J. Chem. Eng. Data **2009,** *54,* 1359–1366

Phase Compositions, Molar Mass, and Temperature Effect on Densities, Viscosities, and Liquid−Liquid Equilibrium of Polyethylene Glycol and Salt-Based Aqueous Two-Phase Systems

Muthiah Perumalsamy*,† and Thanapalan Murugesan‡

Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli-620015, India, and Chemical Engineering Programme, Universiti Teknologi PETRONAS, Bandar Seri Iskandar, 31750, Tronoh, Perak, Malaysia

---

Densities and viscosities of aqueous solutions of polyethylene glycol 4000 [mass fraction from (5 to 50) %] have been measured at different temperatures, (298.15, 303.15, 308.15, 313.15, and 318.15) K. The density data show a linear variation with mass fraction of the polymer for all temperatures. The viscosity data of PEG 4000 solutions were correlated as a function of mass fraction, using a nonlinear equation, for the five different temperatures covered in the present work. Densities, viscosities, and liquid−liquid equilibria of polyethylene glycol−sodium citrate two-phase systems have been measured and are correlated with compositions at (298.15, 308.15, and 318.15) K. The effect of tie line lengths on density and viscosity of the aqueous two-phase systems has also been represented. The tie lines were correlated using the Othmer−Tobias and Bancraft equations as well as the virial equation. The coefficients were estimated and reported. The mean deviations between the experimental and calculated equilibrium compositions in the top and bottom phases were < 1 %.

Introduction

Aqueous two-phase systems (ATPS) provide an environmentally benign technique for the separation and purification of biological materials like proteins, enzymes, plant cells and animal cells, viruses, chloroplasts, and nucleic acids.[1] Aqueous two-phase systems are formed when two mutually incompatible water-soluble polymers or one polymer and one inorganic salt are dissolved in water above a threshold concentration.[1] These systems contain mainly water with the first polymer predominating in one phase and the second polymer or salt predominating in the other phase. Due to their high water content and nontoxicity, polyethylene glycol (PEG) based aqueous two-phase systems provide an environmentally safe process for the purification of biological materials and at the same time without affecting its activity. PEG finds a wide range of applications in the automotive, petroleum, textile, and food industries. For the large-scale implementation of ATPS, it is necessary to study the physical properties and phase behavior of the systems. Despite the success of the ATPS separation techniques, data on the physical properties of PEG at various concentrations and temperatures are necessary for the fundamental understanding of the phase forming ability and also for the development of theoretical models for the prediction of the partitioning behavior of the two-phase system.

Polymer−salt based aqueous two-phase systems have advantages over polymer−polymer based systems because of their low viscosity, low cost, rapid phase separation, etc. Several systems have been studied on polymer−polymer based aqueous two-phase systems and reported by the Albertson research group.[1] Most of the research work on aqueous polymer salt systems made use of polyethylene glycol with salt consisting of phosphate, sulfate, or carbonates as anions. These salts, however, led to a high concentration of sulfate and phosphate salts in the effluent streams, causing environmental problems. Recently, it was found that anion-like citrate can be used as a substitute for phosphate and sulfate salts to form an aqueous two-phase system with PEG.[2−5] Since citrates are biodegradable and nontoxic, PEG + citrate salts could form environmentally safe aqueous two-phase systems, which are more suitable for the extraction of biological materials such as proteins. However, very few researchers have studied the phase equilibrium for PEG−citrate systems. Recently Oliveira et al.[6] studied the effect of temperature and molar mass on phase diagrams of systems composed of polyethylene glycol + sodium citrate + water. Alves et al.[7] Studied the composition of the coexisting liquid phases of the system (water + polyethylene glycol + sodium citrate) at 298.15 K for three polyethylene glycols of different molar masses of about (600, 1500, and 3000) g·mol^{-1}. The new experimental data and literature are described using the VERS model for the excess Gibbs energy of aqueous solutions of neutral polymers and strong electrolytes. Tubío et al.[8] studied the phase diagrams corresponding to systems of sodium citrate and polyethylene glycol of different molar masses of (600, 1000, 1450, 3350, and 8000) g·mol^{-1} determined at (295.15, 310.15, and 323.15) K. Moreover, the pH (5.2, 8.2, and 9.2) effect was also studied. Zafarani-Moattar et al.[4] (2004) studied liquid−liquid equilibrium data for the aqueous PEG 6000−sodium citrate system at different temperatures. Vernau and Kula[2] reported equilibrium compositions for PEG 1550 + sodium citrate + water. The experiments were conducted at room temperature, and the temperature effects were not reported. Liquid−liquid equilibrium data for the aqueous PEG 2000 + sodium citrate + water and PEG 6000 + sodium citrate + water at (298.15, 308.15, and 318.15) K and at (293.15, 303.15, and 313.15) K, respectively, were determined and reported in the previous articles[3,5] by our research group.

---

* Corresponding author. E-mail: mpsamy@nitt.edu.
† National Institute of Technology.
‡ Universiti Teknologi PETRONAS.

10.1021/je801004n CCC: $40.75

Table 1. Densities and Viscosities of the PEG 4000 (p) + Water System at Various Temperatures

w_p	ρ/kg·m^{-3}	$100(\rho - \rho_{cal})/\rho$	η/mPa·s	$100(\eta - \eta_{cal})/\eta$
		T = 298.15 K		
0.00	997	0.00	0.894	0.00
0.05	1004.87	−0.09	1.689	−1.03
0.10	1013.27	−0.12	2.271	−0.56
0.15	1022.19	−0.10	2.921	−1.36
0.20	1031.60	−0.03	4.161	2.12
0.25	1040.37	−0.03	6.120	2.66
0.30	1049.24	−0.01	9.170	2.42
0.35	1058.54	0.04	13.640	1.90
0.40	1067.56	0.07	19.378	−1.11
0.45	1076.12	0.05	27.431	−1.77
0.50	1083.94	−0.03	39.297	1.54
		T = 308.15 K		
0.00	995.60	0.00	0.768	0.00
0.05	1003.14	−0.11	1.661	1.20
0.10	1011.68	−0.13	2.166	0.46
0.15	1020.82	−0.08	2.653	0.75
0.20	1029.76	−0.05	3.399	0.18
0.25	1038.30	−0.07	4.857	2.06
0.30	1047.54	−0.01	7.076	0.42
0.35	1056.38	0.00	10.721	1.31
0.40	1065.62	0.05	15.882	1.26
0.45	1074.46	0.07	22.992	1.39
0.50	1082.50	0.01	32.599	2.24
		T = 318.15 K		
0.00	994	0.00	0.723	0.00
0.05	1001.35	−0.10	1.560	0.44
0.10	1009.39	−0.14	1.991	0.29
0.15	1017.87	−0.13	2.325	0.62
0.20	1026.73	−0.09	2.879	2.11
0.25	1035.01	−0.10	3.869	1.90
0.30	1044.12	−0.03	5.638	1.84
0.35	1052.84	0.00	8.254	−0.83
0.40	1062.09	0.08	12.186	−2.17
0.45	1070.97	0.13	17.849	−2.01
0.50	1077.89	−0.01	26.414	2.01

