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The Effect of Salts on the Liquid−Liquid Phase Equilibria of PEG600 + Salt Aqueous Two-Phase Systems

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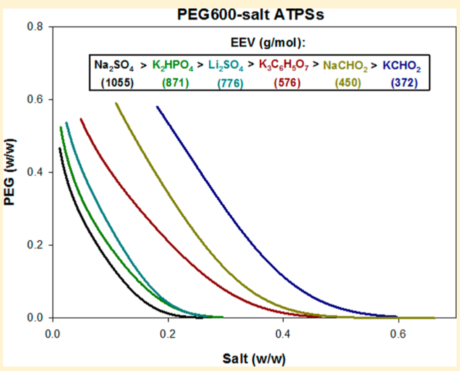
S *Supporting Information*

ABSTRACT: Six new ATPSs were prepared by combining polyethylene glycol PEG600 with potassium citrate, dipotassium hydrogen phosphate, sodium formate, potassium formate, sodium sulfate, and lithium sulfate. Complete phase diagrams, including the binodal curve and three tie-lines, were determined at 23 °C. The experimental data obtained for the binodal curve were successfully adjusted to the Merchuk equation, and the reliability of tie-line data was confirmed using the equations suggested by Othmer−Tobias and Bancroft. The ability of each ion to induce ATPS formation was investigated. Na^+ proved to be more effective in ATPS formation than K^+ and Li^+. For potassium salts, the order observed for the effectiveness of the anions was: $HPO_4^{2-} > C_6H_5O_7^{3-} > HCO_2^-$. Regarding the sodium salts, it was found that SO_4^{2-} is clearly more effective than HCO_2^-. The position of the ions in the Hofmeister series and their free energy of hydration (ΔG_{hyd}) were used to explain the ability of the ions to induce PEG salting-out. Furthermore, the effective excluded volume (EEV) of the salts was determined and the following order was found: $Na_2SO_4 > K_2HPO_4 > Li_2SO_4 > K_3C_6H_5O_7 > NaCHO_2 > KCHO_2$. Similar order was obtained when analyzing the size of the heterogeneous regions, suggesting the practical use of EEV as a comparison parameter between different ATPSs.



1. INTRODUCTION

Aqueous two-phase systems (ATPSs) can be obtained by mixing two aqueous solutions of different constituents that become partially immiscible above certain critical conditions, like temperature or concentration.[1] Both phases have high percentages of water and each one is enriched in a different component. The predominant component in each phase may vary according to the type of ATPS prepared. Since their discovery at the end of 19th century, many ATPSs have been characterized. ATPSs formed by a polymer−polymer or polymer−salt represent the traditional systems.[1] However, alternative biphasic systems can be obtained using surfactants or ionic liquids.[2,3]

The different types of ATPSs offer different features that can be used to selectively separate several kinds of solutes, namely biological compounds. Since water is the solvent in both phases, ATPSs can supply suitable and nondestructive conditions for separation and recovery of labile biomolecules (proteins, enzymes, DNA, or RNA)[4−6] and also biological particles (cells, organelles, bacteria, or viruses).[7,8] Besides the mild conditions, there are other important features of ATPSs like the easiness of operation and scale-up, robustness, and the possibility of direct application from different biological sources (biological suspensions, fermentations broths, and commercial sources).[9]

At the industrial level, polymer−salt ATPSs can offer some advantages over polymer−polymer ATPSs, namely, lower costs and viscosities (which improve mass-transfer). Polyethylene glycol (PEG) is a relatively inexpensive polymer, with high solubility in water, and is considered nontoxic, nonflammable, and nonvolatile.[10] This polymer is unquestionably the most used for the preparation of polymer−salt systems. Indeed, numerous references can be found in the literature describing and characterizing PEG−salt biphasic systems.[1,11] Phosphate and sulfate salts are the most widely used in PEG−salt ATPSs formation. Nevertheless, some attention has to be paid to these salts, especially when used at industrial level, because they can cause environmental problems if directly released into natural ecosystems. Salt recycling can solve this problem and, at the same time, reduce the costs associated with wastewater treatment. However, in some cases salt recycling is not economically feasible. For this reason, the use of salts which are described as biodegradable and do not cause additional problems in wastewater treatment has been proposed. Examples of these salts are citrates, which are easily degraded,[12] and formates, also described as contributors to better performance regarding environmental and toxicity issues.[13]

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In this work, the phase diagrams for six new PEG−salt ATPSs were experimentally determined at 23 °C. The ATPSs were obtained combining PEG600 with typical (phosphates, sulfates) and biodegradable (citrates, formates) salts: potassium citrate, dipotassium hydrogen phosphate, sodium formate, potassium formate, sodium sulfate and lithium sulfate. The binodal curve and three tie-lines are reported for each biphasic system. The results obtained allow the comparison of the effect of anions and cations on the size of the heterogeneous region and in the slope of the tie-lines of ATPSs. The salting-out effect of the salts was described by means of the Gibbs energy of hydration (ΔG_{hyd}) and the effective excluded volume (EEV).

