Liquid−Liquid Equilibrium of Aqueous Two-Phase System Composed of Poly(ethylene glycol) 400 and Sulfate Salts

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Phase diagrams of aqueous two-phase systems (ATPS) composed of 400 $\text{g}\cdot\text{mol}^{-1}$ poly(ethylene glycol) (PEG) + sodium sulfate or magnesium sulfate were determined at (298.15, 308.15, and 318.15) K. The temperature effect on the biphasic area was not relevant, indicating a small enthalpic contribution associated with the phase separation. The ability of the salts in inducing the formation of the ATPS with PEG 400 followed the order sodium sulfate < magnesium sulfate. The liquid−liquid equilibrium experimental data were correlated using the NRTL (nonrandom two-liquid) activity coefficient model, and new interaction parameters were estimated. The mean deviations between calculated and experimental compositions were lower than 0.99 %, showing the good descriptive quality and applicability of the NRTL model.

Introduction

Aqueous two-phase systems (ATPS) are constituted of chemical species that separate into two different phases when mixed in certain ranges of composition and temperature. The formation of an ATPS can be achieved by using two different polymers in water or even an inorganic salt and a polymer in water.[1,2] ATPS have typically been used in the separation and purification of proteins and other biomaterials.[3,4] ATPS have also been used in the extraction of metallic ions,[5,6] extractive crystallization of inorganic salts,[7,8] extraction of sulfide minerals,[8] separation of cell particles, and recovery of nanoparticles.[9]

ATPS of major practical interest are composed of poly(ethylene glycol) (PEG) and inorganic salts. These systems, when compared to those containing only polymers presents some advantages like lower cost, lower viscosity, lower interfacial tension, rapid phase disengagement, and easy scale-up. PEG is one of the most useful constituents in ATPS, given that this polymer is low in cost, nontoxic, noninflammable, and easy to handle.[10] Among the biphase-forming salts, sulfate salts are preferred over others because of their ability to promote hydrophobic interactions between the phases.[11,12] This hydrophobic effect is important in protein partitioning in ATPS, where sulfate has a slight salting-in effect on the peptide group and a strong salting-out effect on the nonpolar groups, which leads to the fact that sulfate stabilizes the native structure of proteins and hence distinguishes itself from the usual protein denaturants.[13,14]

The selective distribution of constituents of ATPS may be affected by different factors like the nature and size of the biocompound, molecular structure and size of the polymer chain, type of salt, pH, system overall composition, and temperature.[1,2] Liquid−liquid equilibrium (LLE) data at different temperatures and compositions are essential for the development, optimization, and scale-up of extraction process using ATPS. Moreover, useful information in phase diagrams can be attained by a thermodynamic model that can adequately describe these multiphase systems. An activity coefficient model, such as the NRTL (nonrandom two-liquid) model,[15] has been successfully applied to correlate the LLE data for ATPS.[16,17]

Equilibrium data for aqueous systems with PEG of different average molar masses and salts are reported in the literature.[18−21] However, for systems containing PEG 400 + sulfate salts, equilibrium data are scarce. Thus, this work presents experimental data of ATPS composed of PEG 400 + sodium sulfate + water and PEG 400 + magnesium sulfate + water, at (298.15, 308.15, and 318.15) K. The effect of the salt nature and temperature on phase diagrams were investigated. The experimental data were correlated with the NRTL model, with the estimation of binary interaction parameters.

Experimental Section

Materials. The following reagents were used: triblock copolymer PEG with an average molar mass of 400 $\text{g}\cdot\text{mol}^{-1}$ (Sigma-Aldrich, U.S.), sodium sulfate (Vetec, Brazil), and magnesium sulfate (Vetec, Brazil), all of analytical grade. Deionized water (Milli-Q, Millipore) was utilized in all experiments ($R \geq 18.2\ \text{M}\Omega\cdot\text{cm}^{-1}$). The polymer and salts were used as received.

ATPS. Ternary phase diagrams were prepared by weighing appropriate quantities of PEG 400, sulfate salts (sodium or magnesium), and water on an analytical balance (Gehaka, AG200, Brazil), with an accuracy of ± 0.0001 g. Liquid−liquid glass tubes were used to carry out phase equilibrium determinations. Typically, 12 g of system was prepared. After vigorously stirring of the system, until it became turbid, it was placed in a temperature-controlled bath (Microquímica, MQBTC 99-20,

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with an accuracy of ± 0.1 K) for 24 h at (298.15, 308.15, or 318.15) K. The equilibrium state was characterized by the absence of turbidity in both top and bottom phases. Samples of the top and bottom phases were collected for analysis.