Since the polyethylene glycol plays a vital role in the formation of aqueous two-phase systems, in the present work densities and viscosities of aqueous PEG 4000 solutions with mass fractions from (5 to 50) % and of PEG 4000 with varying sodium citrate compositions, in both the single and two-phase systems, have been measured, and the temperature effects were studied. The obtained data were correlated with suitable empirical models. The binodal data for the PEG 4000 + sodium citrate + water system at different temperatures of (298.15, 308.15, and 318.15) K were measured and represented with a simple polynomial equation. The tie line data were determined and correlated using the Othmer−Tobias and Bancraft equations as well as the virial equation. The molar mass effect on liquid−liquid equilibria of the ATPS is also discussed.

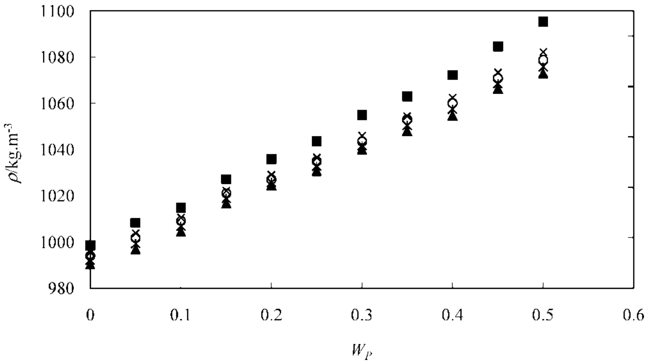

Figure 1. Effect of mass fraction of PEG 4000 (w_p) on densities of aqueous PEG 4000 solutions: ■, 298.15 K; ×, 303.15 K; ○, 308.15 K; *, 313.15 K; ▲, 318.15 K.

Table 2. Coefficients of Equation 2

	T/K				
constants	298.15	303.15	308.15	313.15	318.15
A/kg·m^{-3}	172	170	167	166.4	165
ρ_0/kg·m^{-3}	997.0	995.6	994.0	992.2	990.2

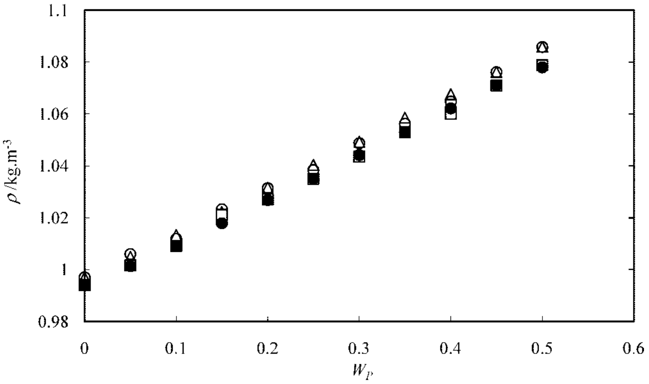

Figure 2. Effect of molar mass of PEG on densities of aqueous PEG solutions: ○, 298.15 K; ●, 308.15 K, PEG 2000 (Murugesan and Perumalsamy, 2005); Δ, 298.15 K; □, 308.15 K, PEG 4000 (Experimental).

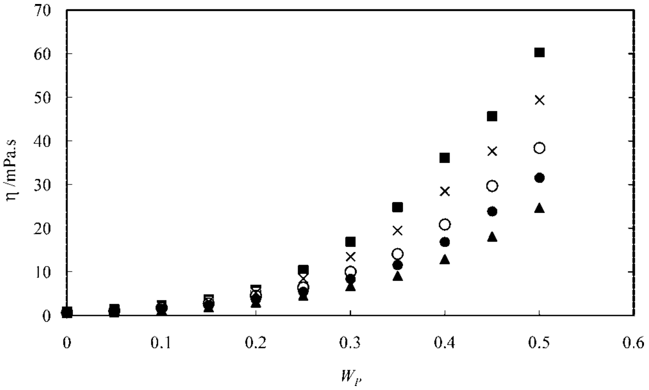

Figure 3. Effect of mass fraction of PEG 4000 (w_p) on viscosities of aqueous PEG 4000 solutions: ■, 298.15 K; ×, 303.15 K; ○, 308.15 K; ●, 313.15 K; ▲, 318.15 K.

Table 3. Coefficients of Equation 3

T/K	A/mPa·s	B/mPa·s	η_0/mPa·s	R^2
298.15	316.80	−33.540	0.893	0.997
303.15	246.89	−28.868	0.808	0.997
308.15	210.50	−24.190	0.722	0.991
313.15	157.12	−19.463	0.661	0.993
318.15	130.70	−14.720	0.598	0.989

Experimental Section

Analytical grade (Merck) polyethylene glycol (poly(oxyethylene)) with a molar mass average of 4000 (cat. no: 8.07490.1000) and tribasic sodium citrate dehydrate (trisodium 2-hydroxypropane-1,2,3-tricarboxylate dehydrate) with minimum 99 % purity (cat. no: 61770905001046) were used. Polymer was in powder form, and the polydispersity index was 1.05. Double distilled, deionized water was used for the preparation of aqueous two-phase systems.

Apparatus and Procedures. The binodal curve was determined by the cloud point method and is discussed in the previous article in detail.[3,5] The aqueous solutions were prepared by mass, using an analytical balance (OHAUS-Essae-Teraoka Ltd., Japan, model AR2140) with an accuracy

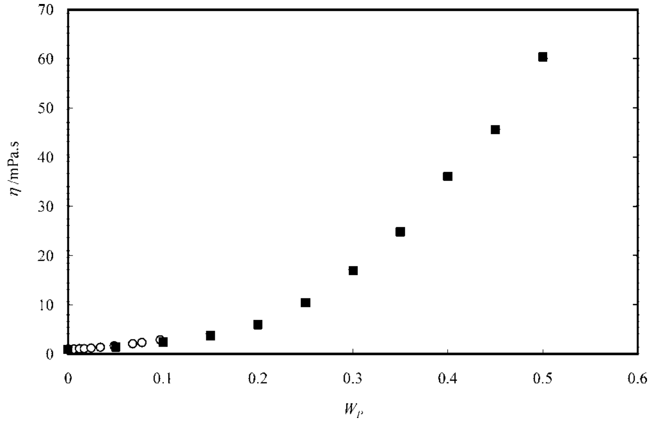

Figure 4. Comparison of experimental data with literature data on viscosity: ■, experimental; ○, Stanislava Kirincic and Cveto klofutar (1999).