2. MATERIALS AND METHODS

2.1. Chemicals. Polyethylene glycol PEG600 (for synthesis, average molecular weight 600) was obtained from Merck (Germany). Potassium citrate tribasic monohydrate ($K_3C_6H_5O_7 \cdot H_2O$, purum p.a., ≥ 99.0%) and lithium sulfate (Li_2SO_4, purum p.a., > 98%) were purchased from Fluka Analytical (USA). Potassium formate ($KCHO_2$, purum p.a.) and sodium formate ($NaCHO_2$, puriss. p.a. ACS; ≥ 99.0) were supplied by Sigma-Aldrich (USA). Sodium sulfate (Na_2SO_4, anhydrous, GR for analysis, > 99.0%), was obtained from Merck (Germany). Dipotassium hydrogen phosphate (K_2HPO_4, anhydrous, PA) was purchased from Panreac (Spain). All the chemicals were used as received, without further purification.

Stock solutions for all salts were prepared in deionized water (*ca.* 100 w = 50 for potassium citrate, 100 w = 30 for lithium sulfate, 100 w = 70 for potassium formate, 100 w = 50 for sodium formate, 100 w = 25 for sodium sulfate, and 100 w = 30 for dipotassium hydrogen phosphate). Exact concentrations were obtained gravimetrically after evaporation on a heating plate (Stuart hot plate SB300). PEG600 is a low viscosity liquid at 23 °C, thus it was used as received and no stock solution was prepared. All weighing was carried out on an Adam Equipment balance model AAA250L, precise to within ± 0.2 mg. Deionized water (electrical conductivity < 1 μS·cm^{-1}) was used for all diluting purposes.

2.2. 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:[14]

$$W_{\text{PEG}} = a \exp[b(W_{\text{salt}})^{0.5} - c(W_{\text{salt}})^3] \tag{1}$$

where W_{PEG} and W_{salt} 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).

Additionally, the experimental data obtained by the cloud point method were fitted to the statistical geometrical relationships described by Guan et al.[15,16] for aqueous two-phase systems:

$$\ln\left(V^* \frac{w_{\text{PEG}}}{M_{\text{PEG}}}\right) + V^* \frac{w_{\text{salt}}}{M_{\text{salt}}} = 0 \tag{2}$$

$$\ln\left(V^* \frac{w_{\text{PEG}}}{M_{\text{PEG}}} + f\right) + V^* \frac{w_{\text{salt}}}{M_{\text{salt}}} = 0 \tag{3}$$

where M_{PEG} and M_{salt} are the molecular weight of the polymer and the salt, respectively. The V^* represents the effective excluded volume (EEV) of the salt into the PEG aqueous solution, and f corresponds to the volume fraction of unfilled effective available volume after tight packing of the salt into the network of the polymer aqueous solution.

2.3. Tie-Lines. Several tie-lines were determined for each PEG−salt system. Tie-line determination was performed using a methodology previously described.[17] 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, in order to determine the composition of the equilibrium phases. 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 100 w = (0.01 to 0.2). Polymer interference in the electrical conductivity measurements was tested for the range 100 w = (0.01−0.5), and no polymer interferences were observed (average errors in salt concentration, calculated using the calibration lines, were below 4 % 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. 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

$$\text{TLL} = \sqrt{(W_{\text{PEG}}^{\text{T}} - W_{\text{PEG}}^{\text{B}})^2 + (W_{salt}^{T} - W_{salt}^{B})^2} \tag{4}$$

where $W_{\text{PEG}}^{\text{T}}$, $W_{\text{salt}}^{\text{T}}$, $W_{\text{PEG}}^{\text{B}}$ and $W_{\text{salt}}^{\text{B}}$ are the top (T) and bottom (B) equilibrium mass fractions of PEG and salts. The slopes of the tie-lines (STL) were obtained from the linear regression of the top, bottom, and feed compositions of the tie-lines.

3. RESULTS AND DISCUSSION

3.1. Phase Diagrams. PEG600 was combined with different salts (potassium citrate, dipotassium hydrogen citrate, sodium formate, potassium formate, sodium sulfate and lithium

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 (eq 1) and Respective Coefficients of Determination (r^2), Standard Deviation (sd) and F-Test

system	N	a	b	c	r^2	sd*	F
PEG600−$K_3C_6H_5O_7$	16	1.03 ± 0.03	−2.8 ± 0.1	39 ± 2	0.9992	0.005	8013
PEG600−K_2HPO_4	15	0.850 ± 0.014	−4.2 ± 0.1	158 ± 8	0.9995	0.004	12348
PEG600−$NaCHO_2$	16	1.49 ± 0.04	−2.6 ± 0.1	35 ± 1	0.9998	0.003	37982
PEG600−$KCHO_2$	14	1.31 ± 0.09	−1.60 ± 0.17	22 ± 1	0.9998	0.003	28898
PEG600−Na_2SO_4	13	0.741 ± 0.006	−4.3 ± 0.1	274 ± 6	0.9999	0.001	55672
PEG600−Li_2SO_4	15	0.927 ± 0.014	−3.6 ± 0.1	188 ± 6	0.9998	0.003	24076