Quantitative Analysis. The salt concentration (sodium or magnesium) was determined by conductivity (Schott CG853, Germany) of the electrolyte in the range of 10^{-4} to 10^{-3} M. The salt solutions showed the same conductivity in water or in the diluted polymer solution (0.6 to 0.01) %. The standard deviation of the salt mass fraction from this method was ± 0.12 %. A refractometer (analytic Jena AG Abbe refractometer 09-2001, Germany) was used to measure PEG 400 quantity at 298.15 K. Since the refractive index of the phase depends on the polymer and salt concentrations, PEG 400 content was obtained by subtracting the salt concentrations obtained by conductivity from the total solution composition (refractive index). The standard deviation of the PEG 400 mass fractions was on the order of 0.07 %. Analytical curves were obtained for the salt and PEG 400 measurements, and water content was determined by mass balance. All analytical measurements were performed in triplicate.

Thermodynamic Modeling. The experimental data were used to adjust the NRTL binary interaction parameters. Mole fractions have traditionally been used in this model, but mass fractions provide a more convenient composition unit because of the large difference in the molar masses of the components involved in polymeric systems. Stragevitch,[22] Sé and Aznar,[23] Batista et al.,[24] and Lintomen et al.[25] used this approach with the NRTL model. When mass fractions are used, the model is expressed as:

$$\ln \gamma_i^w = \left(\frac{\displaystyle\sum_{j=1}^{K} \frac{\tau_{ji} G_{ji} w_j}{\bar{M}_j}}{\displaystyle\sum_{j=1}^{K} \frac{G_{ji} w_j}{\bar{M}_j}} + \sum_{j=1}^{K} \left[\frac{w_j G_{ij}}{\bar{M}_j \displaystyle\sum_{k=1}^{K} \frac{G_{kj} w_k}{\bar{M}_k}} \left(\tau_{ij} - \frac{\displaystyle\sum_{k=1}^{K} \frac{\tau_{kj} G_{kj} w_k}{\bar{M}_k}}{\displaystyle\sum_{k=1}^{K} \frac{G_{kj} w_k}{\bar{M}_k}} \right) \right] \right) \Bigg/ \bar{M}_i \sum_{j=1}^{K} \left(\frac{w_j}{\bar{M}_j} \right) \qquad (1)$$

where γ_i^w is the corresponding activity coefficient of component i expressed on the mass fraction, K is the total number of components in the data group, and $\bar{M}$ and w are the average molar mass and mass fraction, respectively, of the components. The quantities τ_{ij} and G_{ij} are given by

$$\tau_{ij} = \frac{\Delta g_{ij}}{RT} = \frac{A_{ij}}{T} \qquad (2)$$

$$G_{ij} = \exp(-\alpha_{ij}\tau_{ij}) \qquad (3)$$

$$\alpha_{ij} = \alpha_{ji} \qquad (4)$$

This model has three adjustable parameters for each binary pair (Δg_{ij}, Δg_{ji}, and α_{ij}). The parameters Δg_{ij} and Δg_{ji} are related to the characteristic energy of interaction between the molecules of type i and j; α_{ij} is related to the nonrandomness of the mixture. A_{ij} and A_{ji} are characteristic energy parameters of the i and j interactions; T is the absolute temperature. The NRTL interaction parameters can also be adjusted as functions of the absolute temperature and characteristic energy interaction parameters (with subscripts 0 and 1), as defined by the expression:

$$A_{ij} = A_{0ij} + A_{1ij}T \qquad (5)$$

Estimation of interaction parameters was based on the minimization of the objective function of composition, presented in eq 6, according to the procedure developed by Stragevitch and d'Avila.[26]

$$\mathrm{OF}(w) = \sum_{m=1}^{D} \sum_{n=1}^{N} \sum_{i=1}^{K-1} \left[\left(\frac{w_{i,n,m}^{\mathrm{TP,exptl}} - w_{i,n,m}^{\mathrm{TP,calcd}}}{\sigma_{w_{i,n,m}^{\mathrm{TP}}}} \right)^2 + \left(\frac{w_{i,n,m}^{\mathrm{BP,exptl}} - w_{i,n,m}^{\mathrm{BP,calcd}}}{\sigma_{w_{i,n,m}^{\mathrm{BP}}}} \right)^2 \right] \qquad (6)$$

where D is the total number of data groups; N is the total number of tie lines in the data group; K is the total number of components in the data group; w is the mass fraction; the subscripts i, n, and m are component, tie line, and group numbers, respectively; the superscripts TP and BP stand for the top and bottom phases, respectively; and exptl and calcd refer to experimental and calculated compositions. $\sigma_{w_{i,n,m}^{\mathrm{TP}}}$ and $\sigma_{w_{i,n,m}^{\mathrm{BP}}}$ represent the standard deviations observed in the compositions of the two liquid phases. The average deviations between experimental and calculated compositions were quoted according to eq 7.