of ± 0.1 mg. Aqueous two-phase systems were prepared in a 50 cm^3 centrifuge tube and brought into a Schott-Gerate CT 52 (Germany) thermostatic bath to maintain appropriate temperature with an uncertainty of 0.1 K. The systems were stirred and well mixed and allowed to settle, to ensure the proper phase separation at a desired temperature, as indicated by the absence of turbidity in each phase. Samples of the top phase were taken using a pipet, and samples of the bottom phase were also taken through the top phase using a pipet by maintaining a small positive pressure to avoid contamination. Then, the densities and viscosities of both the phases were measured. The density measurements were carried out at (298.15, 308.15, and 318.15) K using a 5 cm^3 glass pycnometer.[9] The pycnometer was filled with the solution and immersed in the water bath. After thermal equilibrium was achieved, the pycnometer was removed from the water bath and then cleaned and dried quickly. Densities were determined from the measurements of the mass of the samples and the pycnometer. The volume of the pycnometer was calibrated as a function of temperature using deionized water. The density of water was taken from Perry's Chemical Engineering Handbook (Perry and Green,[10] 2007). The uncertainty of density measurements was estimated to be ± 0.1 Kg·m^{-3}

Viscosities of the aqueous PEG 4000 solutions and that of the top phase of the aqueous two-phase systems were determined using an Ostwald viscometer at (298.15, 303.15, 308.15, 313.15, and 318.15) K in a water bath. The uncertainty of measurements was ± 0.002 mPa·s. The viscosities of the bottom phase of the aqueous two-phase system were nearly the same as that of water, and hence it was not measured. For the determination of tie line compositions, the concentrations of sodium citrate in the top and bottom phases were determined using flame photometry.[3,5] The concentrations of PEG 4000 in both phases were determined by refractive index measurements. The relation between the refractive index, n, and the mass fraction of polymer, w_p, and that of salt, w_s is given by

$$n = a_0 + a_1 w_p + a_2 w_s \tag{1}$$

which was originally suggested by Cheluget et al.[11] for the polyethylene glycol + NaCl + water system, and later it was successfully used for the polyethylene glycol 2000 + sodium citrate + water system[2] and also used by Zafarani-Moattar et al.[4] for the polyethylene glycol 6000 + sodium citrate + water system. Hence the above equation was applied for the present PEG 4000 + sodium citrate + water system. The parameters

Table 4. Densities of the PEG 4000 (p) + Sodium Citrate (s) + Water System at Various Temperatures

		T = 298.15 K		T = 308.15 K		T = 318.15 K	
w_p	w_s	ρ/kg·m^{-3}	$100(\rho - \rho_{cal})/\rho$	ρ/kg·m^{-3}	$100(\rho - \rho_{cal})/\rho$	ρ/kg·m^{-3}	$100(\rho - \rho_{cal})/\rho$
0.00	0.02	1011.17	−0.09	1006.82	−0.10	1002.82	−0.08
0.05	0.02	1019.33	0.01	1015.58	−0.03	1012.06	0.06
0.10	0.02	1028.35	0.02	1024.93	0.01	1020.67	0.06
0.15	0.02	1037.51	0.16	1034.84	0.21	1029.11	0.23
0.20	0.02	1045.88	0.18	1043.64	0.20	1037.75	0.34
0.25	0.02	1056.29	0.12	1052.13	0.16	1046.21	0.22
0.30	0.02	1064.94	0.10	1061.58	0.21	1054.81	0.03
0.35	0.02	1072.36	−0.05	1069.70	0.05	1062.57	−0.04
0.40	0.02	1083.44	0.15	1078.02	0.00	1069.80	−0.17
0.00	0.04	1022.64	−0.01	1018.18	−0.17	1014.15	−0.23
0.05	0.04	1030.26	−0.14	1028.82	0.04	1024.15	0.01
0.10	0.04	1040.71	0.01	1036.21	−0.10	1033.12	0.04
0.15	0.04	1048.43	−0.11	1045.41	−0.07	1040.73	−0.05
0.20	0.04	1057.67	−0.08	1054.27	−0.07	1048.63	−0.12
0.25	0.04	1066.47	−0.09	1064.23	0.03	1059.26	0.08
0.30	0.04	1075.68	−0.07	1071.52	−0.12	1065.76	−0.12
0.35	0.04	1084.22	−0.10	1081.48	−0.32	1073.25	−0.22
0.00	0.06	1034.37	−0.08	1030.93	−0.10	1026.46	−0.16
0.05	0.06	1043.09	−0.10	1039.58	−0.12	1035.59	−0.10
0.10	0.06	1052.71	−0.04	1049.23	−0.04	1044.88	−0.03
0.15	0.06	1061.27	−0.07	1059.24	0.06	1054.86	0.10
0.20	0.06	1070.83	−0.01	1067.73	0.03	1063.45	0.10
0.25	0.06	1080.27	0.03	1076.12	−0.02	1070.78	−0.02
0.00	0.08	1048.05	0.04	1043.24	−0.11	1039.68	−0.28
0.05	0.08	1056.72	0.01	1053.19	−0.01	1047.26	−0.19
0.10	0.08	1065.59	0.00	1063.21	0.10	1057.83	0.00
0.15	0.08	1074.88	0.03	1072.39	0.13	1067.70	0.12
0.20	0.08	1083.42	0.00	1080.33	0.04	1075.43	0.04
0.25	0.08	1089.85	−0.23	1087.05	−0.25	1081.46	−0.20
0.00	0.10	1061.93	0.17	1058.92	0.20	1054.67	0.15
0.05	0.10	1070.35	0.12	1066.71	0.09	1063.39	0.15
0.10	0.10	1078.78	0.07	1074.87	0.03	1071.82	0.13
0.15	0.10	1087.44	0.04	1083.89	0.04	1080.46	0.14