*Standard deviation is calculated as

$$\mathrm{sd} = \left(\sum_{i=1}^{N} \frac{(100W_{\mathrm{PEG}}^{\mathrm{cal}} - 100W_{\mathrm{PEG}}^{\mathrm{exp}})^2}{N} \right)^{0.5}$$

Table 2. Feed, Top, And Bottom Compositions (In Mass Fraction) Measured for Tie-Lines of All ATPSs 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
			PEG600−$K_3C_6H_5O_7$				
0.2501	0.2002	0.087	0.429	0.387	0.018	−1.37	0.508
0.2651	0.2153	0.073	0.477	0.417	0.010	−1.36	0.581
0.2803	0.2300	0.061	0.524	0.446	0.005	−1.35	0.646
			PEG600−K_2HPO_4				
0.1401	0.1600	0.053	0.309	0.225	0.019	−1.69	0.336
0.1552	0.1750	0.038	0.366	0.255	0.007	−1.65	0.419
0.1700	0.1899	0.029	0.414	0.282	0.003	−1.63	0.483
			PEG600−$NaCHO_2$				
0.2799	0.2600	0.137	0.510	0.415	0.022	−1.76	0.562
0.2950	0.2751	0.118	0.582	0.448	0.010	−1.74	0.660
0.3100	0.2902	0.100	0.644	0.476	0.005	−1.70	0.742
			PEG600−$KCHO_2$				
0.3801	0.2100	0.244	0.414	0.491	0.030	−1.55	0.455
0.3952	0.2250	0.220	0.483	0.530	0.015	−1.51	0.562
0.4099	0.2401	0.202	0.545	0.561	0.008	−1.49	0.647
			PEG600−Na_2SO_4				
0.1201	0.1600	0.039	0.318	0.203	0.011	−1.88	0.348
0.1300	0.1699	0.030	0.359	0.224	0.005	−1.83	0.405
0.1401	0.1802	0.024	0.392	0.242	0.002	−1.79	0.447
			PEG600−Li_2SO_4				
0.1201	0.2400	0.052	0.410	0.215	0.027	−2.34	0.416
0.1299	0.2503	0.038	0.475	0.236	0.014	−2.32	0.502
0.1403	0.2601	0.029	0.521	0.254	0.007	−2.28	0.561

sulfate) in order to obtain new phase diagrams not available in the literature. Besides, the effect of each salt in the phase diagram can be discussed in detail. For each biphasic system, the experimental data obtained for the binodal curve were successfully adjusted to the Merchuk equation (eq 1). This equation was chosen because its successful application in polymer-salt ATPSs was already demonstrated in previous works.[13,17,18] The equation is empirical, so there is no physical meaning for the a, b, and c parameters. The values determined for a, b, and c, the correlation coefficient (r^2), the standard deviation (sd) and F-test statistics (F) are presented in Table 1. The r^2 were in all cases higher than 0.999, the sd $\leq$ 0.005, and the $F \geq 8000$, confirming the satisfactory fitting of the Merchuk equation.

Salt and polymer compositions determined for the ends of the tie-lines are presented in Table 2, together with the feed composition, the TLL calculated from eq 4, and the STL. Salt concentration was achieved by electrical conductivity measurements for both phases, whereas polymer concentration in top phase was obtained by lyophilization (after subtraction of the salt concentration previously determined). Owing to the low concentration of polymer present in the bottom phase and the errors associated to salt quantification by electrical conductivity (precise to within ± 0.5 %), the values obtained for polymer concentration by lyophilization were not satisfactory in these cases. For this reason, the polymer concentration in the bottom phases was calculated, in all cases, using the salt concentration previously determined (by electrical conductivity) and the Merchuk equation (eq 1). According to Table 2, the STL values are not constant and slight variations are observed for the different tie-lines of a given ATPS. Furthermore, it was found that the absolute value of the STL decreases as the TLL increases for all biphasic systems. Among the ATPSs studied, PEG−Li_2SO_4 provided the highest absolute values of STL, while PEG−$K_3C_6H_5O_7$ presented the smallest ones.