$$\Delta w = \sqrt{\frac{\displaystyle\sum_{n=1}^{N} \sum_{i=1}^{K} [(w_{i,n}^{\mathrm{TP,exptl}} - w_{i,n}^{\mathrm{TP,calcd}})^2 + (w_{i,n}^{\mathrm{BP,exptl}} - w_{i,n}^{\mathrm{BP,calcd}})^2]}{2NK}} \qquad (7)$$

Results and Discussion

LLE data for systems composed of PEG 400 + sulfate salts + water are shown in Tables 1 and 2. Phase analysis revealed that the top phase was rich in PEG 400 and poor in electrolyte, while the bottom phase contained most of the electrolyte and

Table 1. LLE Data for Systems Composed of PEG 400 (w_{PEG}) + Na_2SO_4 (w_{S}) + H_2O (w_{W}), from (298.15 to 318.15) K

		overall composition			top phase			bottom phase		
tie line	TLL	100 w_{PEG}	100 w_{S}	100 w_{W}	100 w_{PEG}	100 w_{S}	100 w_{W}	100 w_{PEG}	100 w_{S}	100 w_{W}
					T = 298.15 K					
1	0.3757	19.78	13.28	66.94	35.88	3.50	60.62	3.87	23.16	72.97
2	0.4103	20.09	14.09	65.82	37.28	2.93	59.79	2.96	25.41	71.63
3	0.4569	22.49	14.50	63.01	41.97	2.45	55.58	3.12	26.50	70.38
4	0.5031	23.52	15.45	61.03	44.90	2.02	53.08	2.28	28.76	68.58
					T = 308.15 K					
1	0.3718	20.17	13.21	66.62	36.19	3.75	60.06	4.24	22.77	72.99
2	0.4053	21.42	13.34	65.24	38.93	3.04	58.03	4.05	23.68	72.27
3	0.4642	22.79	14.35	62.86	42.64	2.43	54.93	2.98	26.55	70.47
4	0.5131	24.45	15.41	60.14	46.28	2.05	51.67	2.52	28.85	68.63
					T = 318.15 K					
1	0.3569	19.41	12.65	67.94	34.73	3.61	61.66	4.01	21.78	74.21
2	0.4264	21.43	13.75	64.82	39.63	2.78	57.59	3.14	24.84	72.02
3	0.4637	22.46	14.74	62.80	42.13	2.49	55.38	2.70	26.89	70.41
4	0.5044	23.36	15.48	61.16	44.66	2.06	53.28	1.93	28.86	69.21

Table 2. LLE Data for Systems Composed of PEG 400 (w_{PEG}) + Mg_2SO_4 (w_S) + H_2O (w_W), from (298.15 to 318.15) K

		overall composition			top phase			bottom phase		
tie line	TLL	100 w_{PEG}	100 w_S	100 w_W	100 w_{PEG}	100 w_S	100 w_W	100 w_{PEG}	100 w_S	100 w_W
					T = 298.15 K					
1	0.2317	14.11	10.77	75.12	24.22	5.07	70.71	4.08	16.53	79.39
2	0.3060	15.55	10.95	73.50	28.59	2.91	68.50	2.61	19.07	78.32
3	0.3654	17.45	11.29	71.26	33.15	1.92	64.93	1.85	20.78	77.37
4	0.4205	17.79	12.69	69.52	35.61	1.46	62.93	0.10	23.99	76.01
					T = 308.15 K					
1	0.2345	13.84	10.65	75.51	23.89	4.61	71.50	3.82	16.74	79.44
2	0.3278	15.54	11.43	73.03	29.47	2.74	67.79	1.65	20.08	78.27
3	0.3758	16.64	11.88	71.48	32.52	1.84	65.64	0.80	21.99	77.21
4	0.4211	17.77	12.39	69.84	35.75	1.25	63.00	0.03	23.55	76.42
					T = 318.15 K					
1	0.2886	13.92	10.81	75.27	26.39	3.47	70.14	1.55	18.17	80.28
2	0.3546	15.33	11.65	73.02	30.37	2.34	67.29	0.20	20.98	79.02
3	0.3803	16.18	11.77	72.05	32.20	1.59	66.21	0.10	21.99	78.01
4	0.4208	17.94	12.20	69.86	35.79	1.14	63.07	0.00	23.27	76.73

small PEG 400 quantities. This is a traditional behavior for ATPS, and all works in literature report a mutual exclusion between the polymer and the electrolyte.[2−12,16−21,27]

