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Liquid−Liquid Equilibria of an Aqueous Two-Phase System Formed by a Triblock Copolymer and Sodium Salts at Different Temperatures

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Phase diagrams of aqueous two-phase systems composed of a triblock copolymer (L35) 1900 g·mol^{-1}, sodium tartarate, sodium citrate, or sodium nitrate were determined at (283.15, 298.15, and 313.15) K. The temperature effect on the position of the binodal curves was not relevant, indicating a small enthalpic contribution associating to the phase segregation. The ability of three salts to induce the formation of the biphasic system with L35 followed the order sodium citrate > sodium tartarate > sodium nitrate. The preference of a salt-inducing phase segregation follows the Hofmeister series.

Introduction

Aqueous two-phase systems (ATPSs) have been widely used in preconcentration and separation of chemical substances, such as, metal ions,[1,2] proteins,[3,4] and organic molecules.[5] These systems can be formed by combining either two chemically different polymers or a polymer and a salt (organic or inorganic) in water above a certain critical concentration and temperature.[6,7]

The most used ATPS are composed of poly(ethylene oxide) (PEO) + inorganic salt + water or PEO + dextran + water.[8] However, these systems present some limitations, such as low extractant solubility and a lower complex partition coefficient, for the partitioning of hydrophobic solutes or use of water-insoluble extractant agents. This problem can be solved through the creation of micellar media using a triblock copolymer.[9] A typical example of a triblock copolymer is formed by ethylene oxide (EO) and propylene oxide units (PO) according to the representation: $(EO)_n-(PO)_m-(EO)_n$. The nomenclature of triblock copolymers is based on the physical state (liquid, paste, or flakes) and EO/PO rate of the macromolecule.[10] This type of triblock copolymer self-associates upon increasing concentration (L35 cmc = 2.40 % w/w) or temperature into micellar-like aggregates consisting of a core occupied by PO units surrounded by a corona of EO chains.[11]

The literature reported a small number of equilibrium data for different ATPSs formed by triblock copolymers of different EO/PO ratios (or different average molecular mass) and inorganic salts[12−15] or dextran + triblock copolymer.[16] Despite the existence of equilibrium data for the triblock copolymers + salt + water, studies involving these types of macromolecules are very rare, and that is a restriction for selecting the appropriate ATPS for the solute that one wants to separate or purify.

For the application of ATPS formed by triblock copolymers, it is necessary to obtain the phase diagram data. In this work, ATPSs composed of triblock copolymer L35 ($(PEO)_{11}-(PPO)_{16}-(PEO)_{11}$) + sodium salt + water were determined, and phase composition was measured. Equilibrium data at (283.15, 298.15, and 313.15) K were determined for the systems consisting of L35 + sodium citrate + water, L35 + sodium tartarate + water, and L35 + sodium nitrate + water. The influences of the anion electrolyte nature and temperature on the phase diagram were also investigated.

Experimental Section

Materials. The following reagents were used: triblock copolymer L35 (50 % EO), $(PEO)_{11}-(PPO)_{16}-(PEO)_{11}$, with average molar mass of 1900 g·mol^{-1} (Sigma) and sodium citrate, sodium tartarate, and sodium nitrate (Vetec, Brazil), all of analytical grade. Millipore water was used in all experiments ($R \geq 18.2$ MΩ·cm^{-1}).

Aqueous Two-Phase Systems. We prepared ternary phase diagrams by weighing appropriate quantities of L35, sodium salts (citrate, tartarate, or nitrate), and water on an analytical balance (Gehaka, AG200, Brazil) with a given uncertainty of ± 0.0001 g. Liquid−liquid glass tubes were used to carry out phase equilibrium determinations. Typically, 10 g of system was prepared. After the system was vigorously stirred until it became turbid, it was placed in a temperature-controlled bath (Microquímica, MQBTC 99-20, with an uncertainty of ± 0.1 K) for 72 h at (283.15, 298.15, or 313.15) K. The equilibrium state was characterized by the absence of turbidity in both top and bottom phases. Samples of the top and bottom phase were collected for analysis.