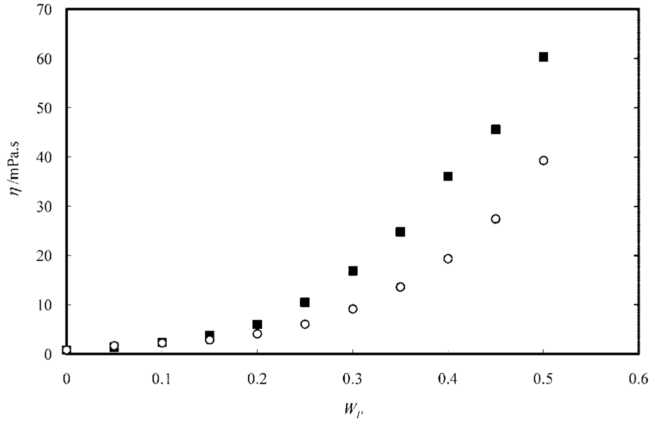

Figure 5. Effect of molar mass of PEG on viscosities of aqueous PEG solutions at 298.15 K: ■, PEG 4000 (Experimental); ○, PEG 2000 (Murugesan and Perumalsamy, 2005).

of eq 1 were evaluated by the same method of calibration plots used by Cheluget et al.[11] The estimated values of coefficients a_0, a_1, and a_2 for the present system were 1.3332, 0.1411, and 0.1478, respectively. The uncertainties of the estimated mass fraction of sodium citrate and PEG were ± 0.001 and ± 0.002, respectively.

Results and Discussion

Effect of Phase Compositions, Temperature, and Molar Mass on Densities and Viscosities of Aqueous PEG 4000 Solution. The densities and viscosities of aqueous solutions of PEG 4000 at various temperatures are given in Table 1 and are plotted in Figure 1. The densities are found to increase with an increase in PEG 4000 concentrations and decrease with an increase in temperature. Teng et al. have measured the densities of PEG 2000 solutions at 298.15 K and observed the same trend.[12] In the previous article, the densities and viscosities of aqueous PEG 2000 at different temperatures were presented, and it was observed that densities are increasing with an increase in PEG concentration and decreasing with an increase in temperature.[9] The densities of aqueous PEG 4000 solutions could be correlated by using the following equation

$$\rho = \rho_0 + Aw_p \tag{2}$$

where ρ is the density of the solution; ρ_0 is the density of the pure water at the corresponding temperature; and w_p is the mass fraction of PEG 4000. The determined constant A and ρ_0 are given in Table 2. The average relative deviation is less than 1 %. The effect of molar mass on density is represented in Figure 2. From the figure, it is observed that the density values are independent of the PEG molar mass ranges from (2000 to 6000) g·mol^{-1} and vary in a linear manner with solute concentration. Gonzalez-Tello et al.[13] observed a similar result that density values do not vary with molar mass ranges from (1000 to 8000) g·mol^{-1}.

The viscosity data for aqueous solution of PEG 4000 are presented in Table 1 and are plotted in Figure 3. It is observed that the viscosities increase with an increase in PEG 4000 concentration and decrease with an increase in temperature. Kirincic et al.[14] have successfully used a third-order polynomial for representing their viscosity data with respect to mass fraction of the PEG 4000 and nonelectrolyte system. Hence in the present work, a simple second-order polynomial of the following form is used to represent the present viscosity data on the PEG 4000−sodium citrate−water system.

$$\eta = Aw_p^2 + Bw_p + \eta_0 \tag{3}$$

where η is the absolute viscosity of the solution and η_0 is the viscosity of water at the corresponding temperature. The constants A, B, and C are the coefficients of the polynomial and are determined by regression analysis. These values along with the average absolute relative deviation (AARD) are given in Table 3. Figure 4 shows a close agreement between the present and literature data (Kirincic et al.[14]) on viscosity. The viscosities increase with an increase in molar mass of PEG at all temperatures and are shown in Figure 5, as an example at 298.15 K. A similar trend was observed by Le-He-Mei et al.[15] for the range of PEG molar mass from (1000 to 20 000) g·cm^{-3}.

Effect of Phase Compositions, Temperature, and Molar Mass on Densities and Viscosities of the Aqueous PEG 4000

Table 5. Coefficients of Equation 4

T/K	A/kg·m^{-3}	B/kg·m^{-3}	ρ_0/kg·m^{-3}
298.15	178.4	624.43	997.8
308.15	1782	627.30	994.2
318.15	1700	636.22	990.1

Table 6. Binodal Data for PEG 4000 (p) + Sodium Citrate (s) + Water at Different Temperatures

298.15 K		308.15 K		318.15 K	
$100w_p$	$100w_s$	$100w_p$	$100w_s$	$100w_p$	$100w_s$
PEG-rich phase		PEG-rich phase		PEG-rich phase	
44.00	2.15	41.20	2.16	43.45	1.64
40.85	2.56	38.74	2.54	39.25	2.26
37.54	3.17	36.56	2.93	36.62	2.53
36.11	3.35	32.95	3.43	33.58	2.88
32.41	4.05	28.15	4.34	31.34	3.29
31.65	4.36	25.21	5.15	28.55	3.76
29.17	4.99	22.91	5.60	26.26	4.20
25.95	5.68	20.24	6.20	24.37	4.68
25.93	5.78	18.31	6.82	22.83	5.06
24.55	6.05	16.28	7.65	21.18	5.40
22.44	6.63	14.41	8.33	19.57	5.93
20.80	7.12	12.78	8.94	18.45	6.33
19.69	7.61	11.89	9.22	17.62	6.58
17.74	8.34	11.11	9.74	16.58	6.72
16.64	8.65	10.71	9.95	15.83	6.93
15.57	9.17	salt-rich phase		15.02	7.15
13.95	9.76	9.17	10.73	14.48	7.37
salt-rich phase		8.29	11.16	13.80	7.52
12.93	10.23	7.82	11.25	13.24	7.70
10.95	11.06	6.46	12.03	12.79	7.78
10.35	11.28	5.68	12.43	12.22	7.96
10.20	11.36	5.12	12.76	11.34	8.19
9.42	11.87	4.55	13.01	10.40	8.50
9.02	12.18	1.89	18.00	9.56	8.75
8.51	12.49	1.35	20.50	8.81	8.98
7.93	12.99	1.35	20.50	8.24	9.37
7.43	13.44	0.96	22.00	7.57	9.78
6.86	13.79			salt-rich phase	
6.21	14.05			7.04	10.17
5.78	14.42			5.90	10.66
4.03	16.05			4.85	11.17
3.31	17.19			3.88	11.67
3.47	18.41			3.10	11.96
2.70	20.41			2.38	12.44
2.35	22.65			1.85	12.97
1.47	25.43			1.78	13.64
				1.86	15.90
				1.75	17.20
				1.48	19.00
				0.57	21.80

