassium formate	37	1.79 ± 0.17	−2.51 ± 0.23	41 ± 1	0.9995

EXPERIMENTAL SECTION

Chemicals. Poly(ethylene glycol) PEG1500 (average molecular weight 1500, for synthesis) was obtained from Merck. Potassium citrate tribasic monohydrate ($K_3C_6H_5O_7 \cdot H_2O$, purum p.a., ≥ 99.0 %) was purchased from Fluka Analytical. Poly(ethylene glycol) PEG8000 (average molecular weight 8000), sodium citrate tribasic dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, ACS reagent, ≥ 99.0 %), potassium formate ($KCHO_2$, purum p.a.), and sodium formate ($NaCHO_2$, puriss. p.a. ACS; ≥ 99.0) were supplied by Sigma-Aldrich. All of the chemicals were used as received, without further purification.

Stock solutions for all chemicals were prepared in deionized water (ca. 40 wt % for potassium and sodium citrates, 70 wt % for potassium formate, 50 wt % for sodium formate, 80 wt % for PEG1500, and 55 wt % for PEG8000). Exact concentrations were obtained gravimetrically after evaporation on a heating plate (Stuart hot plate SB300) for salts or after lyophilization (Scan Vac, model CoolSafe 55-4) for polymers. All weighing was carried out on an Adam Equipment balance model AAA250L, precise to within ± 0.2 mg. Deionized water (electrical conductivity <1 $\mu S \cdot cm^{-1}$) was used for all diluting purposes.

Methods. *Binodal.* The binodal curves were obtained experimentally using the cloud point method. For this purpose, different biphasic systems, with known polymer and salt compositions, were prepared by weight in assay tubes. The tubes were vigorously shaken in a vortex mixer (VWR, model VV3) and placed into a thermostatic bath (Techne, Tempette TE 8D) at 23 ± 0.2 °C. Known amounts of water were successively added to the tubes until one homogeneous phase was obtained. After each addition of water, the tubes were shaken and placed back into the thermostatic bath for phase separation. The binodal curves were determined for several compositions corresponding to the formation of homogeneous systems. Each experimental binodal curve was adjusted to the empirical equation suggested by Merchuk and co-workers:[15]

$$Y = a \exp(b \cdot X^{0.5} - c \cdot X^3) \tag{1}$$

where *Y* and *X* are the polymer and salt compositions in mass fraction, respectively, and *a*, *b*, and *c* are adjustable parameters. These parameters were obtained by nonlinear regression (least-squares) using the SigmaPlot v5.0 (SPSS Inc.) software.

Tie-Lines. Several tie-lines were determined for each PEG−salt system. The tie-line determination was performed using a methodology previously described.[16] Biphasic systems with known compositions of PEG and salt were prepared by weight in 15 mL tubes, vigorously shaken, and then allowed to equilibrate for 48 h, in a thermostatic bath (Grant, LTC1, GD120) at 23 ± 0.1 °C. Then, for each tie-line, samples from the top and bottom phases were withdrawn and conveniently diluted for analysis, to determine the composition of the equilibrium phases. The salt concentration was measured in both phases, at 23 °C, by electrical conductivity using a WTW LF538 conductivity meter with a standard conductivity cell TetraCon 325 (a 4-electrode conductivity cell) precise to within ± 0.5 %. Calibration curves were obtained for each salt, from the electrical conductivities of standard solutions with known concentrations within the range (0.01 to 0.2) wt %. Polymer interference in the electrical conductivity measurements was tested for the range (0.01 to 0.5) wt %, and no polymer interferences were observed (average errors in salt concentration, calculated using the calibration lines, were below 3 % for all salts used). The final salt concentration for each phase was obtained from the average of three measurements (standard deviations less than 0.5 %), after convenient correction with the dilution factor. The polymer concentration in the top phase was assessed gravimetrically after lyophilization: three samples of the top phase were taken, diluted with water (1:4), and frozen at −18 °C for 24 h. The samples were then freeze-dried, and the average dry weight was determined (standard deviation less than 0.2 %). The final polymer concentration for the top phase was calculated by subtraction of the salt concentration from the average dry weight. The polymer concentration in the bottom phase was obtained using the salt concentration previously determined and the Merchuk equation. The tie-line lengths (TLL) were calculated using the equation:

$$\mathrm{TLL} = [(X_{\mathrm{top}} - X_{\mathrm{bottom}})^2 + (Y_{\mathrm{top}} - Y_{\mathrm{bottom}})^2]^{1/2} \tag{2}$$

The slopes of the tie-lines (STL) were obtained from linear regression of the top, bottom, and feed compositions of the tie-lines.

