456 *J. Chem. Eng. Data* **2008,** *53,* 456–461

Effect of Temperature on the Phase Equilibrium of the Aqueous Two-Phase Poly(propylene glycol) + Tripotassium Citrate System

Mohammed Taghi Zafarani-Moattar,* Somayeh Emamian, and Sholeh Hamzehzadeh

Physical Chemistry Department, University of Tabriz, Tabriz, Iran

Liquid–liquid equilibria (LLE) for the aqueous poly(propylene glycol) (PPG) + tripotassium citrate system have been determined experimentally at $T = (293.15, 298.15, 308.15, \text{and } 318.15)$ K. An empirical nonlinear three-parameter expression developed by Merchuk was used for reproducing the experimental binodal data, and the fitting parameters were obtained for the corresponding temperatures. The effect of temperature on the phase-forming ability in the investigated system has been studied based on a salting-out coefficient obtained from fitting the tie-line data to a Setschenow-type equation for each temperature. The values of the salting-out coefficients reflect the extent of preferential hydration of the propylene oxide units in the polymer chain.

Introduction

Liquid–liquid extraction utilizing aqueous two-phase systems (ATPSs) has been used to separate and purify biological products from the complex mixtures in which they are produced. ATPSs can be formed by combining either two incompatible polymers or a polymer and a salt in water above a certain critical concentration.[1–3] Poly(ethylene glycol) (PEG), which is a hydrophilic polymer, is often used in aqueous two-phase partitioning studies. In laboratory-scale separations, the most commonly used systems are comprised of the polymers poly(ethylene glycol) (PEG) and dextran, while for large-scale enzyme extraction, PEG−salt systems are used. The latter systems are more attractive because of their greater selectivity, lower viscosity, lower cost, rapid phase disengagement, and availability of commercial separators, which allow a faster and continuous protein separation.

Poly(propylene glycol) (PPG) is a polymer that is structurally closely related to PEG. Low molecular weights of PPG are completely soluble in water, while high molecular weights are only partially soluble. This polymer can also be used for the separation of biomolecules, since its aqueous solutions with a suitable polymer or a salt form a two-phase system. However, liquid–liquid equilibrium (LLE) data of the aqueous PPG−polymer and PPG−salt systems are relatively scarce. In regard to PPG−salt systems, the liquid–liquid equilibrium data for PPG_{425} + NaCl,[4] PPG_{425} + $MgSO_4$,[5] PPG_{425} + $(NH_4)_2SO_4$,[6] PPG_{425} + Na_2SO_4,[6] PPG_{400} + Na_2SO_4,[7] PPG_{400} + Na_2CO_3,[7] and PPG_{400} + $NaNO_3$[7] aqueous two-phase systems have been reported.

The most common inorganic salts employed by different research groups on the aqueous two-phase extraction technique consist of selective cations (ammonium, potassium, or sodium) and anions (phosphate, sulfate, or carbonate). These salts, however, lead to high salt concentrations in effluent streams and, therefore, to environmental concern. In this respect, as an alternative approach Vernau and Kula[8] have investigated citrates as a substitute for inorganic salts. Citrate is biodegradable and nontoxic and could be discharged into biological wastewater treatment plants. Recently, Salabat et al.[9] have determined LLE, density, and viscosity for the aqueous PPG + trisodium citrate system at 25 °C.

This work is devoted to studying the phase behavior of the PPG_{400} + tripotassium citrate aqueous two-phase system. Here, we report liquid–liquid equilibrium data for the aqueous PPG_{400} + tripotassium citrate system at $T = (293.15, 298.15, 308.15, \text{and } 318.15)$ K that have not been previously published. The obtained results are necessary for design and optimization of extraction processes, understanding of general factors determining partition of solutes and particles in such ATPSs, and development and testing of both thermodynamic and mass transfer models of aqueous two-phase systems. The effect of temperature on the phase-forming ability in the investigated aqueous polymer + salt two-phase system has also been studied based on a salting-out coefficient obtained from fitting the tie-line data to a Setschenow-type[10] equation. This is because the phenomenon of two-phase forming in a polymer + salt aqueous two-phase system is essentially due to the effect of an electrolyte (i.e., salt) on the solubility of a nonelectrolyte (i.e., polymer) which can be studied by the help of the Setschenow-type[10] equation. In this respect, our interest in this work lies in examining the Setschenow-type behavior of phase diagrams for the studied PPG + salt system at different temperatures and the possibility of using the recent derived Setschenow-type equation[10] in correlation to experimental liquid–liquid equilibrium data of the investigated system.

Experimental Section

Materials. Poly(propylene glycol), of molecular weight 400, was obtained from Aldrich. The average molar mass M_n of this polymer was previously determined[7] to be 403 $g \cdot mol^{-1}$. Tripotassium citrate with purity of min. 99 % by mass was supplied by Merck. The polymer and salt were used without further purification, and double-distilled–deionized water was used.

Apparatus and Procedure. The experimental apparatus employed is essentially similar to the one used previously.[11–13] The temperature was controlled to within ± 0.05 K. The

---

* Corresponding author. Fax: +98 411 3340191. E-mail: zafarani47@yahoo.com.

