Liquid−Liquid Phase Equilibrium of Triblock Copolymer L64, Poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide), with Sulfate Salts from (278.15 to 298.15) K

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Liquid−liquid equilibrium data of aqueous two-phase systems (ATPS) formed by mixture of aqueous solutions of triblock copolymer poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide), L64, and sulfate salts were obtained. L64 + lithium sulfate + water, L64 + magnesium sulfate + water, L64 + sodium sulfate + water, and L64 + zinc sulfate + water were formed at (278.15, 288.15, and 298.15) K. The influence of temperature in the binodal position was pronounced. The phase separation process was endothermic for the L64 + Li_2SO_4 system and exothermic for the L64 + $MgSO_4$, L64 + Na_2SO_4 and L64 + $ZnSO_4$ systems. Also, the slope of the tie line tended to increase with an increase in temperature. Also, the capability of the salts to induce the formation of the biphasic system followed the order $ZnSO_4 = MgSO_4 > Na_2SO_4 > Li_2SO_4$.

Introduction

Aqueous two phase systems (ATPS) have been applied, especially in the separation, preconcentration, and purification of biocompounds such as cell organelles,[1,2] proteins,[3] membranes,[4] DNA,[5] antibodies,[6] nanoparticles,[7] dye molecules,[8] and metallic ions.[9,10]

ATPS can be formed under specific thermodynamics conditions[11] by the mixture of aqueous solutions of either polymers, one polymer and one salt, or two salts.[12] The resulting ternary mixtures are systems with two immiscible phases, a polymer-enriched top phase (or enriched with a salt) and a salt-enriched bottom phase (or enriched with another polymer). In almost all ATPS is observed a segregation process between polymer and salts resulting in systems where water is the major component in both phases.

Many equilibrium data for ATPS composed of poly(ethylene glycol) (PEG) have been reported in the literature,[13−16] in which the influence of the electrolyte nature and temperature in the phase separation process has been verified. However, the applications of these systems as separation processes are limited for water-soluble compounds (hydrophilic solute).

As alternative to this limitation, ATPS formed by triblock copolymers are excellent options in the extraction of hydrophobic solute into the polymer-enriched phase.[17] This type of polymer, in aqueous solution and under critical temperature and concentration conditions, goes through an aggregation process forming micelles with a crown of hydrophilic units poly(ethylene oxide) (PEO) and a core of hydrophobic units poly(propylene oxide) (PPO),[18] in which water-insoluble compounds can be solubilized.

However, liquid−liquid equilibrium data for systems constituted by triblock copolymers are scarce.[11,19,20] To our best knowledge, there is no work found in literature that describes phase diagrams of ATPS composed of the triblock copolymer L64 and sulfate salts. In this work, biphasic systems composed of $(EO)_{13}(PO)_{30}(EO)_{13}$ (L64) + salt + water were prepared, and phase compositions were measured. To determine the effect of the salt cations, we used the electrolytes lithium sulfate, magnesium sulfate, sodium sulfate, or zinc sulfate. Equilibrium data at (278.15, 288.15, and 298.15) K were determined to study the influence of temperature in the binodal position.

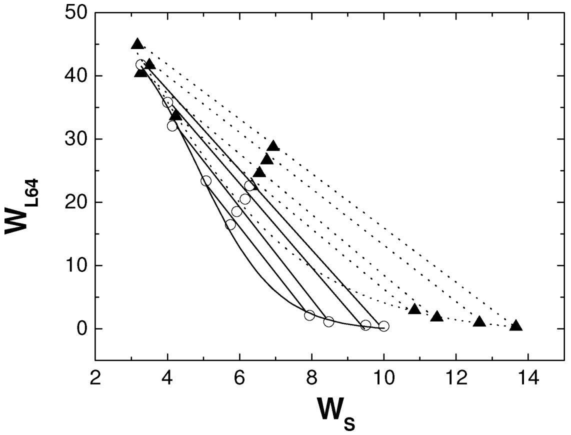

Figure 1. Temperature effect on the phase diagram for the L64 + Li_2SO_4 system: ▲, 278.15 K; ○, 298.15 K.