RESULTS AND DISCUSSION

PEGs with different molecular weights (1500 or 8000) were combined with different salts (sodium or potassium citrates and formats) to study the effect of each ATPS constituent in the phase diagram. For each biphasic system, the experimental data obtained for the binodal curve were successfully adjusted to the Merchuk equation. The values determined for *a*, *b*, and *c* parameters and the correlation coefficient (r^2) are presented in Table 1.

Salt and polymer compositions determined for the ends of the tie-lines are presented in Table 2, together with the feed composition, TLL, and STL. Slight variations in STLs were observed for the different tie-lines of a given ATPS. For all biphasic systems, it was found that the absolute value of the STL decreases as the TLL increases. It was also noticed that

Table 2. Feed, Top, and Bottom Compositions (in Mass Fraction) Measured for Tie-Lines of All ATPS's Studied, together with the Corresponding Tie-Line Length (TLL) and Slope (STL)

feed		top phase		bottom phase			
salt	polymer	salt	polymer	salt	polymer	STL	TLL
			PEG8000−Sodium Citrate				
0.1027	0.1091	0.062	0.193	0.150	0.014	−2.01	0.199
0.1105	0.1201	0.051	0.242	0.170	0.005	−1.98	0.266
0.1206	0.1305	0.041	0.286	0.188	0.002	−1.94	0.320
0.1299	0.1397	0.035	0.319	0.204	0.0005	−1.88	0.361
0.1382	0.1498	0.029	0.345	0.222	0.0001	−1.79	0.395
			PEG8000−Sodium Formate				
0.1796	0.1654	0.116	0.326	0.245	0.011	−2.43	0.341
0.1824	0.1687	0.115	0.337	0.247	0.010	−2.46	0.353
0.1885	0.1749	0.108	0.369	0.258	0.006	−2.40	0.392
			PEG8000−Potassium Citrate				
0.1255	0.1188	0.088	0.193	0.190	0.013	−1.74	0.207
0.1450	0.1295	0.064	0.280	0.210	0.006	−1.88	0.310
0.1645	0.1404	0.054	0.334	0.239	0.001	−1.79	0.381
0.1842	0.1502	0.046	0.384	0.267	0.00023	−1.73	0.443
0.2036	0.1593	0.042	0.418	0.304	0.00015	−1.60	0.493
			PEG8000−Potassium Formate				
0.2605	0.1201	0.209	0.236	0.310	0.024	−2.10	0.235
0.2652	0.1250	0.201	0.266	0.321	0.017	−2.09	0.278
0.2669	0.1313	0.196	0.289	0.329	0.013	−2.08	0.307
			PEG1500−Sodium Citrate				
0.1549	0.1304	0.063	0.284	0.237	0.014	−1.55	0.323
0.1640	0.1377	0.053	0.319	0.255	0.008	−1.54	0.371
0.1723	0.1476	0.043	0.351	0.271	0.005	−1.52	0.414
0.1802	0.1521	0.038	0.372	0.284	0.003	−1.51	0.443
0.1900	0.1601	0.033	0.399	0.305	0.001	−1.49	0.479
			PEG1500−Sodium Formate				
0.2161	0.2065	0.157	0.340	0.301	0.039	−2.07	0.334
0.2232	0.2163	0.138	0.399	0.323	0.023	−2.02	0.419
0.2309	0.2242	0.124	0.446	0.345	0.013	−1.96	0.487
0.2378	0.2342	0.114	0.488	0.358	0.009	−1.96	0.539
0.2448	0.2403	0.105	0.519	0.372	0.006	−1.92	0.578
			PEG1500−Potassium Citrate				
0.1607	0.1651	0.109	0.250	0.246	0.040	−1.52	0.251
0.1647	0.1711	0.095	0.282	0.267	0.026	−1.49	0.309
0.1747	0.1818	0.079	0.334	0.283	0.018	−1.55	0.376
0.1845	0.1911	0.069	0.371	0.311	0.008	−1.50	0.436
0.1923	0.2013	0.062	0.402	0.330	0.005	−1.48	0.479
			PEG1500−Potassium Formate				
0.2991	0.2168	0.206	0.391	0.422	0.016	−1.73	0.432
0.3082	0.2255	0.195	0.431	0.440	0.010	−1.71	0.487
0.3188	0.2330	0.188	0.463	0.454	0.007	−1.72	0.528
0.3274	0.2384	0.181	0.496	0.472	0.004	−1.69	0.572

PEG−formate salt ATPS's provided always higher absolute values of STL than the corresponding PEG−citrate salts.