An important parameter in ATPS is the tie line length (TLL), calculated according to eq 8. This parameter indirectly represents the differences of intensive thermodynamic properties between both phases.

$$\mathrm{TLL} = [(w_{\mathrm{PEG}}^{\mathrm{TP}} - w_{\mathrm{PEG}}^{\mathrm{BP}})^2 + (w_{\mathrm{S}}^{\mathrm{TP}} - w_{\mathrm{S}}^{\mathrm{BP}})^2]^{1/2} \qquad (8)$$

where w_{PEG} and w_S are the PEG and salt mass fractions and the superscripts TP and BP designate the top and bottom phases, respectively. As can be seen in Tables 1 and 2, greater polymer and salt mass fraction differences between the two phases led to higher values of TLL. Figure 1 presents the effect of PEG and sulfate salt overall mass fractions on the TLL at different temperatures. Enhancements in temperature and overall mass fractions of PEG and sulfate salts caused a slight increase in TLL. However, this relationship was not linear, which is typical of behavior observed in ATPS containing PEG + sulfate salts.[18−21,28]

Table 3 presents the values of the slope of the tie line (STL), calculated according to eq 9.

$$\mathrm{STL} = \frac{w_{\mathrm{PEG}}^{\mathrm{TP}} - w_{\mathrm{PEG}}^{\mathrm{BP}}}{w_{\mathrm{S}}^{\mathrm{TP}} - w_{\mathrm{S}}^{\mathrm{BP}}} \qquad (9)$$

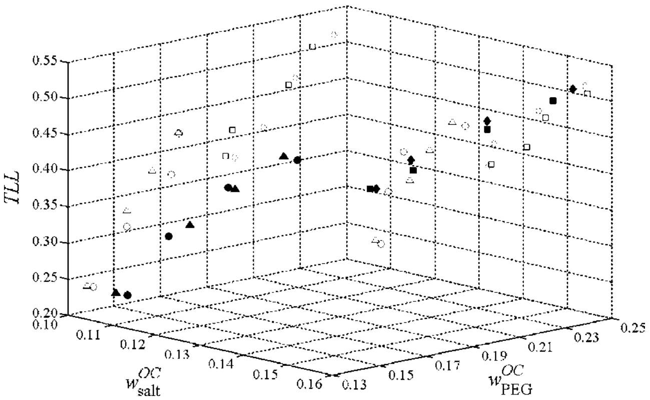

Figure 1. Effect of PEG and salt overall mass fractions on the TLL at different temperatures: ■, Na_2SO_4 at 298.15 K; ♦, Na_2SO_4 at 308.15 K; ●, Mg_2SO_4 at 298.15 K; ▲, Mg_2SO_4 at 308.15 K.

Table 3. STL Values for Systems Composed of PEG 400 + Salt + H_2O, from (298.15 to 318.15) K

		T/K	
tie line	298.15	308.15	318.15
	PEG 400 + Na_2SO_4 + H_2O		
1	−1.63	−1.68	−1.69
2	−1.53	−1.69	−1.65
3	−1.62	−1.64	−1.62
4	−1.59	−1.63	−1.59
	PEG 400 + Mg_2SO_4 + H_2O		
1	−1.76	−1.65	−1.69
2	−1.61	−1.60	−1.62
3	−1.66	−1.57	−1.57
4	−1.58	−1.60	−1.62

Table 4. Adjusted NRTL Parameters in Function of Absolute Temperature, from (298.15 to 318.15) K

$i-j$	$A_{0,ij}$/K	$A_{0,ji}$/K	$A_{1,ij}$	$A_{1,ji}$	α_{ij}
PEG 400−Na_2SO_4	−3197.5	597.94	19.475	0.1356	0.2983
PEG 400−Mg_2SO_4	−3273.8	412.05	18.669	0.5030	0.6994
PEG 400−H_2O	2606.8	−12249.00	−11.428	94.6330	0.5965
Na_2SO_4−H_2O	4938.3	−196.43	−21.794	6.7471	0.2216
Mg_2SO_4−H_2O	3453.8	−271.42	−18.200	10.2830	0.1623