Experimental Section

Materials. Poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) copolymer with the basic backbone $(EO)_n(PO)_m(EO)_n$ was used. The L64, $(EO)_{13}(PO)_{30}(EO)_{13}$, with average molar mass (M_w) of 2900 $g \cdot mol^{-1}$ with mass fraction of 40 % EO was purchased from Aldrich. The analytical grade reagents Li_2SO_4, $MgSO_4$, Na_2SO_4, and $ZnSO_4$ were obtained from Vetec (Brazil). Milli-Q II water (Millipore) was used to prepare all of the aqueous solutions.

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Table 1. Equilibrium Data for the L64 (w_{L64}) + Lithium Sulfate (w_S) + Water (w_W) System from (278.15 to 298.15) K

	overall			top phase			bottom phase			
system	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	TLL
					T = 278.15 K					
1	22.57	6.35	71.08	33.61	4.23	62.16	2.92	10.85	86.23	31.39
2	24.61	6.55	68.84	40.42	3.26	56.32	1.75	11.47	86.78	39.53
3	26.63	6.76	66.61	41.68	3.50	54.82	0.97	12.65	86.38	41.73
4	28.73	6.93	64.35	44.88	3.17	51.95	0.35	13.66	85.98	45.75
					T = 288.15 K					
1	22.73	6.31	70.95	35.91	4.15	59.94	4.20	9.57	86.23	32.17
2	24.63	6.58	68.79	38.72	3.82	57.46	2.59	10.63	86.78	36.77
3	26.84	6.74	66.42	44.97	3.07	51.96	2.46	11.16	86.38	43.27
4	28.80	6.95	64.25	49.90	2.45	47.65	1.94	12.08	85.98	48.92
					T = 298.15 K					
1	16.45	5.75	77.80	23.40	5.07	71.53	2.09	7.95	89.96	21.51
2	18.55	5.93	75.52	32.08	4.14	63.78	1.12	8.48	90.39	31.25
3	20.49	6.16	73.34	35.77	4.01	60.23	0.56	9.50	89.94	35.63
4	22.62	6.28	71.10	41.72	3.27	55.00	0.41	10.01	89.57	41.86

Table 2. Equilibrium Data for the L64 (w_{L64}) + Magnesium Sulfate (w_S) + Water (w_W) System from (278.15 to 298.15) K

	overall			top phase			bottom phase			
system	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	TLL
					T = 278.15 K					
1	25.28	4.67	70.05	35.40	1.20	63.40	0.49	14.55	84.95	37.37
2	27.88	5.1	67.02	39.51	0.87	59.62	0.21	15.82	83.97	42.05
3	30.15	5.7	64.15	44.32	0.64	55.04	0.46	16.73	82.82	46.72
4	31.57	6.41	62.02	48.61	0.59	50.80	0.52	17.84	81.64	51.10
5	34.04	7.07	58.89	53.00	0.35	46.64	0.67	18.90	80.43	55.52
					T = 288.15 K					
1	20.30	4.51	75.19	32.56	1.94	65.50	0.88	9.40	89.72	32.55
2	25.32	4.72	69.96	40.05	1.24	58.71	0.45	11.37	88.18	40.88
3	27.87	5.12	67.01	45.15	0.98	53.87	0.02	12.90	87.09	46.69
4	29.97	5.69	64.34	49.10	0.71	50.19	0.16	13.91	85.93	50.69
5	31.58	6.38	62.04	53.13	0.54	46.32	0.33	15.14	84.53	54.78
					T = 298.15 K					
1	21.87	4.63	73.50	35.15	2.87	61.98	5.56	6.09	88.35	29.76
2	25.56	4.94	69.50	45.58	2.20	52.22	5.15	7.18	87.66	40.73
3	29.10	5.30	65.60	50.87	2.01	47.12	4.97	8.20	86.83	46.32
4	32.74	5.77	61.49	55.03	1.91	43.07	5.30	9.53	85.17	50.31
5	37.02	5.96	57.02	58.19	1.82	39.99	5.16	11.03	83.81	53.83

Aqueous Two-Phase Systems. We prepared ATPS by adding appropriate quantities of L64, sulfate salt (Li_2SO_4, $MgSO_4$, Na_2SO_4, or $ZnSO_4$), and water on an analytical balance (Gehaka, AG 200) with a given uncertainty ± 0.0001 g. Liquid−liquid equilibrium cells were used to carry out phase equilibrium determinations. Typically, 10 g of the mixture was prepared. The mixture was vigorously stirred until the system became turbid. It was allowed to settle for (24 to 72) h at the operational temperature (278.15, 288.15, or 298.15) K in a temperature-controlled bath (Microquímica, MQBTC 99-20, with a temperature uncertainty of ± 0.1 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 with a syringe for analysis.