10.1021/je700549u CCC: $40.75

binodal curves were determined by a titration method. A salt solution of known concentration was titrated with the polymer solution or vice versa, until the solution turned turbid, which indicated the formation of two liquid phases. In accordance with the amount of titrant added until turbidity was observed, the composition of the mixture for each point on the binodal curve was calculated by mass using an analytical balance (Shimatzu, 321-34553, Shimatzu Co., Japan) with a precision of $\pm 1 \cdot 10^{-7}$ kg. For the determination of the tie-lines, feed samples (about $2 \cdot 10^{-5}$ m^3) were prepared by mixing appropriate amounts of polymer, salt, and water in the vessel. The thermostat was set at a desired temperature, and the sample was stirred for 1 h. Then the mixture was allowed to settle for at least 72 h. After separation of the two phases, the concentrations of tripotassium citrate in the top and bottom phases were determined by flame photometry. Following Cheluget et al.,[4] the concentration of PPG in both phases was determined by refractive index measurements performed at 298.15 K using a refractometer (QUARTZ RS-232, Ceti, Belgium) with a precision of ± 0.0001. For dilute aqueous solutions containing a polymer and a salt, the relation between the refractive index, n_D, and the mass fractions of polymer, w_1, and salt, w_2, is given by[4]

$$n_D = n_0 + a_1 w_1 + a_2 w_2 \tag{1}$$

Here n_0 is the refractive index of pure water which is set to 1.3325 at 298.15 K. The values of coefficients a_1 and a_2 for the applied system were obtained at 298.15 K as 0.1380 and 0.1537, respectively. However, it should be noted that this equation is only valid for dilute solutions ($w_1 \leq 0.1$ and $w_2 \leq 0.05$) for which linear calibration plots of the refractive index of ternary solutions with a fixed mass fraction of salt or polymer against polymer or salt are obtained. Therefore, before the refractive index measurements, it was necessary to dilute the samples to the above mass fraction range. The uncertainty of the mass fraction of PPG achieved using eq 1 was better than 0.002.

Results and Discussion

Phase Diagrams. For the aqueous PPG$_{400}$ + tripotassium citrate system, the binodal data and the tie-line compositions determined experimentally at T = (293.15, 298.15, 308.15, and 318.15) K are given in Tables 1 and 2, respectively.

For the studied system, the experimental binodal data were fitted by a least-squares regression method to the following empirical nonlinear expression developed by Merchuk[14]

$$w_1 = a \exp\left(b w_2^{0.5} - c w_2^3\right) \tag{2}$$

where a, b, and c represent fit parameters and w_1 and w_2 represent the concentrations (in weight percent) of polymer and salt, respectively. Recently, the above equation has been successfully used for the correlation of binodal data of some aqueous PEG + salt systems.[15,16] Using eq 2, the fitting parameters a, b, and c obtained from the correlation of experimental binodal data along with the corresponding standard deviations are given in Table 3. The binodals reproduced from eq 2 at different temperatures are also shown in Figure 1. On the basis of the obtained standard deviations, we conclude that eq 2 can be satisfactorily used to reproduce the binodal curves of the investigated system.

Table 1. Binodal Data for the PPG$_{400}$ (1) + Tripotassium Citrate (2) + H$_2$O (3) System at T = (293.15, 298.15, 308.15, and 318.15) K

293.15 K		298.15 K		308.15 K		318.15 K	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
60.88	0.66	67.18	0.26	69.11	0.19	49.44	0.32
57.92	0.87	66.14	0.31	65.90	0.25	44.85	0.34
49.00	1.78	62.00	0.47	61.67	0.42	35.11	0.33
46.06	2.29	57.88	0.73	54.85	0.71	30.59	0.41
42.72	2.86	51.58	1.27	49.67	0.97	26.40	0.56
38.11	3.78	48.52	1.60	44.37	1.36	22.57	1.00
33.24	4.91	44.75	2.11	38.28	1.81	19.82	1.35
27.85	6.50	41.33	2.62	31.42	2.37	17.28	1.81
20.90	8.62	37.65	3.28	26.56	2.88	15.60	2.19
		34.18	3.90	22.41	3.51	14.10	2.69
		31.22	4.51	20.04	3.94	12.03	3.48
		28.05	5.07	17.11	4.65	10.67	4.00
		25.67	5.61	14.45	5.49	9.76	4.42
		23.34	6.10	10.55	6.98	7.83	5.51
		16.67	7.97				
		16.15	8.02				
		15.76	8.14				
		14.58	8.46				
		13.59	8.97				
		12.83	9.20				
		11.17	9.82				