Table 5. Mean Deviations in Phase Compositions for Systems Composed of PEG 400 + Sulfate Salts + H_2O, from (298.15 to 318.15) K

system	100 Δw
PEG 400 + Na_2SO_4 + H_2O at 298.15 K	0.66
PEG 400 + Na_2SO_4 + H_2O at 308.15 K	0.54
PEG 400 + Na_2SO_4 + H_2O at 318.15 K	0.51
PEG 400 + Mg_2SO_4 + H_2O at 298.15 K	0.99
PEG 400 + Mg_2SO_4 + H_2O at 308.15 K	0.97
PEG 400 + Mg_2SO_4 + H_2O at 318.15 K	0.66
average overall deviation	0.75

Despite the small temperature dependence of the STL values determined, results reported in literature show that, by increasing the temperature, STL values tend to increase, reducing the amount of salt needed to form a biphasic system with a given amount of PEG.[19] However, for lower molar mass PEG, this effect seems to be indistinguishable at different temperatures.[21] The average STL for a system composed of PEG 400 + sodium sulfate at different temperatures was equal to (−1.63 ± 0.05). The same value was found for systems containing PEG 400 + magnesium sulfate.

The adjusted NRTL parameters as functions of absolute temperature are presented in Table 4. Deviations between experimental and calculated compositions in both phases were quoted according to eq 7 and are shown in Table 5. From these results, it was observed that the NRTL model accurately described the phase compositions of the investigated systems,

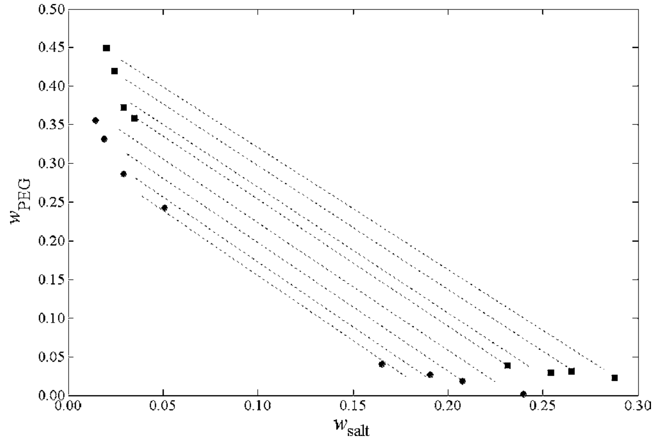

Figure 2. LLE for systems composed of PEG 400 + sulfate salts + water at 298.15 K: ■, Na_2SO_4; ●, Mg_2SO_4; dashed line, NRTL.

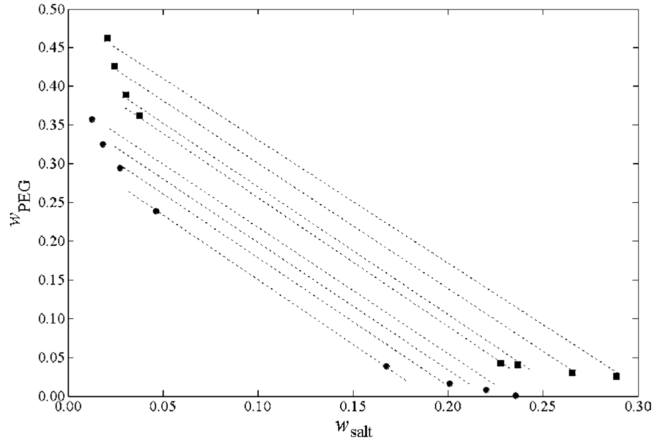

Figure 3. LLE for systems composed of PEG 400 + sulfate salts + water at 308.15 K: ■, Na_2SO_4; ●, Mg_2SO_4; dashed line, NRTL.

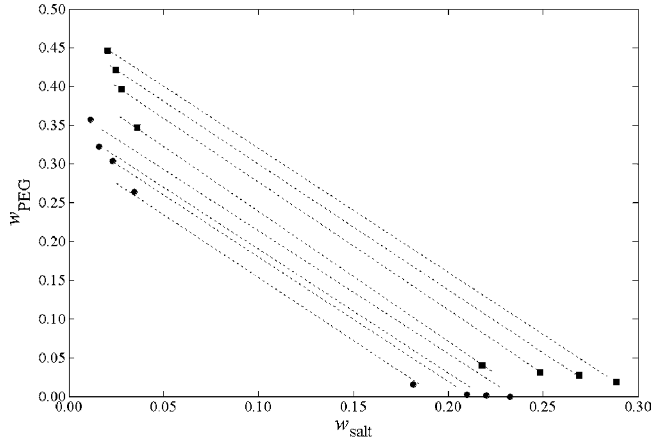

Figure 4. LLE for systems composed of PEG 400 + sulfate salts + water at 318.15 K: ■, Na_2SO_4; ●, Mg_2SO_4; dashed line, NRTL.

with mean deviations between calculated and experimental results smaller than 0.99 %. Figures 2, 3, and 4 present the experimental and calculated compositions using the NRTL model for the studied systems.