Phase Diagram Determination. The salt composition was determined by conductimetry (Schott CG853, Germany) in the mass fraction range of $(1.00 \cdot 10^{-3}$ to $2.50 \cdot 10^{-2})$ %. The conductivity of salt solutions was independent of the polymer solution composition (diluted regime). The uncertainty of the salt composition by this method was ± 0.10 %. The copolymer was quantified by measurements at 298.15 K using a refractometer (Analytic Jena AG Abbe refractometer 09-2001, Germany). Because the refractive index of the phase samples depends on the copolymer and salt concentration and it is an additive property, we obtained the L64 concentration by subtracting the salt concentration (obtained by conductivity) from the total solution composition (obtained by refractive index).[11] The uncertainty of the copolymer mass fraction was ± 0.005 %. The water mass fraction was determined by freeze drying (Speed Vacuum Concentration HETO VR-1, Denmark) at −20 °C for 24 h. The uncertainty of the water mass fraction was ± 0.06 %. All analytical measurements were performed in triplicate.

Results and Discussion

Tables 1, 2, 3, and 4 show liquid−liquid equilibrium data, expressed in mass percent, for L64 + Li_2SO_4 + water, L64 + $MgSO_4$ + water, L64 + Na_2SO_4 + water, and L64 + $ZnSO_4$ + water ATPS at (278.15, 288.15, and 298.15) K. According to the system, four to six tie-line lengths (TLL) were determined. The tie lines were obtained by linear regression of the corresponding set of overall bottom phase and top phase concentrations. An increase in copolymer and salt segregation with an increase in global composition and consequently in the TLL is noted.[10] This behavior is in agreement with the reported results for other aqueous two-phase systems.[11,16,19,21,22]

The influence of temperature on the binodal position of the ATPS is presented in Figures 1, 2, 3, and 4. For all ATPS, the temperature has a significant effect on the phase equilibrium compositions. For the L64 + Li_2SO_4 system, a decrease in temperature causes a reduction of the two-phase area, indicating that the phase separation process is endothermic. This entropic

Table 3. Equilibrium Data for the L64 (w_{L64}) + Sodium Sulfate (w_S) + Water (w_W) System from (278.15 to 298.15) K

	overall			top phase			bottom phase			
system	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	TLL
					$T = 278.15$ K					
1	19.93	5.18	74.89	29.92	1.88	68.20	1.04	11.37	87.59	30.40
2	23.19	5.47	71.34	35.11	1.50	63.40	0.75	13.09	86.17	36.26
3	26.46	5.78	67.76	40.68	1.22	58.11	1.07	13.83	85.09	41.56
4	29.72	6.03	64.25	45.91	0.98	53.11	0.45	15.46	84.09	47.71
5	33.15	6.34	60.52	49.92	0.89	49.19	0.84	16.87	82.29	51.62
6	35.94	6.62	57.44	52.07	0.78	47.15	0.68	18.75	80.57	54.45
					$T = 288.15$ K					
1	19.95	5.17	74.88	29.50	3.31	67.19	0.02	8.97	91.01	30.02
2	23.29	5.45	71.25	38.32	2.22	59.46	0.01	10.62	89.38	39.23
3	26.42	5.75	67.82	44.07	1.70	54.22	0.01	11.76	88.23	45.20
4	29.78	6.06	64.16	48.99	1.33	49.68	0.02	13.36	86.62	50.42
5	32.99	6.33	60.68	52.26	1.07	46.68	0.01	15.01	84.99	54.09
					$T = 298.15$ K					
1	19.97	5.19	74.84	34.31	3.10	62.59	1.21	8.58	90.20	33.55
2	22.14	5.39	72.47	41.73	2.23	56.05	0.55	9.60	89.85	41.83
3	24.29	5.70	70.01	47.49	1.76	50.75	0.47	10.46	89.08	47.82
4	26.59	5.98	67.42	49.83	1.33	48.84	0.55	11.37	88.08	50.29
5	28.48	6.30	65.22	54.12	1.19	44.69	0.37	12.50	87.13	54.93