Quantitative Analysis. The salt concentration (sodium citrate, sodium tartarate, and sodium nitrate) was determined by conductivity (Schott CG853, Germany) of the electrolyte in the range of (10^{-3} to 10^{-2}) % w/w. The salt solutions showed the same conductivity in water or in the diluted polymer solution [(0.1 to 0.01) %]. The standard deviation of the salt mass percent from this method was ± 0.10 %. A refractometer (Analytic Jena AG Abbe refractometer 09-2001, Germany) was used to measure the L35 quantity at 298.15 K. Because the refractive index of the phase depends on the copolymer and salt concentrations, we obtained the L35

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Table 1. Equilibrium Data for L35 (w_{L35}) + Sodium Citrate (w_S) + Water (w_W) System from T = (283.15 to 313.15) K in Mass Fraction Percent

tie line	tie-line length	overall			top phase			bottom phase		
		w_{L35}	w_S	w_W	w_{L35}	w_S	w_W	w_{L35}	w_S	w_W
283.15 K										
1	33.74	18.68	9.10	72.21	34.74	3.21	62.05	4.19	17.52	78.29
2	38.39	21.97	9.68	68.34	37.60	2.29	60.11	3.77	20.43	75.80
3	42.49	24.67	10.79	64.53	42.75	1.57	55.68	5.82	22.59	71.59
4	49.11	27.40	12.06	60.52	48.69	1.20	50.11	6.28	25.97	67.75
5	53.85	30.43	12.94	56.61	53.60	0.86	45.54	6.99	27.83	65.18
298.15 K										
1	34.78	27.32	5.11	67.67	34.94	2.92	62.14	2.43	15.28	82.29
2	42.16	31.06	5.62	63.32	41.68	2.11	56.21	2.33	17.24	80.43
3	48.18	34.82	6.11	59.07	46.93	1.55	51.52	2.43	20.02	77.55
4	54.84	38.57	6.62	54.81	52.51	1.10	46.39	2.25	23.03	74.72
5	59.48	42.31	7.12	50.57	57.56	0.75	41.69	3.32	25.16	71.52
313.15 K										
1	44.82	18.67	9.10	72.23	45.39	1.67	52.94	2.68	15.25	82.07
2	50.58	22.01	9.67	68.32	50.88	1.25	47.87	2.81	16.97	80.22
3	55.64	24.68	10.78	64.54	55.78	0.93	43.29	3.17	19.03	77.80
4	59.20	27.38	12.06	60.56	59.80	0.67	39.53	4.43	21.63	73.94
5	62.71	30.41	12.94	56.65	63.24	0.49	36.27	5.18	24.20	70.62

Table 2. Equilibrium Data for L35 (w_{L35}) + Sodium Tartarate (w_S) + Water (w_W) System from T = (283.15 to 313.15) K in Mass Fraction Percent

tie line	tie-line length	overall			top phase			bottom phase		
		w_{L35}	w_S	w_W	w_{L35}	w_S	w_W	w_{L35}	w_S	w_W
283.15 K										
1	23.68	20.00	8.21	71.79	26.72	4.48	68.8	6.51	16.82	76.67
2	39.43	22.11	10.21	67.68	37.85	2.57	59.58	2.88	20.78	76.34
3	46.22	25.28	11.32	63.40	42.59	1.89	55.52	2.41	24.73	72.86
4	51.55	28.20	12.13	59.67	46.78	1.49	51.73	2.33	27.59	70.08
5	55.84	30.59	13.39	56.02	52.83	1.18	45.99	4.51	29.16	66.33
298.15 K										
1	40.85	35.99	4.55	59.46	41.06	2.54	56.40	3.02	17.44	79.54
2	48.20	39.92	5.34	54.74	47.57	1.77	50.66	3.13	20.44	76.25
3	52.77	45.04	5.63	49.33	52.67	1.24	46.09	4.55	22.89	72.56
4	58.43	49.66	6.33	44.01	57.39	1.12	41.49	4.24	25.39	70.37
5	69.28	54.62	6.59	38.79	68.25	0.56	31.19	5.82	30.59	63.59
313.15 K										
1	53.65	25.78	9.93	64.29	53.20	1.52	45.28	2.03	17.64	80.33
2	57.19	25.58	11.16	63.26	58.02	1.13	40.85	3.63	18.82	77.55
3	62.29	29.02	12.02	58.96	61.86	0.85	37.29	3.12	21.57	75.31
4	69.07	32.69	12.66	54.65	67.89	0.62	31.49	3.26	24.99	71.75
5	70.63	38.89	14.22	46.89	70.63	0.36	29.01	6.10	29.08	64.82