Table 2. Experimental Phase Equilibrium Compositions for the PPG$_{400}$ (1) + Tripotassium Citrate (2) + H$_2$O (3) System at T = (293.15, 298.15, 308.15, and 318.15) K

top phase: polymer-rich phase		bottom phase: salt-rich phase	
100 w_1	100 w_2	100 w_1	100 w_2
	293.15 K		
57.57	1.30	10.37	13.95
63.73	0.40	9.31	15.14
71.53	0.19	6.23	19.70
76.57	0.09	5.11	22.55
	298.15 K		
57.35	0.89	10.17	10.56
60.34	0.63	8.30	12.92
65.46	0.26	5.79	15.55
70.53	0.19	4.38	18.63
84.17	0.05	0.54	30.53
	308.15 K		
68.10	0.12	7.55	11.05
74.21	0.07	5.23	14.62
78.54	0.05	5.01	16.03
86.66	0.00_4	5.35	18.19
91.28	0.01	3.59	21.48
	318.15 K		
72.77	0.07	15.04	2.62
75.02	0.03	10.93	3.90
75.78	0.13	9.48	4.58
78.05	0.04	7.41	6.22
79.09	0.05	7.10	6.55

Table 3. Values of Parameters of Equation 2 for PPG (1) + Tripotassium Citrate (2) + H$_2$O (3) at T = (293.15, 298.15, 308.15, and 318.15) K

T/K	a	b	$10^4 \cdot c$	sd[a]
293.15	83.91	−0.3965	3.50	0.16
298.15	84.14	−0.4359	7.63	0.39
308.15	95.00	−0.6895	17.46	1.49
318.15	78.35	−1.1795	−39.11	3.97

[a] sd $= \left(\sum_{i=1}^{N} (w_1^{\text{calcd}} - w_1^{\text{exptl}})^2 / N\right)^{0.5}$, where w_1 and N represent the concentration (in weight percent) of polymer and the number of binodal data, respectively.

The effect of temperature on the phase-forming ability for the studied system is also illustrated in Figure 1. The locus for the experimental binodals shown in Figure 1 demonstrates that the two-phase area is expanded with an increase in temperature which is attributed to the decrease in solubility of the polymer or the increase in phase-forming ability in the studied system. Additionally, to show the effect of

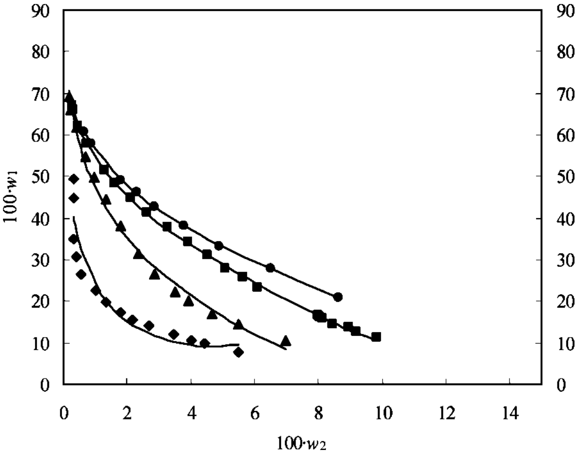

Figure 1. Binodal curves for the PPG_{400} (1) + tripotassium citrate (2) + H_2O (3) two-phase system at different temperatures: •, 293.15 K; ■, 298.15 K; ▲, 308.15 K; ♦, 318.15 K; —, calculated from eq 2.

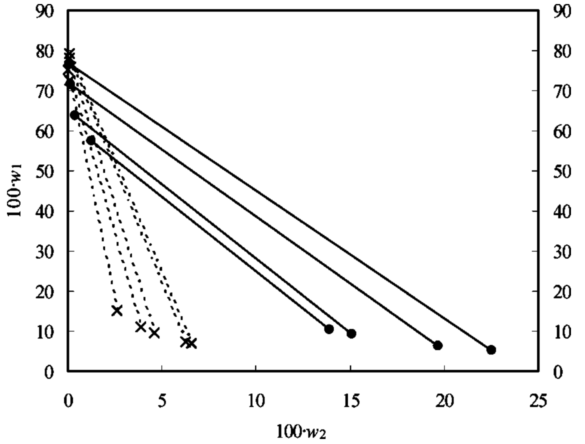

Figure 2. Effect of temperature on the equilibrium phase compositions for the PPG_{400} (1) + tripotassium citrate (2) + H_2O (3) system: •, 293.15 K; ×, 318.15 K; —, tie-lines at 293.15 K; ---, tie-lines at 318.15 K. These tie-lines were obtained by connecting the experimental equilibrium phase composition data.

Table 4. Values of Parameters from Least-Squares Linear Fits to the Setschenow-Type Equation (Equation 4) for PPG (1) + Tripotassium Citrate (2) + H_2O (3) at T = (293.15, 298.15, 308.15, and 318.15) K

T/K	k_S/(kg·mol^{-1})	intercept	R^2	10^2·Dev^a
293.15	3.03	0.81	0.999	0.00_3
298.15	3.94	0.82	0.998	0.05
308.15	4.43	1.19	0.994	0.02
318.15	7.52	2.05	0.972	0.07

[a] Dev = $(\sum_p \sum_l \sum_j (w_{p,l,j}^{\text{calcd}} - w_{p,l,j}^{\text{exptl}})^2/6N)$, where $w_{p,l,j}$ is the weight fraction of the component j (i.e., polymer, salt, or water) in the phase p for lth tie-line and the superscripts "calcd" and "exptl" refer to the calculated and experimental values, respectively. N is the number of tie-line data.