From these results, it was verified that the effect of temperature was not significant. All phase diagrams obtained show a very small dependence on the temperature. An increase in temperature causes a short expansion of the two-phase region, indicating only a small change in ΔC_p and ΔH associated with the phase segregation process. The same behavior is observed for all ATPS formed by PEG + sulfate salts.[18−20,28]

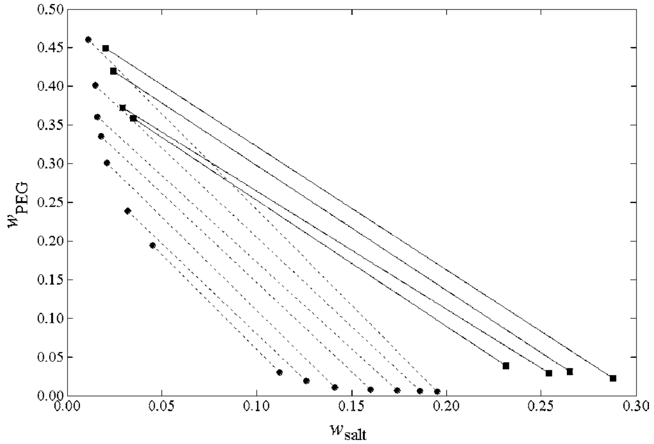

Figure 5. LLE for systems composed of PEG + sodium sulfate + water at 298.15 K: ■, PEG 400; ●, PEG 6000;[30] continuous line, PEG 400 tie lines; dashed line, PEG 6000 tie lines.[30]

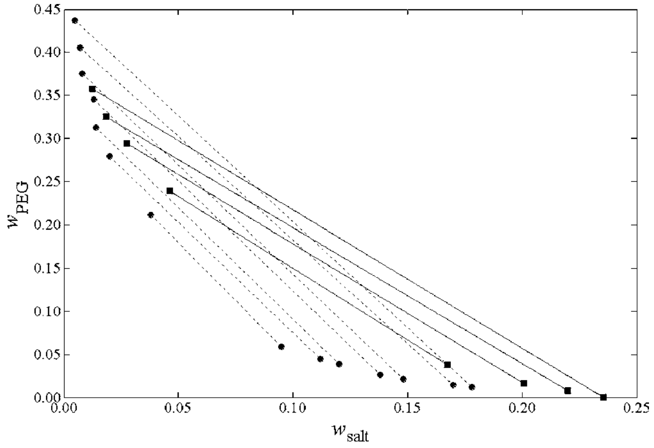

Figure 6. LLE for systems composed of PEG + magnesium sulfate + water at 298.15 K: ■, PEG 400; ●, PEG 6000,;[30] continuous line, PEG 400 tie lines; dashed line, PEG 6000 tie lines.[30]

From the results shown in Figures 2 to 4 it is also possible to appreciate the influence of an electrolyte in inducing phase segregation. The preference of the two investigated salts in inducing phase segregation in ATPS follows the order Na_2SO_4 < $MgSO_4$. A model proposed by da Silva and Loh,[29] based on calorimetric measurements, attributed the trend in the efficacy of sodium sulfates for inducing ATPS formation to the cation−polymer interactions. Their proposed model is that, when the PEG and sulfate salts are mixed, the cations interact with ethylene oxide polymer groups, decreasing interactions between PEG and solvating water molecules, in a process that is driven by entropy increase. This cation binding continues as more electrolyte is added, until a saturation point, after which no more entropy gain may be attained and phase splitting becomes more favorable. In general, interactions of ethylene oxide groups with different cations promote different biphasic areas. The same behavior was observed for systems composed of PEG 6000 or PEG 1500 + sulfate salts.[20,28]

To have a better insight on the effect of PEG molar mass in phase diagrams, LLE data for systems composed of PEG 6000 + sulfate salts + at 298.15 K, reported by Salabat,[30] are presented in Figures 5 and 6. Although the overall compositions of this work and those utilized by Salabat[30] are different, it is possible to conclude that phase segregation was favored because of the higher PEG molar mass, being also evident through increases in the STL values.