Table 3. Equilibrium Data for L35 (w_{L35}) + Sodium Nitrate (w_S) + Water (w_W) System from T = (283.15 to 313.15) K in Mass Fraction Percent

tie line	tie-line length	overall			top phase			bottom phase		
		w_{L35}	w_S	w_W	w_{L35}	w_S	w_W	w_{L35}	w_S	w_W
283.15 K										
1	40.51	21.11	25.13	53.76	38.56	15.79	45.65	2.08	33.41	64.51
2	46.99	23.04	25.61	51.35	43.14	15.20	41.66	0.79	35.55	63.66
3	52.72	25.05	26.22	48.73	48.22	14.39	37.39	1.08	37.99	60.93
4	56.30	26.84	26.78	46.38	51.17	14.00	34.83	0.82	39.18	60.00
5	60.51	28.67	27.34	43.99	53.36	13.80	32.84	0.01	42.35	57.64
298.15 K										
1	46.35	19.16	24.60	56.24	44.88	14.03	41.09	1.71	30.91	67.58
2	50.95	21.28	25.44	53.28	48.71	13.19	38.10	1.71	32.86	65.43
3	56.24	23.12	25.67	51.21	53.11	12.63	34.26	1.30	34.50	64.20
4	61.35	25.14	26.11	48.75	57.35	12.02	30.63	1.01	36.31	62.68
5	62.56	27.14	26.55	46.31	60.06	11.71	28.23	3.20	37.79	59.01
313.15 K										
1	35.95	19.19	24.83	55.98	33.94	17.28	49.60	1.00	31.67	67.33
2	40.26	21.25	25.12	53.63	39.13	16.64	44.23	2.22	32.72	65.06
3	46.04	22.97	25.29	51.74	43.06	15.28	41.66	1.07	34.17	64.76
4	53.71	25.10	26.21	48.69	48.26	14.35	37.39	0.04	38.00	61.96
5	57.77	27.10	26.69	46.21	53.46	13.68	32.86	1.74	39.41	58.85

content by subtracting the salt concentration obtained by conductivity from the total solution composition (refractive index). The standard deviation of the L35 mass percent was on the order of 0.05 %. Analytical curves were obtained for the salt and copolymer measurements, and water content was determined by mass balance. All analytical measurements were performed in triplicate.

Results and Discussion

The phase compositions for the ATPS formed by L35 and sodium salts are shown in Tables 1, 2, and 3, where all concentrations are expressed in mass percent. The systems formed by PEO 2000 + salts (citrate and tartarate)[17,18] showed the same biphasic area, suggesting that the dispersing molecular interaction associated with the polarizable organic anion determine the ATPS phase behavior more than macromolecular specific interactions. However, the system formed by PEO 2000 + sodium nitrate[19] has a small biphasic area, as compared with the system formed by L35 + sodium nitrate, showing, in the case of the inorganic anion, a macromolecular hydrophobic effect (L35 is more hydrophobic than PEO 2000). An important parameter that expressed the difference in the intensive thermodynamic properties of each phase of the ATPS is the tie-line length (TLL), and it can be described by the equation

$$\mathrm{TLL} = [(W_{L35}^{T} - W_{L35}^{B})^2 + (W_S^T - W_S^B)^2]^{1/2} \qquad (1)$$

where W_{L35} and W_S are the copolymer and salt concentrations, and the superscripts T and B designate the top and bottom phases, respectively.

Tables 1, 2, and 3 show that at constant temperature, for systems containing sodium citrate and sodium tartrate, the copolymer mass fraction of the salt-rich phase increases when the salt fraction increases. This behavior is observed for the systems formed by PEO 2000 + salt (citrate, tartarate, and nitrate).[17−19]

The temperature effect on the phase diagrams can be observed in Figures 1, 2, and 3. The influence of temperature was not significant for all systems. However, the temperature affects the volume and concentration of both phases.