Several equations have been proposed to evaluate the reliability of tie-line data in (liquid + liquid) equilibrium. However, the equations suggested by Othmer−Tobias (eq 5) and Bancroft (eq 6) are the most extensively used:[19]

$$\log\left(\frac{1 - W_{\mathrm{PEG}}^{\mathrm{T}}}{W_{\mathrm{PEG}}^{\mathrm{T}}}\right) = k_1 + n \log\left(\frac{1 - W_{\mathrm{salt}}^{\mathrm{B}}}{W_{\mathrm{salt}}^{\mathrm{B}}}\right) \tag{5}$$

$$\log\left(\frac{W_{\mathrm{water}}^{\mathrm{B}}}{W_{\mathrm{salt}}^{\mathrm{B}}}\right) = k_2 + r \log\left(\frac{W_{\mathrm{water}}^{\mathrm{T}}}{W_{\mathrm{PEG}}^{\mathrm{T}}}\right) \tag{6}$$

where $W_{\mathrm{water}}^{\mathrm{B}}$ and $W_{\mathrm{water}}^{\mathrm{T}}$ are the equilibrium mass fractions of water in the bottom and top phases, respectively. All the other parameters were defined above. Both equations have been successfully applied in the correlation of (liquid + liquid) equilibrium compositions of many polymer-salt ATPSs.[20−22] The application of these equations to the equilibrium data presented in Table 2 gave a satisfactory linearization, as illustrated in Figure 1. The values obtained for the fitting parameters k_1, n, k_2, and r are presented in Table 3, together with the corresponding coefficient of determination (r^2) and F-test (F). In all cases $r^2 \geq 0.997$ and $F \geq 316$ were found.

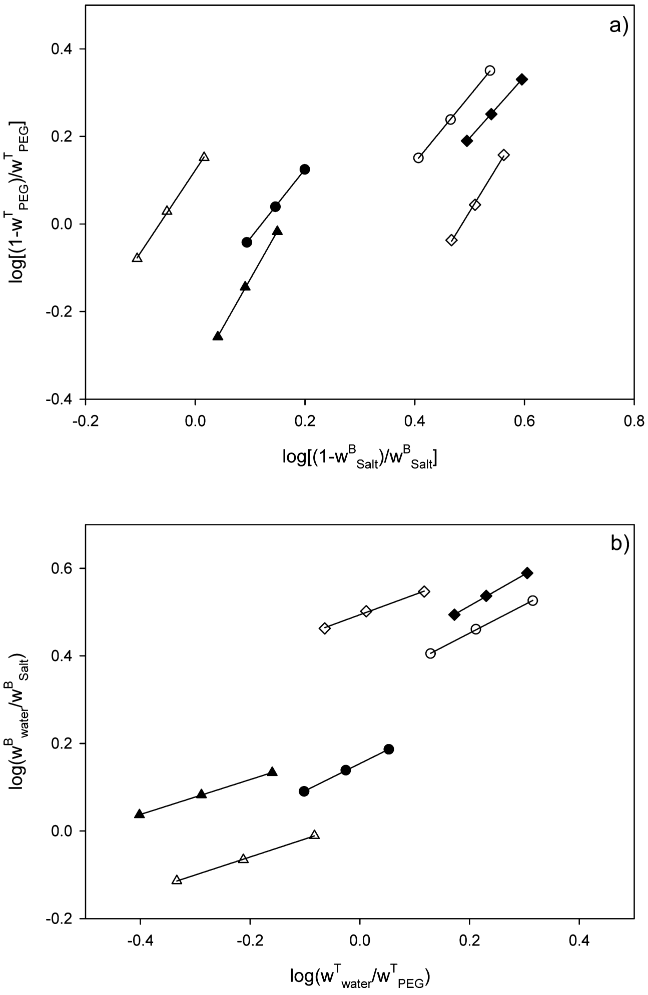

Figure 1. Linearization of the liquid−liquid equilibrium data determined for the PEG600−salt ATPS using: (a) Othmer−Tobias equation and (b) Bancroft equation: potassium citrate (●); dipotassium hydrogen phosphate (○); sodium formate (▲); potassium formate (△); sodium sulfate (◆) and lithium sulfate (◇).

The complete phase diagrams obtained for the six PEG−salt ATPSs are shown in Figures 2 to 4. According to these figures, it is evident that the largest heterogeneous region was obtained with PEG−sodium sulfate ATPS, while PEG−potassium formate ATPS provided the smallest one.

3.2. Effect of Salt Type on the Binodal Curves. Detailed analysis of the six phase diagrams shows that each salt produced a particular effect in the phase diagram. It is also observed that the change of the anion can produce more significant effects than the change of the cation, as previously reported in the literature.[23,24]

A comparison between sodium and potassium formate ATPSs shows that sodium salt ATPSs present a larger heterogeneous region. This means that smaller concentrations of sodium formate are needed to obtain a biphasic system. Therefore, Na^+ is more effective in ATPS formation than K^+. A similar behavior has been reported for other sodium and potassium salts in a wide variety of polymer-salt ATPSs.[13,17,25,26] The effectiveness of Na^+ and K^+ can be explained using a thermodynamic approach based both on the Hofmeister series and the free energy of hydration (ΔG_{hyd}). The Hofmeister series is an empirical classification of ions, ordering them from kosmotropic to chaotropic, based on their capacity to induce protein salting-out.[27] According to this series, Na^+ has a larger salting-out effect than K^+. For that reason, Na^+ is expected to present higher ability to form ATPS than K^+. An equivalent explanation can be achieved analyzing the ΔG_{hyd} values: −295 kJ·mol^{-1} for K^+ and −365 kJ·mol^{-1} for Na^+.[28] More negative ΔG_{hyd} corresponds to a larger hydration shell, which decreases the amount of water available to hydrate the polymer, thus contributing to its salting-out.