Conclusions

Equilibrium data for systems composed of PEG 400 + sodium sulfate + water and PEG 400 + magnesium sulfate + water were determined at different temperatures of (298.15 to 318.15) K. The data were correlated using the NRTL model, with the estimation of new interaction parameters. The model was able to correlate the data with excellent precision, yielding an average global mean deviation of 0.75 % between experimental and calculated equilibrium compositions. Moreover, the temperature effect on the biphasic area was not relevant. The cation nature had an effect on the phase diagram of these ATPS. The efficacy of the salts in inducing phase segregation follows the order sodium sulfate < magnesium sulfate.

Literature Cited

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

(2) Zaslavsky, B. Y. *Aqueous Two Phase Partitioning, Physical Chemistry and Bioanalytical Applications*; Marcel Dekker: New York, 1995.

(3) Silva, C. A. S.; Coimbra, J. S. R.; Rojas, E. E. G.; Minim, L. A.; Silva, L. H. M. Partitioning of Caseinomacropeptide in Aqueous Two-Phase Systems. *J. Chromatogr., B* **2007**, *858*, 205–210.

(4) Rosa, P. A. J.; Azevedo, A. M.; Aires-Barros, M. R. Application of Central Composite Design to the Optimisation of Aqueous Two-Phase Extraction of Human Antibodies. *J. Chromatogr., A* **2007**, *1141*, 50–60.

(5) Silva, L. H. M.; Silva, M. C. H.; Amim Júnior, J.; Martins, J. P.; Coimbra, J. S. R.; Minim, L. A. Hydrophobic Effect on the Partitioning of $[Fe(CN)_5(NO)]^{2-}$ and $[Fe(CN)_6]^{3-}$ Anions in Aqueous Two-Phase Systems Formed by Triblock Copolymers and Phosphate Salts. *Sep. Purif. Technol.* **2008**, *60*, 103–112.

(6) Silva, L. H. M.; Silva, M. C. H.; Aquino, R. A. N.; Francisco, K. R.; Cardoso, M. V. C.; Minim, L. A.; Coimbra, J. S. R. Nitroprusside-PEO Enthalpic Interaction as Driving Force for Partitioning of the $[Fe(CN)_5NO]^{2-}$ Anion in Aqueous Two-Phase Systems Formed by Poly (Ethylene Oxide) and Sulfate Salts. *J. Phys. Chem. B* **2006**, *110*, 23540–23546.

(7) Taboada, M. E.; Palma, P. A.; Graber, T. A. Crystallization of Potassium Sulfate by Cooling and Salting-Out Using 1-Propanol in a Calorimetric Reactor. *Cryst. Res. Technol.* **2004**, *38*, 12–19.

(8) Osseo-Azare, K.; Zeng, X. Partition of Pyrite in Aqueous Biphase Systems. *Int. J. Miner. Process* **2000**, *58*, 319–330.

(9) Braas, G.; Walker, S.; Lyddiatt, A. Recovery in Aqueous Two-Phase Systems of Nanoparticulates Applied as Surrogate Mimics for Virial Gene Therapy Vectors. *J. Chromatogr., B* **2000**, *743*, 409–419.

(10) Graber, T. A.; Galvez, M. E.; Galleguillos, H. R.; Alvarez-Benedi, J. Liquid-Liquid Equilibrium of the Aqueous Two-Phase System Water + PEG 4000 + Lithium Sulfate at Different Temperatures. Experimental Determination and Correlation. *J. Chem. Eng. Data* **2004**, *49*, 1661–1664.

(11) Su, C. K.; Chiang, B. H. Partitioning and Purification of Lysozyme from Chicken Egg White Using Aqueous Two-Phase System. *Process Biochem. (Oxford, U.K.)* **2006**, *41*, 257–263.

(12) Eiteman, M. A.; Gainer, J. L. Partition of Isomeric Dipeptides in Poly (Ethylene Glycol)/Magnesium Sulfate Aqueous Two-Phase Systems. *Biochim. Biophys. Acta* **1991**, *1073*, 451–455.

(13) Kumar, D. Apparent Molar Volume of Some Amino Acids in Aqueous Electrolytes Systems. *Can. J. Chem.* **1999**, *77*, 1288–1294.

(14) Liu, C.; Ren, C. Transfer Properties of Amino Acids from Water to Aqueous Sodium Sulfate Solutions at 298.15 K. *J. Chem. Eng. Data* **2009**, DOI: 10.1021/je900218z.

(15) Renon, H.; Prausnitz, J. M. Local Compositions in Thermodynamic Excess Functions for Liquid Mixtures. *AIChE J.* **1968**, *14*, 135–144.