Phase diagrams determined for PEG−salt ATPS's are presented in Figures 1 and 2. It can be seen from these figures that each ATPS constituent produced a particular effect in the phase diagram. The change in PEG molecular weight produced obvious modifications in the phase diagram. For the salts, the change of anion produced more significant effects than the change of cation. These effects of each ATPS constituent are discussed separately ahead. It was found that PEG8000−sodium citrate ATPS's provided the highest heterogeneous region, while PEG1500−potassium formate provided the smallest one. The influence of the PEG molecular weight in the binodal is connected with polymer solubility in water. On the other hand, the effect produced in binodal by anions and cations can be related to their charge and free energy of hydration (ΔG_{hyd}), as will be discussed below.

Cation Effect. The comparison between sodium and potassium ATPS's shows that PEG−potassium salt ATPS's present smaller heterogeneous regions than the corresponding PEG−sodium salts. This means that higher concentrations of potassium salts are needed to obtain the ATPS. Therefore, Na^+ is more effective in ATPS formation than K^+. A similar behavior was previously reported in literature.[16−18] A thermodynamic approach using the Hofmeister series and the ΔG_{hyd} has been used to explain this fact. In the Hofmeister series, salts are ordered from kosmotropic to chaotropic, based on their capacity to induce salting-out.[19] According to this series, the

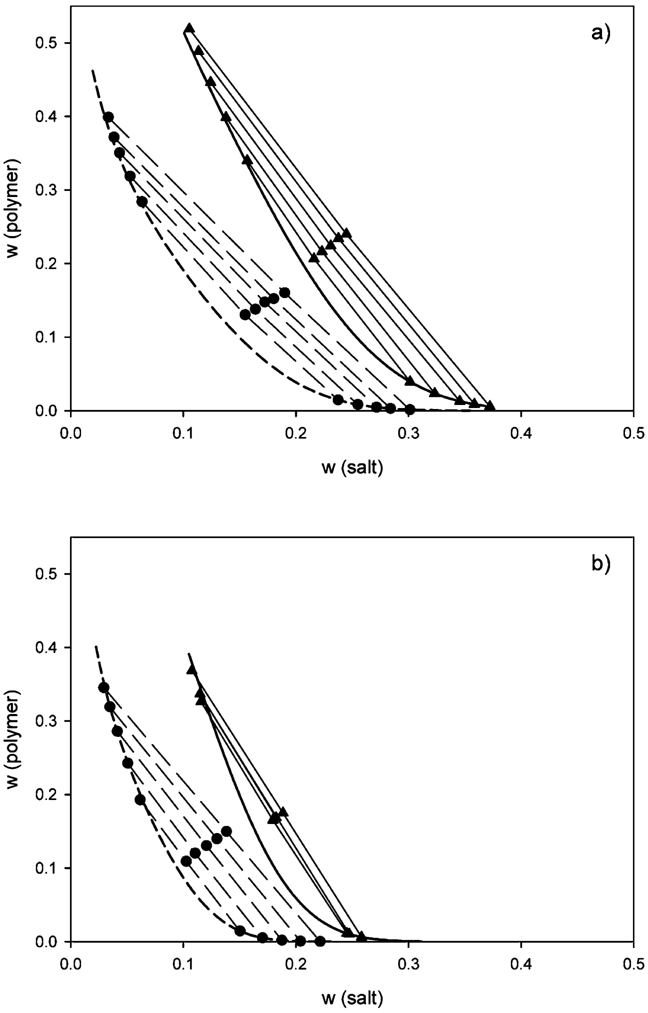

Figure 1. Binodal curve and tie-lines obtained for the systems PEG–sodium formate (–▲–) and PEG–sodium citrate (- - ● - -) at 23 °C: (a) PEG 1500; (b) PEG 8000. All compositions are in mass fraction.