However, this thermodynamic approach fails when sodium sulfate is compared with lithium sulfate. According to the Hofmeister series, the salting-out ability of Li^+ is larger than that of Na^+.[27] Analyzing the ΔG_{hyd} values, it is found that Li^+ has a more negative ΔG_{hyd} (−475 kJ·mol^{-1})[28] than Na^+. Thus, it is expected that Li^+ presents a larger ability to form ATPS than Na^+. Nevertheless, this fact was not observed and Na^+ proved to be more effective in ATPS formation than Li^+ (Figure 4). This unexpected behavior was previously reported by Ananthapadmanabhan and Goddard[23] for PEG3350−(sodium or lithium) sulfate ATPSs, ascribing the motives of such phenomenon to the strong specific interaction between Li^+ and the ethylene oxide units of PEG. Complexation of lithium cations with the oxygens in the ether groups was reported by Schott et al.,[29] showing that polyoxyethylene chains act as polydentate ligands in the complexation with cations. Later, da Silva and Loh[30] attributed the different ability of Li^+ and Na^+ to induce biphasic formation to both the different sizes and strengths of interaction with PEG. When PEG and lithium sulfate are mixed, cation and polymer interact leading to the release of some water molecules that were solvating both. This phenomenon is driven by the entropy increase and continues as more electrolyte is added until a saturation point is reached and no more entropy gain occurred. At this point, phase separation becomes more favorable and ATPS formation takes place. The saturation point does not represent the physical saturation of all the binding sites around the PEG molecule, but the necessary amount of electrolyte present in solution (not interacting with the PEG) to destabilize the system and raise phase separation.

Table 3. Fitting parameters Obtained for Othmer−Tobias Equation (eq 5) and Bancroft Equation (eq 6) and the Corresponding Coefficients of Determination (r^2) and *F*-test

	Othmer−Tobias				Bancroft			
ATPS	n	k_1	r^2	F	r	k_2	r^2	F
PEG600−$K_3C_6H_5O_7$	1.582	−0.191	> 0.999	31814	0.154	0.622	> 0.999	5451
PEG600−K_2HPO_4	1.529	−0.472	> 0.999	7426	0.649	0.322	> 0.999	2442
PEG600−$NaCHO_2$	2.217	−0.348	> 0.999	3434	0.399	0.197	> 0.999	14545
PEG600−$KCHO_2$	1.886	0.123	> 0.999	1456	0.411	0.023	> 0.999	3106
PEG600−Na_2SO_4	1.404	−0.506	> 0.999	7337	0.715	0.371	> 0.999	3100
PEG600−Li_2SO_4	2.046	−0.999	0.998	617	0.460	0.494	0.997	316

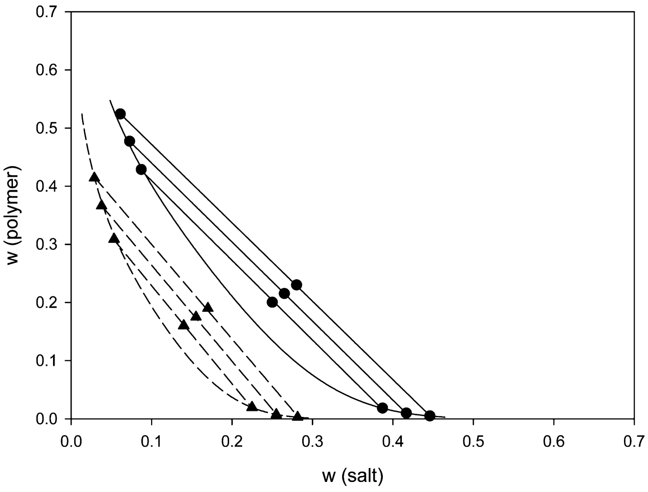

Figure 2. Binodal curve and tie-lines obtained for the systems PEG600−dipotassium hydrogen phosphate (--▲--) and PEG600−potassium citrate (−●−) at 23 °C. All compositions are in mass fraction.