Table 4 shows the values of the tie-line slope (STL) for the studied systems at all temperatures. The STL can be expressed by

$$\mathrm{STL} = \frac{W_{L35}^{T} - W_{L35}^{B}}{W_S^T - W_S^B} \qquad (2)$$

where W_{L35} and W_S are the polymer and salt concentrations and the superscripts T and B designate the top and bottom phases, respectively.

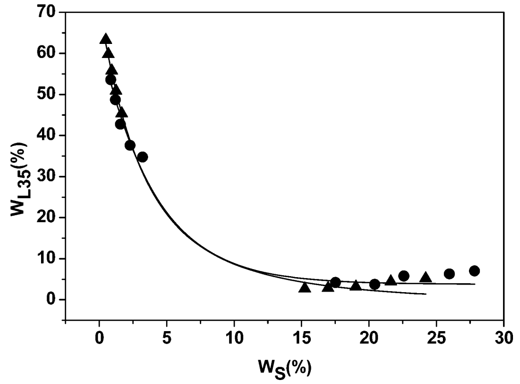

Figure 1. Temperature effect on the phase diagram for the L35 + sodium citrate system: ●, 283.15 K; ▲, 313.15 K.

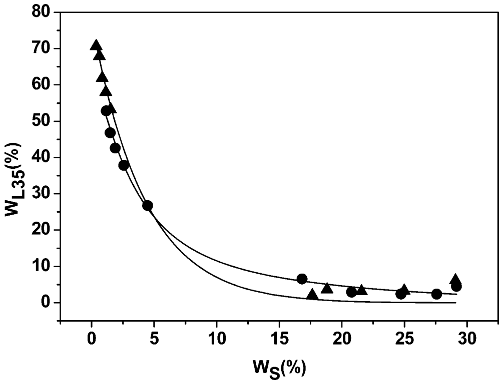

Figure 2. Temperature effect on the phase diagram for the L35 + sodium tartarate system: ●, 283.15 K; ▲, 313.15 K.

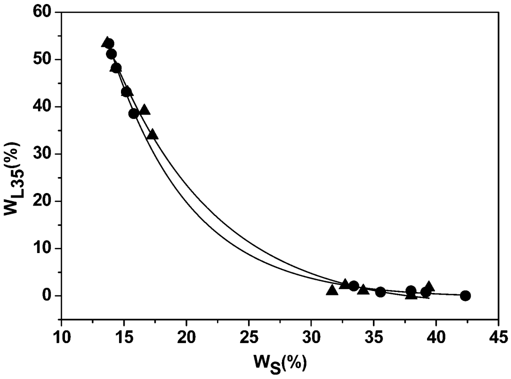

Figure 3. Temperature effect on the phase diagram for the L35 + sodium nitrate system: ●, 283.15 K; ▲, 313.15 K.

Table 4. STL Values For L35 + Salt + Water Systems

tie line	L35 + sodium citrate + water		
	T/K		
	283.15	298.15	313.15
1	−2.11	−2.57	−3.67
2	−1.85	−2.57	−3.28
3	−1.75	−2.27	−2.77
5	−1.72	−2.21	−2.65
4	−1.70	−2.21	−2.45

tie line	L35 + sodium tartarate + water		
	T/K		
	283.15	298.15	313.15
1	−1.62	−2.56	−3.18
2	−1.91	−2.40	−3.06
3	−1.76	−2.26	−2.84
4	−1.70	−2.24	−2.65
5	−1.72	−2.07	−2.25

tie line	L35 + sodium nitrate + water		
	T/K		
	283.15	298.15	313.15
1	−2.07	−2.28	−2.54
2	−2.08	−2.29	−2.37
3	−2.00	−2.22	−2.36
4	−2.00	−2.04	−2.32
5	−1.87	−2.01	−2.18

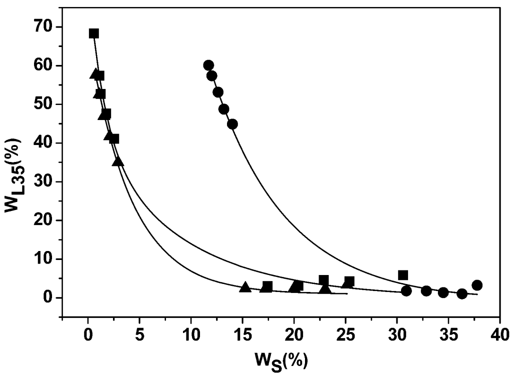

Figure 4. Anion effect on the phase diagram for the L35 + salt + water: ▲, sodium citrate; ■, sodium tartarate; ●, sodium nitrate.