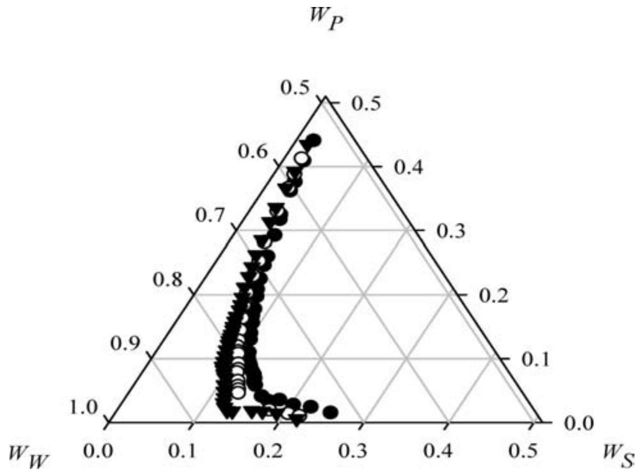

Figure 6. Binodal curves for PEG 4000 (w_p) + sodium citrate (w_s) + water (w_w) at different temperatures: ●, 298.15 K; ○, 308.15 K; ▼, 318.15 K.

Table 7. Parameters of Equation 5 for PEG 4000 + Sodium Citrate + Water at Different Temperatures

T/K	A	B	C	D	R^2
298.15	−43.740	29.570	−6.851	0.564	0.999
308.15	−62.840	37.330	−7.660	0.555	0.998
318.15	−70.370	42.170	−8.329	0.552	0.997

+ Sodium Citrate System. The experimental densities of PEG 4000 + sodium citrate + water solutions at various temperatures are given in Table 4. It is observed that the densities increase with increasing both PEG 4000 and sodium citrate concentration and decrease with an increase in temperature. These data could be correlated as follows

$$\rho = Aw_p + Bw_s + \rho_0 \tag{4}$$

where w_p and w_s are the mass fractions of polymer and salt, respectively. The constants A and B are dependent on the temperature and are given in Table 5.

Effect of Phase Compositions, Temperature and Molar Mass on Liquid−liquid Equilibrium of Aqueous PEG + Sodium Citrate System. The phase equilibrium for an aqueous two-phase system can be represented as a binodal curve relating the concentration of the salt and polyethylene glycol in the corresponding phases. The binodal data for PEG 4000 + sodium citrate + water is given in Table 6. The binodal curves at different temperature are represented in Figure 6. From the figure, it is observed that the two-phase area is expanded with increasing temperature, which is in good agreement with the reported experimental results for the other aqueous two-phase systems.[2−6] In the previous studies, Graber et al.[16] and Taboada et al.[17] have used two nonlinear expressions to fit the binodal data of aqueous two-phase systems. It was observed that the binodal is found to vary with temperature. An attempt was made to fit the binodal data using different expressions, including those used previously; however, the better results were obtained with the following third-order polynomial equation

$$w_p = Aw_s^3 + Bw_s^2 + Cw_s + D \tag{5}$$

where w_p and w_s are the mass fractions of PEG 4000 and sodium citrate, respectively. The parameters of eq 5 at different temperatures are given in Table 7. In the previous article,[3] the following equation was used to fit the binodal data for the PEG 2000 + sodium citrate + water system.

$$w_p = A + Bw_s^{0.5} + Cw_s \tag{6}$$

The constants A, B, and C were found to be functions of temperature and were correlated with temperature by empirical equations. Experimental data were fitted well with the equations.

Tie line data for the PEG 4000 + sodium citrate + water system is given in Table 8 along with tie line length, L_T, viscosities of the top phase, and densities of the top and bottom phases. The tie line lengths are estimated using the following relationship[5]

$$L_T = [(w_{p(T)} - w_{p(B)})^2 + (w_{s(T)} - w_{s(B)})^2]^{1/2} \tag{7}$$

It is observed that the tie line length, L_T, increases with increasing temperature and PEG compositions, which are in good agreement with the observations of Voros et al.,[18] Zafarani-Moattar, and Sadeghi.[19] The effect of temperature on tie line composition for the total composition of $w_p = 0.2$, $w_s = 0.125$ at different temperatures of (298.15, 308.15, and 318.15) K is shown in Figure 7. From the figure, it was observed that the slope of the tie line increases with increasing temperature as reported by Voros et al.,[18] Murugesan and Perumalsamy,[3] and Zafarani-Moattar et al.[4] for other aqueous PEG + salt systems.

Table 8. Tie Line Data for PEG 4000 (p) + Sodium Citrate (s) + Water at Different Temperatures

top phase		bottom phase			ρ/kg·m^{-3}			
w_p	w_s	w_p	w_s	L_T	ρ_T/kg·m^{-3}	ρ_B/kg·m^{-3}	$\Delta\rho$/kg·m^{-3}	η_T/mPa·s
				T = 298.15 K				
0.2615	0.0610	0.0337	0.1841	0.2589	1098.3	1145.6	45.3	9.722
0.3060	0.0500	0.0270	0.2041	0.3187	1098.5	1160.7	60.2	13.923
0.3502	0.0395	0.0235	0.2265	0.3764	1100.2	1171.8	70.6	16.197
0.3863	0.0310	0.0147	0.2543	0.4335	1100.8	1189.0	86.2	19.255
				T = 308.15 K				
0.2647	0.0500	0.0295	0.1480	0.2548	1089.9	1117.9	28.0	6.668
0.3150	0.0400	0.0189	0.1800	0.3275	1089.8	1135.9	46.1	8.899
0.3515	0.0350	0.0135	0.2050	0.3783	1091.7	1145.6	53.9	12.045
0.3901	0.0275	0.0096	0.2200	0.4264	1091.7	1158.9	67.2	14.258
				T = 318.15 K				
0.2876	0.0390	0.0186	0.1590	0.2946	1076.8	1111.2	27.4	5.178
0.3250	0.0330	0.0175	0.1720	0.3375	1078.6	1124.1	38.5	6.931
0.3706	0.0270	0.0148	0.1900	0.3914	1079.2	1132.2	46.0	9.725
0.4176	0.0210	0.0057	0.2180	0.4566	1081.3	1169.8	63.5	12.083

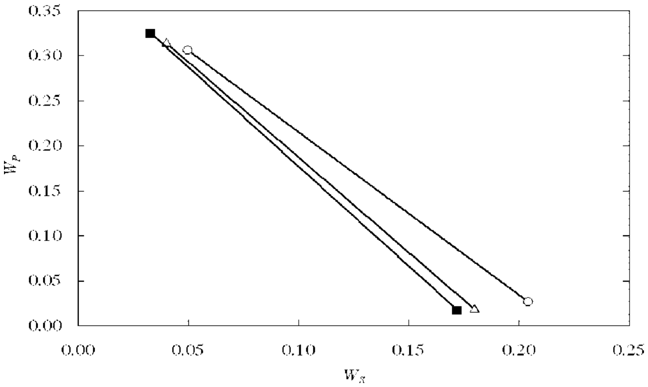

Figure 7. Effect of temperature on the equilibrium phase compositions for PEG 4000 (w_p) + sodium citrate (w_s) + water. These tie lines were obtained by connecting the experimental equilibrium phase composition for the total composition ($w_p = 0.2$, $w_s = 0.125$): −○−, 298.15 K; −Δ−, 308.15 K; −◆−, 318.15 K.