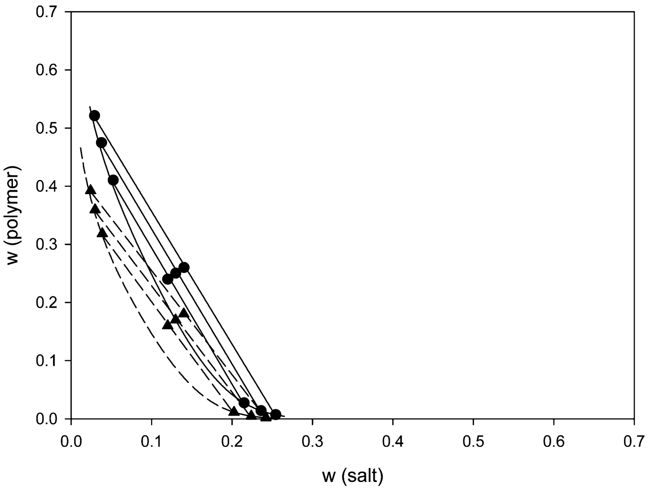

Figure 4. Binodal curve and tie-lines obtained for the systems PEG600−sodium sulfate (--▲--) and PEG600−lithium sulfate (−●−) at 23 °C. All compositions are in mass fraction.

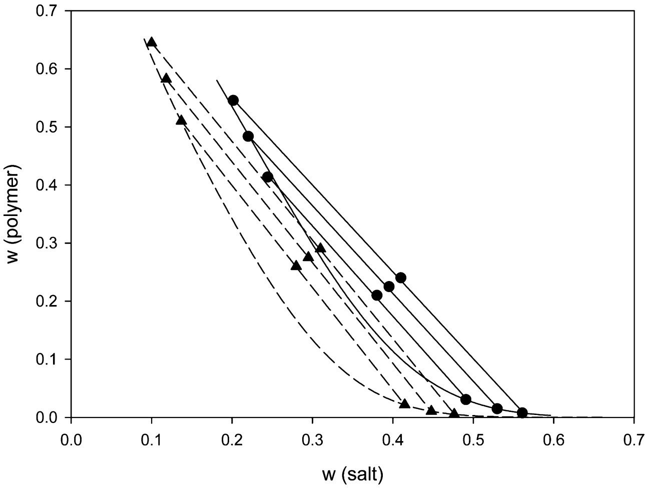

Figure 3. Binodal curve and tie-lines obtained for the systems PEG600−sodium formate (--▲--) and PEG600−potassium formate (−●−) at 23 °C. All compositions are in mass fraction.

The same binding process happens with sodium sulfate, but in this case cation−anion interactions around the polymer are greater, and a larger fraction of sodium is in the bulk solution. Therefore the amount of free electrolyte necessary to cause phase separation is reached sooner, the ATPS obtained being at lower salt concentration.[30] The effectiveness of sodium sulfate over lithium sulfate was also reported in literature for ATPSs composed by PEG1500,[31] PEG4000,[32] PEG6000,[33] PEG8000, and UCON, a poly(ethylene glycol-*co*-propylene glycol) random copolymer.[34]

Comparing the different potassium salts regarding their ability to produce ATPS (Figures 2 and 3), the following order was obtained: HPO_4^{2-} > $C_6H_5O_7^{3-}$ > HCO_2^-. For the sodium salts (Figures 3 and 4), it was found that the sulfate anion was clearly more effective than formate. In fact, among all the salts studied here, sodium sulfate was the most effective in ATPS formation. As discussed above for the cations, the ability to form ATPS can be explained considering the position of the anions in the Hoffmeister series and by means of their ΔG_{hyd} values. Ananthapadmanabham and Goddard[23] also reported that the higher the valence of the anion, the lower the concentration necessary to form ATPS. According to these authors, the anions with a higher valence have a better salting-out capacity because they hydrate more water and consequently reduce the amount of water available to hydrate the polymer. For the anions used in this work, the following order is obtained for the ΔG_{hyd}:$C_6H_5O_7^{3-}$ (ΔG_{hyd} = −2793 kJ/mol)[35] < HPO_4^{2-} (ΔG_{hyd} = −1125 kJ/mol)[21] < SO_4^{2-} (ΔG_{hyd} = −1080 kJ/mol)[28] < HCO_2^- (ΔG_{hyd} = −395 kJ/mol).[28] Thus, both the ΔG_{hyd} and the valence of the anions reflect the effectiveness of the sodium salts found: SO_4^{2-} > HCO_2^-. Nevertheless, this approach fails for the potassium salts, namely with citrate. Although $C_6H_5O_7^{3-}$ has the highest valence and the more negative ΔG_{hyd} value, it provided a heterogeneous region smaller than the divalent anion HPO_4^{2-}. The unexpected behavior of $C_6H_5O_7^{3-}$ was already observed in other ATPSs,[21,36] pointing out that the empirical rule for ATPS formation based either on the ion valence or ΔG_{hyd} is not