The increase in the temperature promoted an STL increase. It is possible that the STL change for the L35 + sodium salt + water systems was due to the transfer of water from the top to the bottom phase. Therefore, the polymer concentration increases in the upper phase, and the salt content decreases in the lower phase. This behavior is observed for the systems formed by triblock copolymer L35 + sulfate salts + water or L35 + phosphate buffer + water.[12,13] However, the effect of temperature on the binodal position and STL is more expressive in the systems formed by L35 + sulfate salt or phosphate buffer.

Figure 4 shows the influence of salt in inducing phase segregation. The formation of the ATPS clearly indicates the mutual exclusion of the salt and polymer and their high affinity for water. This tendency is observed in all systems constituted by triblock copolymers or polymers and inorganic salt.[6,7,12−15] The preference of three salts in inducing the phase segregation in ATPS follows the order: sodium citrate > sodium tartarate > sodium nitrate. This preference can be observed in the Hoffmeister series (HS):[20] citrate^{3-} > SO_4^{2-} = tartarate^{2-} > HPO_4^{2-} > CrO_4^{2-} > acetate$^-$ > HCO_3^- > Cl^- > NO_3^- > ClO_3^-. The binodal area for the system formed by L35 + sodium sulfate[12] is the same for the system formed by L35 + sodium tartarate (present work). The HS originates from the ranking of various ions toward their ability to precipitate (salting-out) a mixture of hen egg white proteins, but the HS can be used to understand the phase segregation occurrence. The ions that decrease the solubility of macromolecules are denoted "kosmotropes" (with a high charge density, such as, citrate), and the ions that increase the solubility of macromolecules are denoted "chaotropes" (with a low charge density, such as, nitrate). The salting-out effect results from interfacial effects of strongly hydrated anions, kosmotropic ions, near the macromolecule surface removing water molecules from the macromolecule solvation layer and dehydrating its surface, which promotes the phase separation. (In general, the $\Delta_{hyd}G$ is more negative for the anions with a high charge density[21].)

Conclusions

Liquid−liquid equilibrium data for the systems L35 + sodium citrate + water, L35 + sodium tartarate + water, and L35 + sodium nitrate + water were determined at different temperatures (283.15 to 313.15) K. The temperature effect on the equilibrium data of L35 + sodium salt system was not relevant, but the anion nature has a major effect on the phase diagram of these ATPS. The efficacy of the salts in inducing phase segregation follows the order: sodium citrate > sodium tartarate > sodium nitrate. This order follows the Hofmeister series effect.

Literature Cited

(1) Zhang, T.; Li, W.; Zhou, W.; Gao, H.; Wu, J.; Xu, G.; Chen, J.; Liu, H.; Chen, J. Extraction and separation of gold(I) cyanide in polyethylene glycol-based aqueous biphasic systems. *Hydrometallurgy* **2001**, *62*, 41–46.

(2) da Silva, L. H. M.; da Silva, M. C. H.; de 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.

(3) Rodrigues, L. R.; Venâncio, A.; Teixeira, J. A. Partitioning and separation of α-lactalbumin and β-lactoglobulin in polyethylene glycol/ammonium sulphate aqueous two-phase systems. *Biotechnol. Lett.* **2001**, *23*, 1893–1897.

(4) da Silva, L. H. M.; Meirelles, A. J. A. Bovine serum albumin, α-lactoalbumin and β-lactoglobulin partitioning in polyethylene glycol/maltodextrin aqueous-two-phase systems. *Carbohydr. Polym.* **2000**, *42*, 279–282.