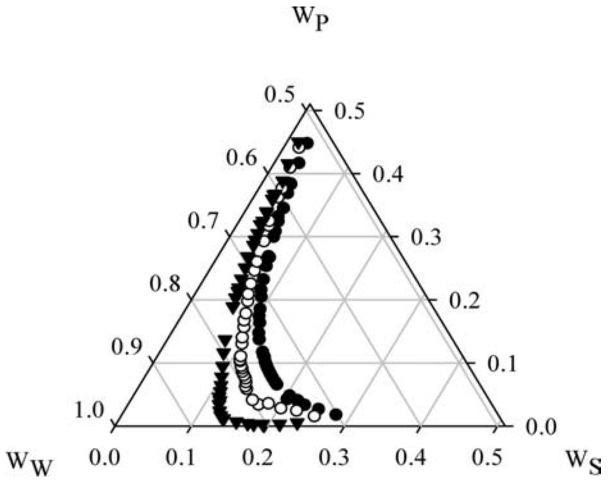

Figure 8. Binodal curves for PEG 4000 (w_p) + sodium citrate (w_s) + water (w_w) for different PEG molar masses: ●, PEG 2000 (Murugesan and Perumalsamy,[3] 2005); ○, PEG 4000 (Experimental); ▼, PEG 6000 (Zafarani-Moattar et al.[4]).

Hence the polyethylene glycol + sodium citrate + water system forms two phases at high temperatures more easily; that is, a smaller mass fraction of polymer or salt is sufficient for phase formation. The effect of molar mass on binodal curves for PEG + sodium citrate ATPS, at 298.15 K, is shown in Figure 8. From the figure, it is observed that the binodal curves shift to lower PEG and salt concentrations as the PEG molar mass increases. The PEG concentration in the bottom phase is decreased as the molar mass increases for the same tie line length system. Similarly the salt concentration is eliminated in the PEG-rich phase as PEG molar mass increases. A similar behavior was also reported by Tubío et al.[8] and Oliveira et al.[6] for PEG + sodium citrate systems. The hydrophobic character of the higher molar PEG increases when compared to smaller molar mass, which increases the incompatibility between the phase-forming components.

The density difference between the phases ($\Delta\rho$) and the viscosities of the top phases (η_T) are also given in Table 8. The density difference between the phases and the viscosities of the top phases increase with an increase in the tie line length and decrease with an increase in temperature. From Figures 9 and 10, it is observed that the density differences between the phases and the viscosities of the top phase show linear relationship with tie line length.

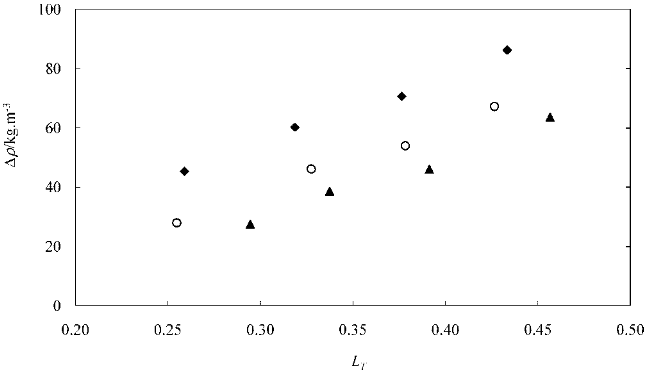

Figure 9. Relationship between density difference ($\Delta\rho$) and tie line length (L_T) for the PEG 4000 + sodium citrate + water two-phase system: ♦, 298.15 K; ○, 308.15 K; ▲, 318.15 K.

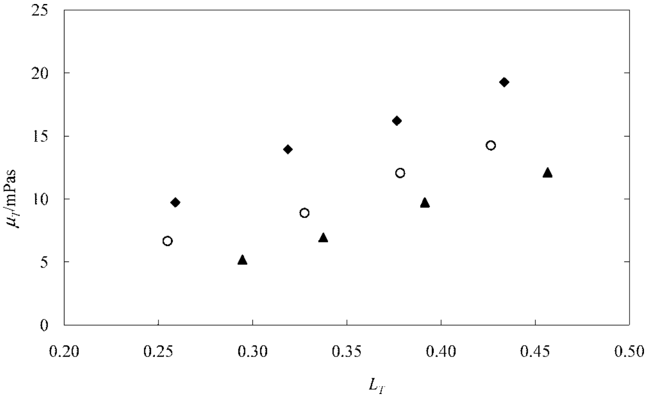

Figure 10. Relationship between viscosity of top phase (η_T) and tie line length (L_T) for the PEG 4000 + sodium citrate + water two-phase system: ♦, 298.15 K; ○, 308.15 K; ▲, 318.15 K.

Table 9. Parameters of Equations 8 and 9 for PEG 4000 (p) + Sodium Citrate (s) + Water at Different Temperatures

T/K	K	n	K_1	r	σ_p	σ_s
298.15	0.3434	1.3997	2.1888	0.7108	0.04	0.20
308.15	0.3783	1.1459	2.4281	0.8573	0.03	0.26
318.15	0.2079	1.4726	2.9680	0.6672	0.06	0.31

The tie-line compositions were correlated using the Othmer−Tobias[20] (eq 4) and Bancroft[21] (eq 5) equations as follows

$$[(1 - w_p^t)/w_p^t] = K[(1 - w_s^b)/w_s^b]^n \tag{8}$$

$$(w_w^b/w_s^b) = K_1(w_w^t/w_p^t)^r \tag{9}$$

where K, n, K_1, and r are the fit parameters. The superscript t and b represents the polymer-rich phase (top phase) and the salt-rich phase (bottom phase), respectively. The subscripts p and s stand for the PEG and salt, respectively. To determine the fit parameters, eqs 8 and 9 are linearized by taking the logarithm on both sides of the equations. The values of the fit parameters, K, n, K_1, and r, along with the mass percent standard deviations σ_p and σ_s are given in Table 9. Figures 11 and 12 show the plots of $\log[(1 - w_p^t)/w_p^t]$ against $\log[(1 - w_s^b)/w_s^b]$ and $\log(w_w^b/w_s^b)$ against $\log(w_w^t/w_p^t)$, respectively. Recently, eqs 8 and 9 have been successfully used for the correlation of tie line compositions of the PEG 2000 + sodium citrate + water system.[3]