Table 4. EEV and *f* Parameters Obtained from eq 3, together with the Number of Solubility Points (*N*), Ratio between Molecular Weight of the Two Phase-Forming Components (*R*) and Respective Coefficients of Determination (r^2), Standard Deviation (sd) and *F*-test

system	*N*	EEV (g/mol)	*f*	*R**	r^2	sd	*F*
PEG600−$K_3C_6H_5O_7$	16	576 ± 63	0.457 ± 0.049	1.96	0.9687	3.15	433
PEG600−K_2HPO_4	15	871 ± 76	0.291 ± 0.047	3.44	0.9667	3.11	378
PEG600−$NaCHO_2$	16	450 ± 7	0.032 ± 0.008	8.82	0.9911	2.19	1562
PEG600−$KCHO_2$	14	372 ± 6	0.084 ± 0.007	7.13	0.9937	1.66	1891
PEG600−Na_2SO_4	13	1055 ± 59	0.188 ± 0.031	4.22	0.9802	2.11	545
PEG600−Li_2SO_4	15	776 ± 15	0.174 ± 0.011	5.46	0.9968	1.02	4083

*$R = M_{PEG}/M_{Salt}$ where M_{PEG} and M_{Salt} are the molecular weights of the polymer and the salt, respectively.

followed by citrates. The anion HCO_2^- followed the ΔG_{hyd} tendency and presented the smallest heterogeneous regions.

3.3. Effective Excluded Volume. The expressions proposed by Guan et al.[15,16] (eq 2 and 3) can also be used to correlate the experimental binodal curve, as far as they provide an adequate representation of the experimental data. Besides, these equations allow the determination of the EEV of the salt. In their original work, Guan et al.[15] successfully used eq 2 to correlate binodal data of polymer−polymer ATPSs and obtain the corresponding EEV. These authors also reported that when there is a great disparity between the molecular weight of the two phase-forming components, only one parameter (the EEV) is necessary to correlate the experimental binodal data. This happens because in these conditions the *f* will be very small and consequently it can be neglected. However, when the two phase-forming components have comparable molecular weights, the additional parameter *f* is needed and eq 3 becomes more adequate to correlate binodal data.[15,16] This fact was investigated in this work. When the experimental binodal data were correlated using eq 2, poor correlations were obtained, with r^2 between 0.818 and 0.981 and standard deviation sd > 3.2 (data not shown). For that reason, eq 3 was also tested, and it was observed that correlations improved significantly. The results obtained for EEV and *f* parameter are presented in Table 4, together with the r^2, sd and *F*-test. According to this table, PEG−$NaCHO_2$ ATPS presented the smallest *f* value while PEG600−$K_3C_6H_5O_7$ ATPS provided the highest one. However, for all the six ATPSs, the *f* parameter is significant enough not to be neglected. Similar conclusions regarding the significance of *f* were reported by Xie et al.[37,38] using phase-forming components with low disparity in size: polypropylene glycol (PPG, M_w = 400) and several potassium salts ($K_3C_6H_5O_7$, $K_2C_4H_4O_6$, $K_2C_2O_4$, K_3HPO_4, K_2CO_3, and K_2HPO_4). Li et al.[39] also found significant *f* values for ATPSs composed by ionic liquid *N*-ethylpyridinium tetrafluoroborate ([EPy][BF_4], M_w = 195) and $Na_3C_6H_5O_7$, $(NH_4)_3C_6H_5O_7$, $Na_2C_4H_4O_4$, or $Na_2C_4H_4O_6$. An important aspect to highlight is the connection observed between the quality of the correlation and the molecular weight ratio of PEG to salt (*R*). It was found that correlations were considerable better for the ATPSs which have higher *R* values. Guan et al.[16] already reported this connection between *R* and the quality of the correlation, considering that the best representations of the experimental binodals of PEG−phosphate ATPSs were obtained when *R* was greater than approximately 4. In this work, correlation improved considerably for R > 5, which correspond to the PEG600−$NaCHO_2$, PEG600−$KCHO_2$, and PEG600−Li_2SO_4 ATPSs. Nevertheless, if we compare the correlation data from Table 4 with those obtained using the Merchuk equation (Table 1), it is clear that the Merchuk equation (with 3 adjustable parameters) provided in all cases better representations of the binodal curves. The use of an additional fitting parameter in the Merchuk equation is justified statistically, as proved by the much larger *F*-value obtained (*F* increases 6- to 100-fold depending on ATPS, see Tables 1 and 4). The same conclusion has been previously found by other authors using these equations in different polymer−salt ATPSs.[18,38,40] The interest in applying eq 3 comes from the possibility to determine the EEV parameter. In a practical approach, this parameter can be related with the salting-out strength of the salts, at constant PEG molecular weight, as pointed out by Huddleston and co-workers.[18] The order found here for the EEV of the salts was Na_2SO_4 > K_2HPO_4 > Li_2SO_4 > $K_3C_6H_5O_7$ > $NaCHO_2$ > $KCHO_2$. The same order is observed for the size of the heterogeneous region, as illustrated in Figure 5, and represents the effectiveness of the salts to form ATPSs. Thus, a higher EEV value is associated with higher salting-out ability of the salt. This suggests that EEV can be considered a suitable parameter for a quantitative (rather than qualitative) analysis of different ATPSs, providing a practical measurement of the size of the heterogeneous region and the salting-out ability of the salts.