temperature on the equilibrium phase compositions for the investigated system, the experimental tie-lines are compared in Figure 2 for the temperatures (293.15 and 318.15) K, as an example. As shown in Figure 2, the slope and length of the tie-lines increase with an increase in temperature, similar to the one observed in our previous work[17] for the aqueous PEG + trisodium citrate system. This is because the PPG becomes more hydrophobic with an increase in temperature.[18,19] Thus, by increasing temperature, water is driven from the PPG-rich phase to the salt-rich phase. So, the PPG concentration at the PPG-rich phase increases, while the salt-rich phase will be somewhat more diluted (i.e., the salt concentration will be decreased). In other words, water becomes a poorer solvent for PPG as the temperature is raised. Depression of the hydrophilicity of PPG with increasing temperature may be related to the salting-out phenomenon because a decrease in the solubility of PPG in water with an increase in temperature, as shown in Figures 1 and 2, is reflected by a decrease in the critical concentration of a salt required to form a two-phase system which in turn indicates the effectiveness of the salt in inducing the formation of two phases (i.e., the salting-out strength of the salt). Salting-out effects are usually quantified by fitting solubility data to the empirical equation of Setschenow[20]

$$\ln \frac{S_0}{S} = k_S c_S \tag{3}$$

where S_0 and S refer to the solubilities of the nonelectrolyte in pure water and in a salt solution of concentration c_s, respectively, and k_S, defined as the salting-out coefficient, is specific for a particular nonelectrolyte—salt pair. In earlier studies, based on eq 3 or the salting-out concept, the Setschenow-type behavior of the phase diagrams for a number of aqueous PEG + salt two-phase systems has been substantiated by some authors,[21,22] but with only limited success. In this regard, in recent years, Hey et al.[10] proposed a new derivation of Setschenow-type equations from which a characteristic parameter, k_S, for the salting-out effect of an electrolyte on ethylene oxide (EO) groups in PEG + salt aqueous two-phase systems can be satisfactorily determined. Therefore, we decided to use this equation to introduce a quantitative measure for the salting-out effects of the studied salt on propylene oxide (PO) units in the investigated PPG + salt aqueous two-phase system at different temperatures. The equation[10] used has the following form

$$\ln\left(\frac{c'_P}{c''_P}\right) = k_P(c''_P - c'_P) + k_S(c''_S - c'_S) \tag{4}$$

where c_p, c_s, k_P, and k_S, represent the molality of PO, the molality of the salt, a parameter relating the activity coefficient of PO to its concentration, and the salting-out coefficient, respectively. Single and double primes also refer to the upper and lower phases, respectively. If the first term on the right-hand side of this equation is small compared to the second term, a Setschenow-type equation is obtained. This would imply that $k_P << k_S$ since the absolute values of $(c''_P - c'_P)$ exceed the $(c''_S - c'_S)$ values.

The experimental tie-line compositions for the aqueous PPG_{400} + tripotassium citrate system given in Table 2 were fitted by a least-squares linear regression method to eq 4 (i.e., the logarithm of the ratio between the molality of PO for the top phase and the one for the bottom phase, $\ln(c'_P / c''_P)$, was considered as a linear function of $(c''_S - c'_S)$, the differences in the salt concentrations between the bottom and top phases, respectively), and the obtained slopes which give the salting-out coefficients, k_S, together with corresponding intercepts and correlation coefficients are listed in Table 4 for each working temperature. To examine more closely the relation between the Setschenow-type behavior and the phase diagrams, the Setschenow-type plots of the tie-line data for the studied system have also been shown in Figure 3. On the basis of correlation coefficients given in Table 4 and Setschenow-type plots shown in Figure 3, we conclude that the equilibrium compositions of

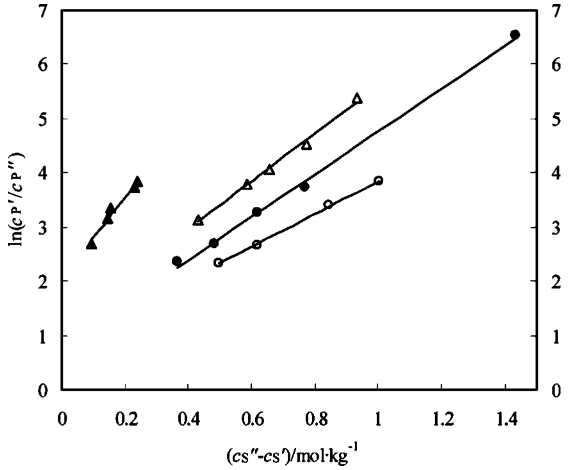

Figure 3. Setschenow-type plots for the tie-line data of the PPG_{400} (1) + tripotassium citrate (2) + H_2O (3) system. Upper and lower phase concentrations are denoted by single and double primes, respectively: ○, 293.15 K; •, 298.15 K; Δ, 308.15 K; ▲, 318.15 K.