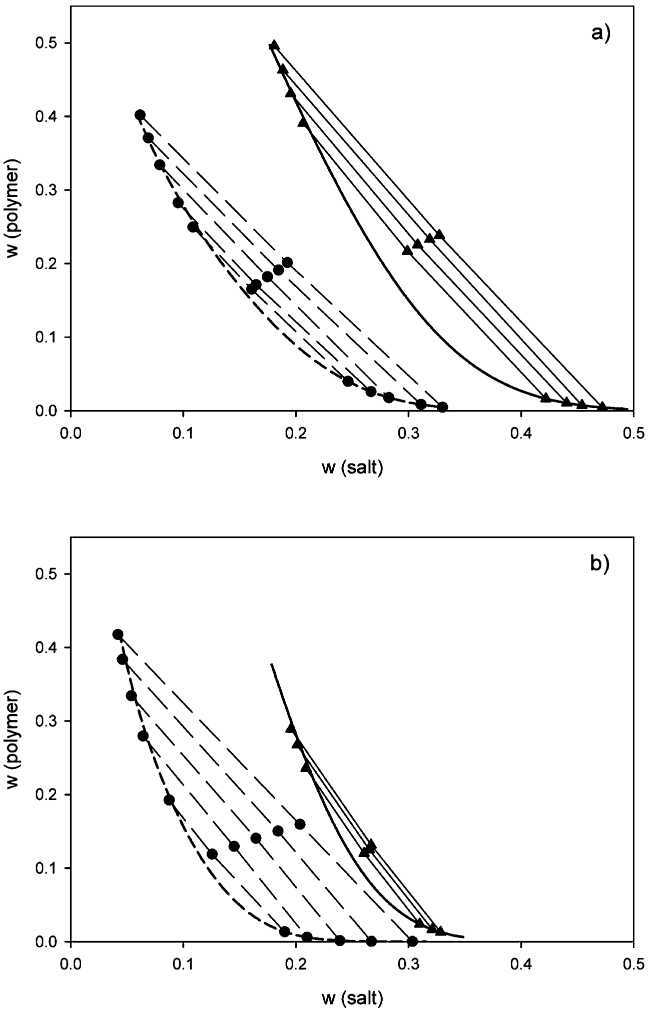

Figure 2. Binodal curve and tie-lines obtained for the systems: PEG–potassium formate (–▲–) and PEG–potassium citrate (- - ● - -) at 23 °C: (a) PEG 1500; (b) PEG 8000. All compositions are in mass fraction.

salting-out effect of Na^+ is larger than K^+. Consequently Na^+ is expected to present more ability to form ATPS's than K^+. An analogous analysis can be done using the ΔG_{hyd} values previously determined by Marcus: −295 kJ·mol^{-1} for K^+ and −365 kJ·mol^{-1} for Na^+.[20] More negative ΔG_{hyd} indicates a larger hydration shell and consequently a reduction in the amount of water available to hydrate the polymer, contributing to the salting-out of PEG.

Anion Effect. As expected, the change of the anion produced a more evident modification in the phase diagram than the change of the cation. Anions are known to affect phase separation more than the cations.[21,22] Inspection of Figures 1 and 2 demonstrates that PEG–formate salt ATPS's presented smaller heterogeneous regions than the corresponding PEG–citrate salts. This means that a lower concentration of citrate salt is needed to obtain the ATPS. For this reason, citrates are more effective in ATPS formation than formates. The tendency of the anions to form ATPS is closely connected with the position of the anion in the Hoffmeister series. Once again, this behavior can be explained using the ΔG_{hyd} values found in the literature for the anions: −2793 kJ·mol^{-1}[23] for $C_6H_5O_7^{3-}$ and −395 kJ·mol^{-1}[20] for CHO_2^-. The more negative ΔG_{hyd} value for citrate indicates that this anion has a larger hydration shell and consequently the salting-out of the PEG is achieved using a lower salt concentration. For the anions, the valence of each anion can also be related to the ability to form ATPS's. It was observed that the higher the valence of the anion, the lower the concentration required to obtain a biphasic system.[21] The extent of a ionic species hydration (and ΔG_{hyd}) depends upon the valence of the ion. Multivalent anions compete more strongly for water, so the salting-out of the polymer will be facilitated.

PEG Molecular Weight Effect. When the molecular weight of the PEG was increased, biphasic formation occurred at lower concentrations of polymer and salt. Larger heterogeneous regions were observed for PEG8000–salt than for PEG1500–salt ATPS's. PEG with higher molecular weights is more hydrophobic, and its solubility in water decreases, leading to the polymer salting-out. Similar conclusions were previously found by other authors.[1,2]

Some references were found in the literature for PEG–citrate salt ATPS's utilizing polymer molecular weights and temperatures close to those used in this work. Marcos and co-workers[24] reported graphically the effect of PEG molecular weight in the phase diagram, at 20 °C. As could be observed in our work, the increase of PEG molecular weight produced larger biphasic regions. Besides, the binodal curve presented for