(5) Willauer, H. D.; Huddleston, J. G.; Rogers, R. D. Solute partitioning in aqueous biphasic systems composed of polyethylene glycol and salt: the partitioning of small neutral organic species. *Ind. Eng. Chem. Res.* **2002**, *41*, 1892–1904.

(6) Oliveira, R. M.; Coimbra, J. S.; Francisco, K.; Minim, L. A.; da Silva, L. H.; Pereira, J. A. 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.

(7) Martins, J. P.; Carvalho, C. P.; da Silva, L. H. M.; Coimbra, J. S. R.; da 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.

(8) de Oliveira, R. M.; Coimbra, J. S. R.; Minim, L. A.; da Silva, L. H. M.; 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.

(9) Rodrigues, G. D.; da Silva, M. C. H.; da Silva, L. H. M.; Paggioli, F. J.; Minim, L. A.; Coimbra, J. S. R. Liquid−liquid extraction metal ions without use of organic solvent. *Sep. Purif. Technol.* **2008**, *62*, 687–693.

(10) da Silva, M. C. H.; da Silva, L. H. M.; Paggioli, F. J. A novel micellar medium using triblock copolymer for cobalt determination. *Anal. Sci.* **2005**, *21*, 933–937.

(11) da Silva, L. H. M.; da Silva, M. C. H.; Amim-Junior, 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.

(12) da Silva, M. D. H.; da Silva, L. H. M.; Amim Junior, J.; Guimaraes, R. O.; Martins, J. P. Liquid−liquid equilibrium of aqueous mixture of triblock copolymers L35 and F68 with Na_2SO_4 or Li_2SO_4 or $MgSO_4$. *J. Chem. Eng. Data* **2006**, *51*, 2260–2264.

(13) da Silva, L. H. M.; da Silva, M. d. C. H.; Mesquita, A. F.; do Nascimento, K. S.; Coimbra, J. S. R.; Minim, L. A. Equilibrium phase behavior of triblock copolymer + salt + water two-phase systems at different temperatures and pH. *J. Chem. Eng. Data* **2005**, *50*, 1457–1462.

(14) Haraguchi, L. H.; Mohamed, R. S.; Loh, W.; Pêssoa Filho, P. A. Phase equilibrium and insulin partitioning in aqueous two-phase systems containing block copolymers and potassium phosphate. *Fluid Phase Equilib.* **2004**, *215*, 1–15.

(15) de Oliveira, M. C.; de Abreu Filho, M. A. N.; Pêssoa Filho, P. A. Phase equilibrium and protein partitioning in aqueous two-phase systems containing ammonium carbamate and block copolymers PEO-PPO-PEO. *Biochem. Eng. J.* **2007**, *37*, 311–318.

(16) Svensson, M.; Berggren, K.; Veide, A.; Tjerneld, F. Aqueous two-phase systems containing self-associating block copolymers: partitioning of hydrophilic and hydrophobic biomolecules. *J. Chromatogr., A* **1999**, *839*, 71–83.

(17) Murugesan, T.; Perumalsamy, M. Liquid−liquid equilibria of poly(ethylene glycol) 2000 + sodium citrate + water at (25, 30, 35, 40, and 45) °C. *J. Chem. Eng. Data* **2005**, *50*, 1392–1395.

(18) Malpiedi, L. C.; Fernández, C.; Picó, G.; Nerli, B. Liquid−liquid equilibrium phase diagrams of polyethyleneglycol + sodium tartarate + water two-phase systems. *J. Chem. Eng. Data* **2008**, *53*, 1175–1178.

(19) Graber, T. A.; Taboada, M. E.; Asenjo, J. A.; Andrews, B. A. Influence of molecular weight of the polymer on the liquid−liquid equilibrium of the poly(ethylene glycol) + $NaNO_3$ + H_2O system at 298.15 K. *J. Chem. Eng. Data* **2001**, *46*, 765–768.

(20) Kunz, W.; Henle, J.; Ninham, B. W. 'Zur Lehre von der Wirkung der Salze' (about the science of the effect of salts: Franz Hofmeister's historical papers. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 19–37.

(21) Marcus, Y. Thermodynamics of solvation of ions. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 2995–2999.

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