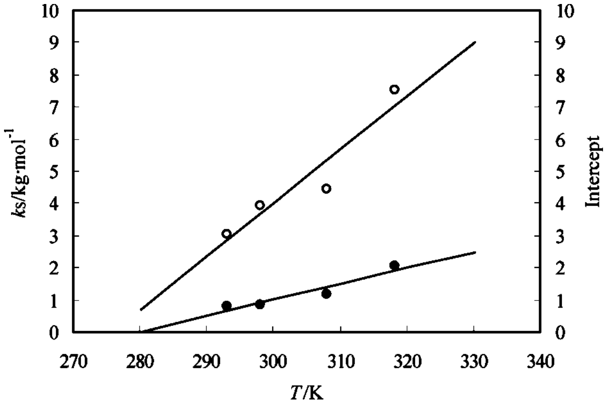

Figure 4. Relationship between the values of the fit parameters, k_S, and the corresponding intercept, listed in Table 4, and the working temperatures: ○, the salting-out coefficients obtained from fitting the tie-line data to eq 4; —, best fit line using a linear function as $k_S = f + gT$, with the obtained fit parameters ($f = -45.83$ and $g = 0.16$) and correlation coefficient, $R^2 = 0.894$; •, the fit parameter specified as intercept; —, best fit line using a similar linear function, with corresponding fit parameters ($f = -13.92$ and $g = 0.05$) and correlation coefficient, $R^2 = 0.904$.

the investigated system at the studied temperatures can be satisfactorily characterized by Setschenow-type behavior.

For the studied system, as shown in Table 4, the values of k_S increase with increasing temperature. The increase of k_S is reflected in the phase diagram by a shift in the position of the binodal curve to the left corresponding to a decrease in the area representing the single-phase mixture as shown in Figure 1, indicating an increasing degree of incompatibility between the electrolyte and PPG or in other words an increasing phase-forming ability. Further, Figure 4 shows the k_S and the corresponding intercept values in relation to the working temperatures. It appears that in the studied system there is a close relationship between the salting-out coefficient of the phase-forming salt and the working temperature.

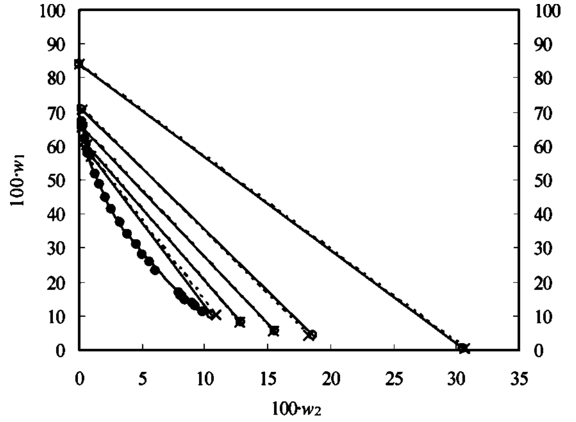

Figure 5. Phase diagram of the PPG_{400} (1) + tripotassium citrate (2) + H_2O (3) two-phase system at 298.15 K: •, experimental binodal; -, calculated binodal from eq 2; —○—, experimental tie-lines; --- × ---, calculated tie-lines using eq 4.

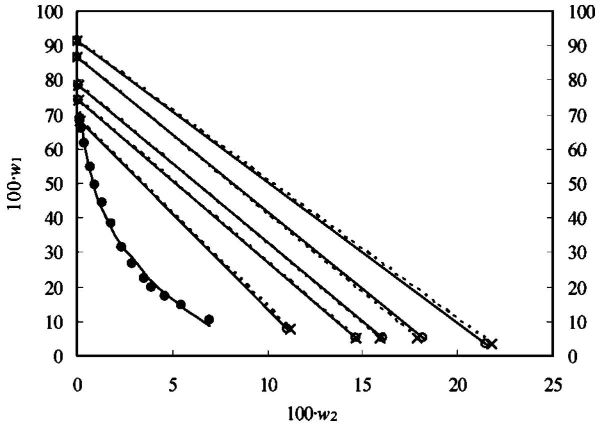

Figure 6. Phase diagram of the PPG_{400} (1) + tripotassium citrate (2) + H_2O (3) two-phase system at 308.15 K: •, experimental binodal; -, calculated binodal from eq 2; —○—, experimental tie-lines; --- × ---, calculated tie-lines using eq 4.

Table 5. Values of Parameters of Equation 5 and the Plait Points for PPG (1) + Tripotassium Citrate (2) + H_2O (3) at T = (293.15, 298.15, 308.15, and 318.15) K

T/K	f	g	R^2	plait point (w_1, w_2, w_3)
293.15	0.3061	2.0601	0.966	(0.3830, 0.0373, 0.5797)
298.15	0.4387	1.3440	0.989	(0.4634, 0.0184, 0.5182)
308.15	0.4136	2.3593	0.972	(0.4424, 0.0122, 0.5454)
318.15	0.6886	1.5261	0.992	(0.6887, 0.0001, 0.3111)

Furthermore, using the parameters given in Table 4, the performance of eq 4 in representing the tie-line data of the investigated system has been examined, and the corresponding standard deviations are also given in Table 4. On the basis of the obtained standard deviations given in Table 4, we conclude that eq 4 can be satisfactorily used to correlate the tie-line data of the investigated system. To show the reliability of the model, comparisons between the experimental and reproduced tie-lines at (298.15 and 308.15) K are also shown, respectively, in Figures 5 and 6, as examples.