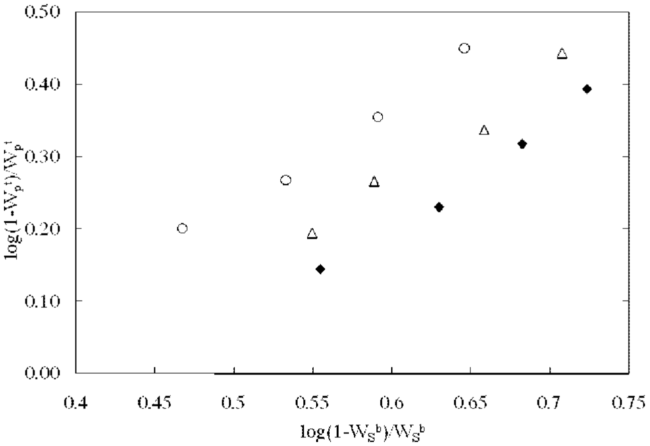

Figure 11. Linear dependency of the Othmer−Tobias equation: ○, 298.15 K; Δ, 308.15 K; ◆, 318.15 K.

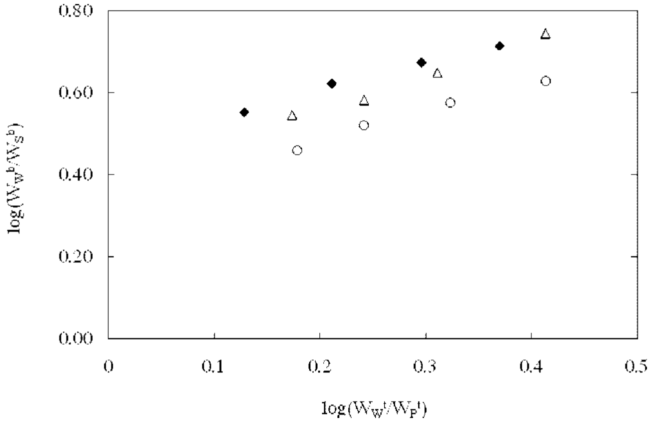

Figure 12. Linear dependency of the Bancroft equation: ○, 298.15 K; Δ, 308.15 K; ◆, 318.15 K.

The tie line data were also correlated using the virial equation as adopted by Edmond and Ogston.[21] These equations were successfully used for the PEG 2000 + sodium citrate + water system[3] and also by Mishima et al.[22] for aqueous two-phase systems. The virial equation relates the chemical potentials of polymer (p) and salt (s) as a function of molality of polymer and salt and can be written as

$$\mu_{\mathrm{p}} = \mu_{\mathrm{p}}^0 + RT(\ln m_{\mathrm{p}} + \beta_{\mathrm{pp}} m_{\mathrm{p}} + \beta_{\mathrm{ps}} m_{\mathrm{s}}) \qquad (10)$$

$$\mu_{\mathrm{s}} = \mu_{\mathrm{s}}^0 + RT(\ln m_{\mathrm{s}} + \beta_{\mathrm{ss}} m_{\mathrm{s}} + \beta_{\mathrm{ps}} m_{\mathrm{p}}) \qquad (11)$$

where β_{pp}, β_{ss}, and β_{ps} are the interaction parameters between a molecule of polymer (p) and salt (s), and μ_{p}^0 and μ_{s}^0 are the standard state chemical potential of polymer and salt, respectively. Using the Gibbs−Duhem relation, the chemical potential of water (w) is then obtained as

$$\mu_{\mathrm{w}} = \mu_{\mathrm{w}}^0 - RTV_3\rho\left(m_{\mathrm{p}} + m_{\mathrm{s}} + \frac{1}{2}(\beta_{\mathrm{pp}})\, m_{\mathrm{p}}^2 + \frac{1}{2}(\beta_{\mathrm{ss}})\, m_{\mathrm{s}}^2 + \beta_{\mathrm{ps}} m_{\mathrm{p}} m_{\mathrm{s}}\right) \qquad (12)$$

where μ_{w}^0 is the standard state chemical potential of pure water. The Gibbs energy is at a minimum when the system is in thermodynamic equilibrium. From the classical thermodynamics, it is obvious that the top and bottom phases of the aqueous two-phase system containing polymer (p), salt (s), and water (w) will obey the following constraints at equilibrium[3]

$$\mu_{\mathrm{p}}^{\mathrm{top}} = \mu_{\mathrm{p}}^{\mathrm{bot}} \qquad (13a)$$

$$\mu_{\mathrm{s}}^{\mathrm{top}} = \mu_{\mathrm{s}}^{\mathrm{bot}} \qquad (13b)$$

$$\mu_{\mathrm{w}}^{\mathrm{top}} = \mu_{\mathrm{w}}^{\mathrm{bot}} \qquad (13c)$$

Table 10. Interaction Parameters of the Virial Equation for the System PEG 4000 (p) + Sodium Citrate (s) + Water at Different Temperatures

T/K	β_{pp}	β_{ps}	β_{ss}
298.15	−45.1127	−11.2627	−5.6411
308.15	26.6533	16.8985	4.9642
318.15	88.4194	45.0596	17.5694

The interaction parameters are obtained by fitting the experimental LLE data to eq 13 using the objective function suggested by Zafarani-Moattar et al.[4] The evaluated interaction parameters are given in Table 10. To prove the reliability of predictions using eq 13, a comparison between the experimental and correlated tie lines for the temperature 298.15 K is shown in Figure 13. Figure 13 shows a good agreement of experimental binodal data with the experimental and predicted tie line data with an error of < 1 %.

Conclusions

The densities and viscosities of PEG 4000 aqueous solution were measured at different temperatures. The density data and viscosity data were represented with linear and nonlinear equations, respectively. The effect of tie line lengths on density and viscosity of the aqueous two-phase systems has also been studied. The binodal data and tie line compositions were measured at (298.15, 308.15, and 318.15) K. From the data, it was observed that the two-phase region is expanded with increasing temperature because of an increase in solubility of the phase-forming components. The binodal data are fitted with a polynomial equation relating the concentration of PEG and sodium citrate at different temperatures. The tie line data for the PEG 4000 + sodium citrate + water system was correlated satisfactorily by Othmer−Tobias and Bancroft equations as well as virial equations.