PEG8000−sodium citrate at 20 °C was in agreement with our binodal at 23 °C. Perumalsamy and co-workers[25] reported the binodal curve and six tie-lines for PEG6000−sodium citrate at 20 °C. The binodal curve is in agreement with ours and Marcos and co-workers,[24] although tie-lines present a similar length (TLL) but slightly different slopes (STL). The same behavior was observed for the binodal and tie-lines presented by Zafarani-Moattar and co-workers[26] for PEG6000−sodium citrate at 25 °C. The similitude found for all these binodal curves is presented in Figure 3 and can be explained by the polydispersity of the polymer, as previously noted for PEG−potassium phosphate[27] and PEG−sodium tartrate[28] ATPS's. Our results obtained for PEG1500−sodium citrate were also compared with the literature. Alves et al.[29] and Patrício et al.[30] determined several tie-lines for PEG1500−sodium citrate at 25 °C. Despite differences in STL, the end of the tie-lines coincided with our binodal curve. On the contrary, tie-lines reported by Oliveira et al.[31] for PEG1500−sodium citrate at 35 °C presented slopes very close to ours but the end of tie-lines are a little distant from our binodal, probably due to the higher temperature used. Murugesan and Perumalsamy[32] presented the binodal and four tie-lines for PEG2000−sodium citrate at 25 °C, and it is possible to observe that our results are very close to theirs both for the binodal and STL. Once more, the polydispersity of the PEG may be the main reason for this coincidence.

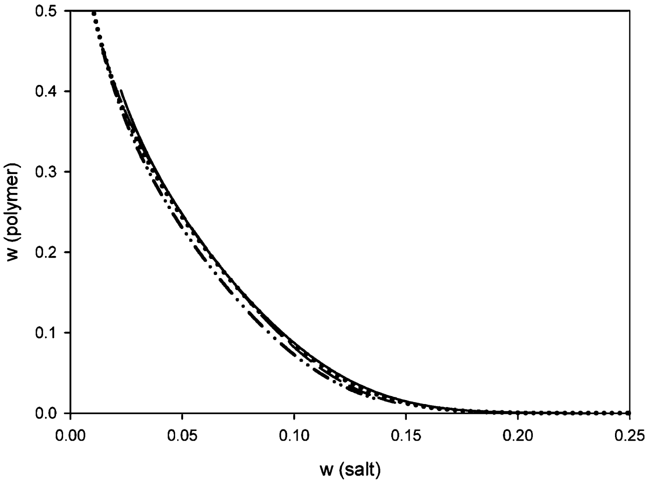

Figure 3. Comparison between binodal curve determined in this work for PEG8000−sodium citrate (—) and binodals obtained from literature: - - -, PEG8000−sodium citrate at 20 °C obtained graphically from ref 24; ···, PEG6000−sodium citrate at 20 °C from ref 25; —·· —, PEG6000−sodium citrate at 25 °C from ref 26. All compositions are in mass fraction.

For PEG8000−potassium citrate, no references were found in literature. However, Zafarani-Moattar et al.[33] reported the binodal and five tie-lines for PEG6000−potassium citrate at 25 °C that can be compared with our results: binodals are similar, but STL presented some differences. Yan-Min et al.[34] presented graphically the binodal curve for PEG−potassium citrate at 30 °C using PEG with different molecular weights. A qualitative comparison between those results obtained for PEG6000−potassium citrate and our binodal for PEG8000−potassium citrate showed that a small shift to the left is noted for PEG6000−potassium citrate. This variation can be due to the higher temperature used in that work and is also evident if we compare our binodal for PEG1500−potassium citrate with those reported for PEG1000 and PEG2000−potassium citrate. Slight differences were also found in literature for the binodal determined by Jayapal et al.[35] for PEG2000−potassium citrate at 25 °C and our results for PEG1500−potassium citrate.

The only reference in literature for PEG−formate salts reports the binodal and five tie-lines obtained for PEG6000−sodium formate at 25 °C.[36] Comparing those results with our binodal and tie-lines for PEG8000−sodium formate, we noticed that binodals are coincident and only slight variations were found for the STL.

■ CONCLUSIONS

Binodal curves and tie-lines were determined for eight different PEG−salt ATPS's, at 23 °C. For all of the ATPS's, the experimental binodal curve was successfully adjusted to the Merchuk equation. The ends of tie-lines were achieved using the typical techniques for ATPS's, like electrical conductivity and lyophilization. Changing the cation or anion of the salts and the molecular weight of the PEG, we found that each constituent caused a particular effect on the phase diagram. The effect of salt type seems to be largely dependent on the anion, which makes the dominant contribution, and it is related to its ΔG_{hyd}. A more negative ΔG_{hyd} indicates a higher capacity to form biphasic systems, and thus a lower salt concentration is needed to obtain ATPS's. For PEG, the increase in the molecular weight produced an increase in the heterogeneous region, and thus lower salt and polymer concentrations were needed to form the ATPS. According to the results, the PEG8000−sodium citrate ATPS provided the highest heterogeneous region, while PEG1500−potassium formate provided the smallest one.

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Notes

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