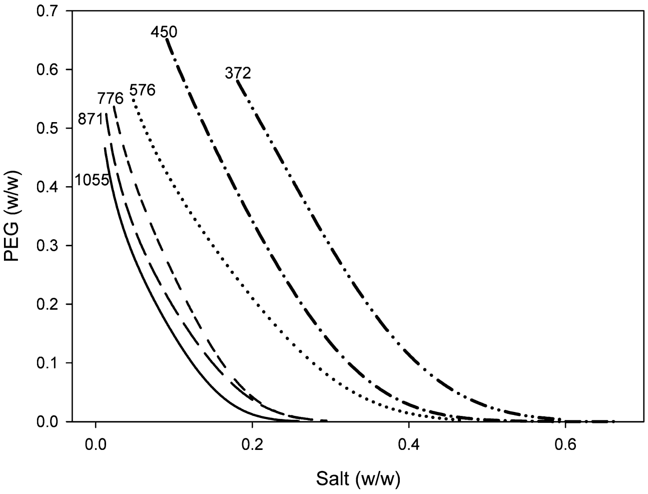

Figure 5. Representation of the binodal curves determined for the six PEG−salt ATPSs and the corresponding EEV values (in g/mol) obtained from eq 3: PEG600−Na_2SO_4 (—); PEG600−K_2HPO_4 (− −−; PEG600−Li_2SO_4 (---); PEG600−$K_3C_6H_5O_7$ (···); PEG600−NaCHO2 (−·−) and PEG600−$KCHO_2$ (−··−).

Additionally, Huddleston and co-workers[18] observed some linearity when plotting the EEV as a function of the molecular weight of the salt, suggesting a close relationship between them. However, the authors stated that it *"can only really be considered* *an artefact*" of the Guan equation,[16] "*since such relationship cannot in general be true*". We have also investigated this relationship, as illustrated in Figure 6. The results obtained show no linear relation between the molecular weight of the salt and the EEV, confirming the reasoning of Huddleston and co-workers.[18]

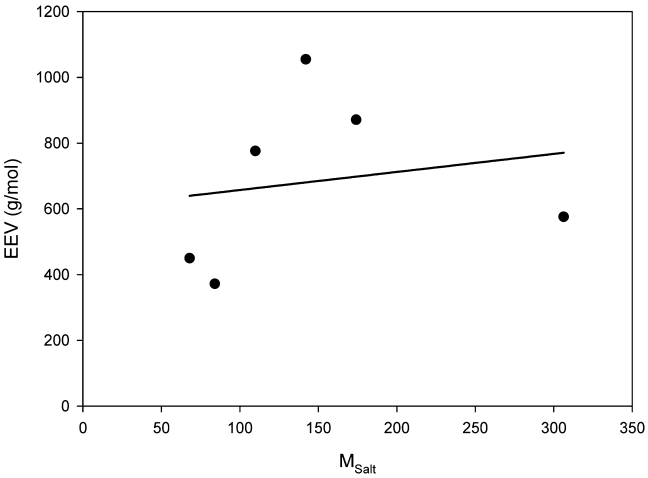

Figure 6. Relationship between the EEV obtained from eq 3 and the molecular weight of the salt.

4. CONCLUSIONS

Six new ATPSs were prepared by combining PEG600 with different salts (potassium citrate, dipotassium hydrogen citrate, sodium formate, potassium formate, sodium sulfate and lithium sulfate) and their complete phase diagrams were determined. The experimental data obtained for the binodal curve were successfully adjusted to the Merchuk equation. The reliability of tie-line data was evaluated using the equations suggested by Othmer–Tobias and Bancroft. A satisfactory linearization was obtained in all cases.

Regarding the ability of the ions to induce ATPS formation, it was observed that Na^+ is more effective in ATPS formation than K^+ and Li^+. For the salts sharing the cation K^+, the following order was observed: $HPO_4^{2-} > C_6H_5O_7^{3-} > HCO_2^-$, while for the salts formed by the cation Na^+ it was found that SO_4^{2-} is clearly more effective than HCO_2^-. Effectiveness of each ion can be explained using the Hofmeister series or their free energy of hydration. However, some exceptions were observed, namely with Li^+ and $C_6H_5O_7^{3-}$.

The effective excluded volume of the salts is related to their salting-out strength and the order found ($Na_2SO_4 > K_2HPO_4 > Li_2SO_4 > K_3C_6H_5O_7 > NaCHO_2 > KCHO_2$) is analogous to the order observed for the size of the heterogeneous regions. This fact suggests the practical use of EEV as a quantitative comparison parameter between different ATPSs.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Complete phase diagrams for ATPS obtained. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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