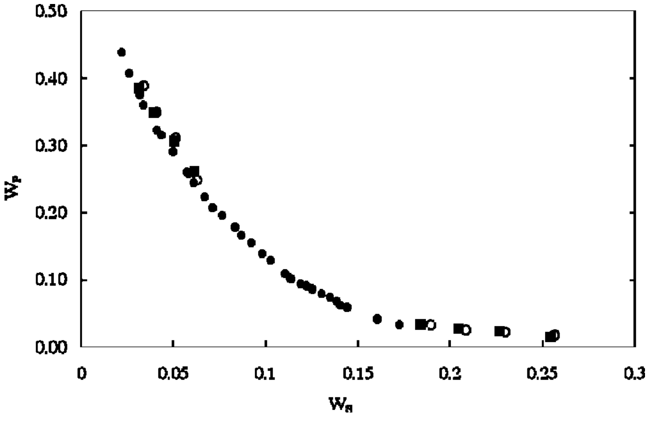

Figure 13. Binodal and tie lines for the PEG 4000 (w_{p}) + sodium citrate (w_{s}) + water system at 298.15 K: ●, Experimental binodal data; ■, experimental tie line data; ○, calculated tie line data using eq 13.

Literature Cited

(1) Albertsson, P. A. *Partition of Cell Particles and Macromolecules*, 3rd ed.; Wiley: New York, 1986.

(2) Vernau, J.; Kula, M. R. Extraction of Proteins from Biological Raw Materials Using Aqueous Poly(ethylene) glycol-Citrate Phase Systems. *Biotechnol. Appl. Biochem.* **1990**, *12*, 397–404.

(3) Murugesan, T.; Perumalsamy, M. Liquid-Liquid Equilibria of polyethylene glycol 2000 + Sodium citrate + Water at (25, 30, 35, 40, and 45) °C. *J. Chem. Eng. Data* **2005**, *50*, 1392–1395.

(4) Zafarani-Moattar, M. T.; Sadeghi, R.; Hamidi, A. A. Liquid-Liquid Equilibrium of an Aqueous Two Phase System containing Polyethylene glycol and Sodium citrate: Experimental and Correlation. *Fluid Phase Equilib.* **2004**, *219*, 149–155.

(5) Perumalsamy, M.; Bathmalakshmi, A.; Murugesan, T. Experimental and Correlation of liquid-liquid Equilibria of an Aqueous Two Phase System containing PEG6000 + Sodium citrate. *J. Chem. Eng. Data* **2007**, *52*, 1186–1188.

(6) Oliveira, R. M.; Coimbra, J. R.; Minim, L. A.; Mendes da Silva, L. H.; Ferreira Fontes, M. P. Liquid-Liquid Equilibria of Biphasic Systems Composed of Sodium Citrate + Polyethylene(glycol) 1500 or 4000 at Different Temperatures. *J. Chem. Eng. Data* **2008**, *53*, 895–899.

(7) Alves, G. L. F.; Brenneisen, J.; Ninni, L.; Meirelles, A. J. A.; Maurer, G. Aqueous Two-Phase Systems of Poly(ethylene glycol) and Sodium Citrate: Experimental Results and Modeling. *J. Chem. Eng. Data* **2008**, *53* (7), 1587–1594.

(8) Tubío, G.; Pellegrini, L.; Nerli, B. B.; Pico, G. A. Liquid-liquid equilibria of aqueous two-phase systems containing poly (ethylene glycols) of different molar mass and sodium citrate. *J. Chem. Eng. Data* **2006**, *51*, 209–212.

(9) Murugesan, T.; Perumalsamy, M. Densities and Viscosities of Polyethylene Glycol 2000 + Salt + Water Systems from (298.15 to 318.15) K. *J. Chem. Eng. Data* **2005**, *50*, 1290–1293.

(10) Perry, R. H.; Green, D. *Chemical Engineers' Handbook*, 7th ed.; McGraw-Hill New York, 1998. Water: Density at Atmospheric pressure and Temperatures from 0 to 100 C *Tables of Standard Handbook Data* Standartov: Moscow, 1978.

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

(12) Teng, P. S.; Teng, T. T. Measurement and Prediction of the Density of Aqueous Multicomponent Solutions Involving Polyethylene Glycol 2000. *J. Chem. Eng. Jpn.* **2004**, *37*, 40–44.

(13) González-Tello, P.; Camacho, F.; Blázquez, G. Density and Viscosity of Concentrated Aqueous Solutions of Polyethylene Glycol. *J. Chem. Eng. Data* **1994**, *39*, 611–614.

(14) Kirincic, S.; Klofutar, C. Viscosity of aqueous solutions of poly (ethylene glycol)s at 298.15K. *Fluid Phase Equilib.* **1999**, *155*, 311–325.

(15) Mei, L.; Lin, D.; Zhu, Z.; Han, Z. Densities and Viscosities of Polyethylene Glycol + Salt + Water Systems at 20 C. *J. Chem. Eng. Data* **1995**, *40*, 1168–1171.

(16) Graber, T. A.; Taboada, M. E.; Carton, A.; Bolado, S. Liquid-Liquid Equilibria of Poly(ethylene glycol) + Sodium Nitrate + Water System at 298.15 K. *J. Chem. Eng. Data* **2000**, *45*, 182–184.

(17) Taboada, M. E.; Asenjo, J. A.; Andrews, B. A. Liquid-Liquid and Liquid-Liquid-Solid Eqilibrium in Na2CO3-PEG-H2O. *Fluid Phase Equilib.* **2001**, *180*, 273–280.

(18) Voros, N.; Proust, P.; Fredenslund, A. Liquid-Liquid Equilibria of Aqueous Two Phase Systems containing Salts and Polyethylene Glycol. *Fluid Phase Equilib.* **1993**, *90*, 333–353.

(19) 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.

(20) Othmer, D. F.; Tobias, P. E. Toluene and Acetaldehyde Systems; Tie Line Correlation; Partial Pressures of Ternary Liquid Systems and the Prediction of Tie Lines. *Ind. Eng. Chem.* **1942**, *34*, 690–700.

(21) Edmond, E.; Ogston, A. G. An approach to the study of phase separation in ternary aqueous systems. *Biochem. J.* **1968**, *109*, 569–576.

(22) Mishima, K.; Nakatani, N.; Matsuyama, K.; Nagatani, M.; Nishikawa, H. Liquid-Liquid Equilibria of Aqueous two phase systems containing Polyethylene glycol and dipotassium hydrogen phosphate. *Fluid Phase Equilib.* **1995**, *107*, 269–276.

Received for review December 30, 2008. Accepted March 5, 2009.

JE801004N