The location of the plait point for the studied system at each working temperature was also estimated by extrapolation from the auxiliary curve satisfactorily fitted with the following linear equation

$$w_1 = f + gw_2 \tag{5}$$

where f and g represent fit parameters. For the studied system, the estimated values for the plait points along with the obtained fitting parameters for eq 5 and the corresponding correlation coefficients are listed in Table 5. As an example, the locus of the estimated plait point for the temperature 298.15 K along with the used procedure is illustrated in Figure 7.

Effect of Temperature on the Exclusion of PPG. The formation of aqueous two-phase systems, as mentioned earlier, clearly indicates the mutual exclusion of the salt and the polymer due to their high affinity for the solvent. The exclusion of solute

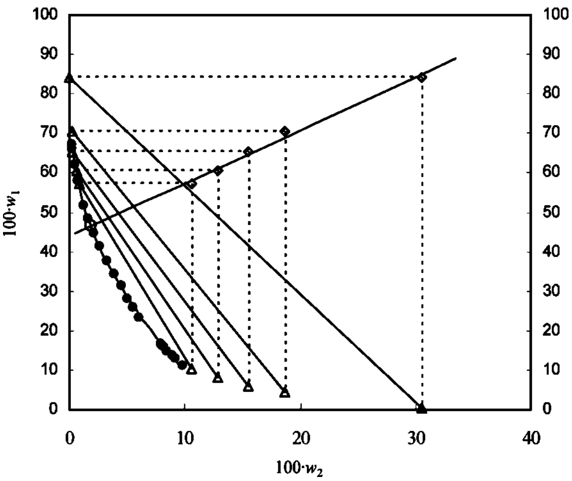

Figure 7. Binodal curve, tie-lines, and plait point for the PPG$_{400}$ (1) + tripotassium citrate (2) + H$_2$O (3) system at 298.15 K: •, binodal curve data; -, calculated binodal from eq 2; Δ, tie-line data; ---, tie-lines; ◇, auxiliary curve data; —, calculated from eq 5; ○, plait point.

from the environs of a polymer molecule can be described in terms of a local excess of water molecules, in analogy with the Gibbs surface excess defined in connection with macroscopic interfaces.[23] Therefore, we decided to estimate the excess number of water molecules adjacent to the PO repeat units to get some information with regard to the effect of temperature on the exclusion of the polymer. As stated by Hey et al.,[10] the excess number of water molecules can be related to the magnitude of the salting-out coefficient, k_S, for a given electrolyte as follows

$$\frac{k_S}{v} = 0.018 N_{ew} \frac{d(\varphi c_S)}{dc_S} \tag{6}$$

where k_S, v, φ, c_s, and N_{ew} represent the salting-out coefficient, the number of moles of ions in the fully dissociated mole of electrolyte, the molal osmotic coefficient, the molality of the salt, and the excess number of water molecule per macromolecule in the salt-depleted region, respectively. N_{ew} is related to the actual number of water molecules, N_w, in this region by[10]

$$N_{ew} = N_w\left(1 - \frac{n_w/n_s}{N_w/N_s}\right) \tag{7}$$

where n_w/n_s and N_w/N_s represent water/electrolyte ratios from regions unaffected by and influenced by the polymer, respectively. The other relevant equations together with the procedure[10] for deducing eq 6 are not given here.

The necessary molal osmotic coefficients, φ, for tripotassium citrate at T = (293.15, 298.15, and 308.15) K have been calculated from the corresponding water activity data[24] using the following equation