(16) Sé, R. A. G.; Aznar, M. Liquid-Liquid Equilibrium of the Aqueous Two-Phase System Water + PEG 4000 + Potassium Phosphate at Four Temperatures: Experimental Determination and Thermodynamic Modeling. *J. Chem. Eng. Data* **2002**, *47*, 1401–1405.

(17) Rasa, H.; Mohsen-Nia, M.; Modarress, H. Phase Separation in Aqueous Two-Phase Systems Containing Poly (Ethylene Glycol) and Magnesium Sulphate at Different Temperatures. *J. Chem. Thermodyn.* **2008**, *40*, 573–579.

(18) Oliveira, R. M.; Coimbra, J. S. R.; Francisco, K. R.; Minim, L. A.; Silva, L. H. M.; Pereira, J. A. M. Liquid-Liquid Equilibrium of Aqueous Two-Phase Systems Containing Poly(ethylene) Glycol 4000 and Zinc Sulfate at Different Temperatures. *J. Chem. Eng. Data* **2008**, *53*, 919–922.

(19) Carvalho, C. P.; Coimbra, J. S. R.; Costa, I. A. F.; Minim, L. A.; Silva, L. H. M.; Maffia, M. C. Equilibrium Data for PEG 4000 + Salt + Water Systems from (278.15 to 318.15) K. *J. Chem. Eng. Data* **2007**, *52*, 351–356.

(20) Martins, J. P.; Carvalho, C. P.; Silva, L. H. M.; Coimbra, J. S. R.; Silva, M. D. H.; Rodrigues, G. D.; Minim, L. A. Liquid-liquid Equilibria of Aqueous Two-Phase System Containing Poly(ethylene) Glycol 1500 and Sulfate Salts at Different Temperatures. *J. Chem. Eng. Data* **2008**, *53*, 238–241.

(21) Oliveira, R. M.; Coimbra, J. S. R.; Minim, L. A.; Silva, L. H. M.; Fontes, M. P. F. Liquid-Liquid Equilibria of Biphasic Systems Composed of Sodium Citrate + Polyethylene 1500 or 4000 at different temperatures. *J. Chem. Eng. Data* **2008**, *53*, 895–899.

(22) Stragevitch, L. *Liquid-Liquid Equilibrium in Non Electrolyte Mixtures* (in Portuguese). D.Sc. Thesis, School of Chemical Engineering, University of Campinas, Campinas, 1997.

(23) Sé, R. A. G.; Aznar, M. Thermodynamic Modeling of Phase Equilibrium of Aqueous Two-Phase Systems Water + Poly(Ethylene Glycol) + Salt. *Braz. J. Chem. Eng.* **2002**, *19*, 255–266.

(24) Batista, E.; Monnerat, S.; Kato, K.; Stragevitch, L.; Meirelles, A. J. A. Liquid-Liquid Equilibrium for Systems of Canola Oil, Oleic Acid and Short-Chain Alcohols. *J. Chem. Eng. Data* **1999**, *44*, 1360–1364.

(25) Lintomen, L.; Pinto, R. T. P.; Batista, E.; Meirelles, A. J. A.; Wolf-Maciel, M. R. Liquid-Liquid Equilibrium of the Water + Acid Citric + 2-Butanol + Sodium Chloride System at 298.15 K. *J. Chem. Eng. Data* **2000**, *45*, 1211–1214.

(26) Stragevitch, L.; d'Ávila, S. G. Application of a Generalized Maximum Likelihood Method in the Reduction of Multicomponent Liquid-Liquid Equilibrium Data. *Braz. J. Chem. Eng.* **1997**, *14*, 41–52.

(27) 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.

(28) Martins, J. P.; Oliveira, F. C.; Coimbra, J. S. R.; Silva, L. H. M.; Silva, M. C. H. Equilibrium Phase Behavior for Ternary Mixtures of Poly (Ethylene) Glycol 6000 + Water + Sulfate Salts at Different Temperatures. *J. Chem. Eng. Data* **2009**, *53*, 2441–2443.

(29) Silva, L. H. M.; Loh, W. Calorimetric Investigation of the Formation of Aqueous Two-Phase Systems in Ternary Mixtures of Water, Poly (Ethylene Oxide) and Electrolytes (or Dextran). *J. Phys. Chem. B* **2000**, *104*, 10069–10073.

(30) Salabat, A. The Influence of Salts on the Phase Composition in Aqueous Two-Phase Systems: Experiments and Predictions. *Fluid Phase Equilib.* **2001**, *188*, 489–498.

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