$$\varphi = -\frac{\ln(a_w)}{vmM_w} \tag{8}$$

where a_w and M_w represent the activity and molecular weight of water, respectively. For the temperature 318.15 K, the necessary molal osmotic coefficients, φ, were predicted employing the Wilson model used successfully by Sadeghi and Ziamajidi[24] for the correlation of the water activity data of the binary tripotassium citrate + water[24] system at T = (293.15, 298.15, 303.15, 308.15, and 313.15) K. The obtained molal osmotic coefficients for aqueous tripotassium citrate were used to determine d(φc_S)/dc_S by plotting of φc_S against c_S at four studied temperatures. The plots are generally linear for concentrations up to about 1 molal. Thus, N_{ew} values at different temperatures can be estimated by substituting the values obtained for d(φc_S)/dc_S and k_S / v into eq 6. The estimated values for N_{ew} along with the corresponding values for d(φc_S)/dc_S and k_S / v are listed in Table 6. In the limiting condition that N_S is negligible, i.e., that the hydration shell of the polymer completely excludes electrolyte ions, these N_{ew} values become equal to N_w, the actual number of water molecules around the polymer chains. Using the N_{ew} values reported in Table 6 for the PPG used in the study with an average degree of polymerization of about 7, the N_{ew} values per PO repeat unit are estimated as 8, 11, 12, and 21 for the temperatures T = (293.15, 298.15, 308.15, and 318.15) K, respectively. The reliability of N_{ew} values may be tested using a few criteria. A good test could be comparison of the obtained results with those reported in the literature or calculated by different methods. But this criterion is rather difficult to apply because there is no N_{ew} value reported in the literature for PPG, and also different methods with different mechanisms always yield different results for N_{ew}.[25] However, in this work, our interest lies in the behavior of the obtained N_{ew} values per PO repeat units in relation to the ability of the investigated system to exclude PPG which may be characterized as the phase forming ability for the studied aqueous two-phase system. It seems that the enhancement of the exclusion of PPG in the investigated ATPS (Figure 1) may be related to the increasing number of water molecules around the repeat units in the PPG chain, as the temperature is raised. In other words, due to the hydration sheath increment per PO repeat units in the PPG chain by increasing temperature, the near-surface region of the polymer may be less accessible to structure-making ions and hence the extent of exclusion will increase as proposed by Sadeghi.[26] Recently, the existence of a salt-deficient region around poly(ethylene oxide), PEO, chains in ternary PEO + structure-making electrolyte + water systems has also been verified by a spectroscopic method. [27] From this spectroscopic study,[27] it is concluded that as the temperature is raised the interactions of PEO with surrounding water molecules become weaker, and thus for a given concentration of a given water-structure-making salt, the water-structuring forces dominate the interaction between the structure-making electrolyte and the polymer leading to the hydration sheath increment around PEO chains and therefore the ability of the system to produce a polymer-rich, salt-deficient local composition increase in the regions of the solution with a size of about a micron. The same interpretation may be given for our system containing PPG since PEO is structurally closely related to PPG.

Table 6. Excess Number of Water Molecules per PPG Chain

T/K	k_S/v (kg·mol^{-1})	d(φc_S)/dc_S	N_{ew}
293.15	0.76	0.75	56
298.15	0.99	0.73	75
308.15	1.11	0.75	81
318.15	1.88	0.74	140

Conclusions

Liquid−liquid equilibrium data for the PPG$_{400}$ (1) + tripotassium citrate (2) + H$_2$O (3) system were obtained at T = (293.15, 298.15, 308.15, and 318.15) K. The experimental binodal data were satisfactorily correlated with the Merchuk equation. The effect of temperature on the phase-forming ability for the studied system was also successfully discussed on the basis of the salting-out coefficient of the salt, k_S, obtained from fitting the tie-line data to a Setschenow-type equation. It was shown that, for the studied system, the value of k_S increases with increasing temperature, and the temperature with higher phase-forming ability has a larger value of k_S. Additionally, the tie-line data for the investigated system can be successfully represented by the Setschenow-type equation. Furthermore, using the k_S values obtained for the studied electrolyte, the number of water molecules adjacent to the propylene oxide repeat units in the PPG chain was estimated, and it was shown that the enhancement of the exclusion of PPG in the investigated ATPS may be related to the increasing number of water molecules around the PO repeat units, as the temperature is raised.

Literature Cited

(1) Albertsson, P. A. *Partitioning of cell particles and macromolecules*, 3rd ed.; Wiley: New York, 1986.

(2) Walter, H.; Brooks, D. E.; Fisher, D. *Partitioning in aqueous two-phase systems*; Academic Press: New York, 1985.

(3) Zaslavsky, B. Y. *Aqueous two-phase partitioning, physical chemistry and bioanalytical applications*; Marcel Dekker: New York, 1995.

(4) Cheluget, E. L.; Gelinas, S.; Vera, J. H.; Weber, M. E. Liquid−liquid Equilibrium of Aqueous Mixtures Poly(propylene glycol) with NaCl. *J. Chem. Eng. Data* **1994**, *39*, 127–130.

(5) Zafarani-Moattar, M. T.; Salabat, A. Thermodynamics of Magnesium Sulfate-Polypropylene glycol Aqueous Two-Phase system. Experiment and Correlation. *Fluid Phase Equilib.* **1998**, *152*, 57–65.

(6) Salabat, A.; Dashti, H. Phase Composition, Viscosities and Densities of Systems $PPG_{425} + Na_2SO_4 + H_2O$ and $PPG_{425} + (NH_4)_2SO_4 + H_2O$ at 298.15 K. *Fluid Phase Equilib.* **2004**, *216*, 153–157.

(7) Zafarani-Moattar, M. T.; Sadeghi, R. Phase Diagram Data for Several PPG + Salt Aqueous Biphasic Systems at 25 °C. *J. Chem. Eng. Data* **2005**, *50*, 947–950.

(8) Vernau, J.; Kula, M. R. Extraction of Proteins from Biological Raw Material Using Aqueous PEG/citrate Phase Systems. *Biotechnol. Appl. Biochem.* **1990**, *12*, 397–404.

(9) Salabat, A.; Shamshiri, L.; Jahanbin Sardarodi, J. Liquid-liquid Equilibrium Data, Viscosities, and Densities of Aqueous Mixtures of Poly(propylene glycol) with Tri-sodium Citrate at 25 °C. *J. Chem. Eng. Data* **2005**, *50*, 154–156.

(10) Hey, M. J.; Jackson, D. P.; Yan, H. The salting-out effect and phase separation in aqueous solutions of electrolytes and poly(ethylene glycol). *Polymer* **2005**, *46*, 2567–2572.

(11) Zafarani-Moattar, M. T.; Sadeghi, R. Liquid-liquid Equilibria of Aqueous Two-Phase Systems Containing Polyethylene Glycol and Sodium Dihydrogen Phosphate or Disodium Hydrogen Phosphate. Experiment and Correlation. *Fluid Phase Equilib.* **2001**, *181*, 95–112.

(12) Zafarani-Moattar, M. T.; Sadeghi, R. Measurement and Correlation of Liquid-liquid Equilibria of the Aqueous Two-Phase System Polyvinylpyrrolidone-Sodiu Dihydrogen Phosphate. *Fluid Phase Equilib.* **2002**, *203*, 177–191.

(13) Zafarani-Moattar, M. T.; Sadeghi, R. Phase Behavior of Aqueous Two-Phase PEG + NaOH System at Different temperatures. *J. Chem. Eng. Data* **2004**, *49*, 297–300.

(14) Merchuk, J. C.; Andrews, B. A.; Asenjo, J. A. Aqueous Two-Phase Systems for Protein Separation: Studies on Phase Inversion. *J. Chromatogr. B* **1998**, *711*, 285–293.

(15) Huddleston, J. G.; Willauer, H. D.; Rogers, R.D. Phase Diagram Data for Several PEG + Salt Aqueous Biphasic Systems at 25°C. *J. Chem. Eng. Data* **2003**, *48*, 1230–1236.

(16) Zafarani-Moattar, M. T.; Hamzehzadeh, S. Liquid-liquid Equilibria of Aqueous Two-Phase Systems Containing Poly(ethylene glycol) and Sodium Succinate or Sodium Formate. *Calphad* **2005**, *29*, 1–6.

(17) Zafarani-Moattar, M. T.; Sadeghi, R.; Hamidi, A. A. Liquid-Liquid Equilibria of an Aqueous Two-Phase System Containing Polyethylene Glycol and Sodium Citrate: Experiment and Correlation. *Fluid Phase Equilib.* **2004**, *219*, 149–155.

(18) Zafarani-Moattar, M. T.; samadi, F.; Sadeghi, R. Volumetric and ultrasonic studies of the system (water + polypropylene glycol 400) at temperatures from (283.15 to 313.15) K. *J. Chem. Thermodyn.* **2004**, *36*, 871–875.

(19) Sadeghi, R.; Ziamajidi, F. Thermodynamic properties of aqueous polypropylene oxide 400 solutions from isopiestic measurements over a range of temperatures. *Fluid Phase Equilib.* **2006**, *249*, 165–172.

(20) Setschenow, J. Über die Konstitution der Salzlösungen auf Grund ihres Verhaltens zu Kohlensäure. *Z. Phys. Chem.* **1889**, *4*, 117–125.

(21) Ananthapadmanabhan, K. P.; Goddard, E. D. Aqueous biphasic formation in polyethylene oxide-inorganic salt systems. *Langmuir* **1987**, *3*, 25–31.

(22) Zaslavsky, B. Y.; Gulaeva, N. D.; Djafarov, S.; Masimov, E. A.; Miheeva, L. M. Phase separation in aqueous poly(ethylene glycol)-$(NH_4)_2SO_4$ systems and some physicochemical properties of the phases. *J. Colloid Interface Sci.* **1990**, *137*, 147–156.

(23) Parsegian, V. A.; Rand, R. P.; Rau, D. C. Osmotic stress, crowding, preferential hydration, and binding: A comparison of perspectives. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3987–3992.

(24) Sadeghi, R.; Ziamajidi, F. Vapor-liquid equilibria of binary tri-potassium citrate + water and ternary polypropylene oxide 400 + tri-potassium citrate + water systems from isopiestic measurements over a range of temperatures. *Fluid Phase Equilib.* **2007**, *255*, 46–54.

(25) Burakowski, A.; Gliński, J. Hydration numbers of non-electrolytes - Application of the acoustic method of Pasynski. *Chem. Phys.* **2007**, *332*, 336–340.

(26) Sadeghi, R. Measurement and correlation of phase equilibria for several PVP + salt aqueous two-phase systems at 303.15K. *Fluid Phase Equilib.* **2005**, *237*, 40–47.

(27) Al-Sagheer, F.; Hey, M. J. Hofmeister anion effects on aqueous solutions of poly (ethylene oxide) studied by attenuated total reflectance FT-IR spectroscopy. *Colloids Surfaces A: Physicochem. Eng. Aspects* **2004**, *245*, 99–103.

Received for review September 22, 2007. Accepted December 15, 2007.

JE700549U