Table 4. Equilibrium Data for the L64 (w_{L64}) + Zinc Sulfate (w_S) + Water (w_W) System from (278.15 to 298.15) K

	overall			top phase			bottom phase			
system	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	$100w_{L64}$	$100w_S$	$100w_w$	TLL
					$T = 278.15$ K					
1	19.75	7.28	72.97	33.33	1.37	65.30	0.27	16.38	83.35	36.31
2	22.33	7.77	69.90	36.46	1.12	62.42	0.50	18.55	80.94	39.95
3	24.94	8.29	66.77	40.27	0.87	58.86	0.90	20.09	79.01	43.82
4	27.49	8.8	63.71	44.82	0.67	54.51	1.17	21.13	77.70	48.20
5	29.1	9.25	61.65	48.07	0.51	51.41	1.34	21.95	76.71	51.42
					$T = 288.15$ K					
1	17.16	6.82	76.02	33.43	2.24	64.33	0.64	12.05	87.31	34.22
2	19.78	7.3	72.92	38.04	1.63	60.33	0.03	13.63	86.34	39.86
3	22.36	7.82	69.82	43.21	1.37	55.42	0.03	15.00	84.97	45.28
4	24.96	8.31	66.73	45.91	1.10	52.98	0.03	16.54	83.43	48.41
5	27.42	8.76	63.82	49.47	0.84	49.69	0.10	17.77	82.13	52.20
					$T = 298.15$ K					
1	17.12	6.83	76.05	34.14	3.21	62.64	0.29	11.22	88.49	34.79
2	19.75	7.31	72.94	41.95	2.00	56.05	0.36	12.34	87.31	42.86
3	22.28	7.85	69.87	47.06	1.47	51.46	0.79	13.36	85.85	47.78
4	24.94	8.29	66.77	50.54	1.27	48.19	0.31	15.17	84.52	52.12
5	25.81	8.67	65.52	51.98	1.12	46.90	0.78	15.74	83.48	53.25

phase separation is a general ATPS process and has already been related by other authors in previous studies.[10] However, L64 + $MgSO_4$, L64 + Na_2SO_4 and L64 + $ZnSO_4$ systems showed an increase in the biphasic area with a decrease in temperature, indicating that the phase separation process is exothermic. To our best knowledge, this work is the first that describes an ATPS enthalpy phase separation.

The temperature effect on the phase equilibrium compositions can be analyzed through the slope of the tie lines (STL) values that are reported in Table 5. The STL is defined as the ratio

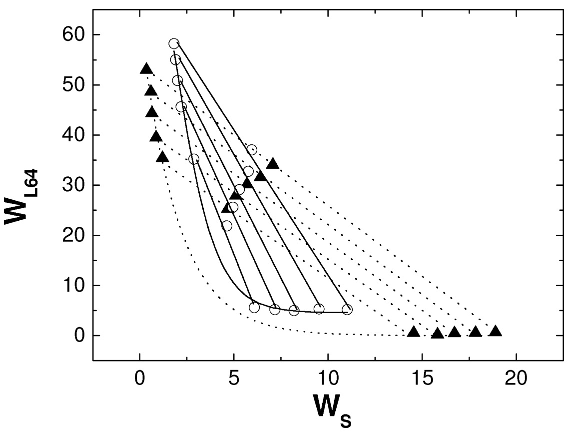

Figure 2. Temperature effect on the phase diagram for the L64 + $MgSO_4$ system: ▲, 278.15 K; ○, 298.15 K.

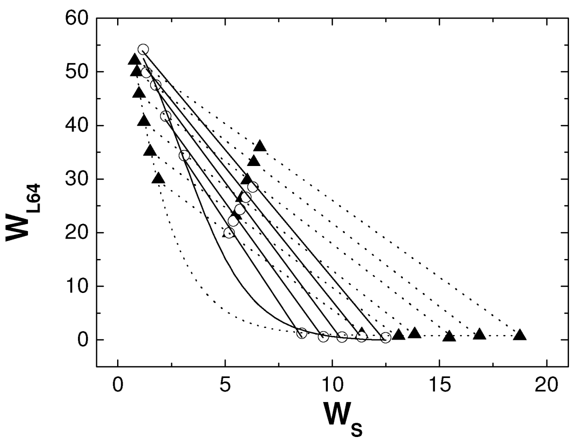

Figure 3. Temperature effect on the phase diagram for the L64 + Na_2SO_4 system: ▲, 278.15 K; ○, 298.15 K.

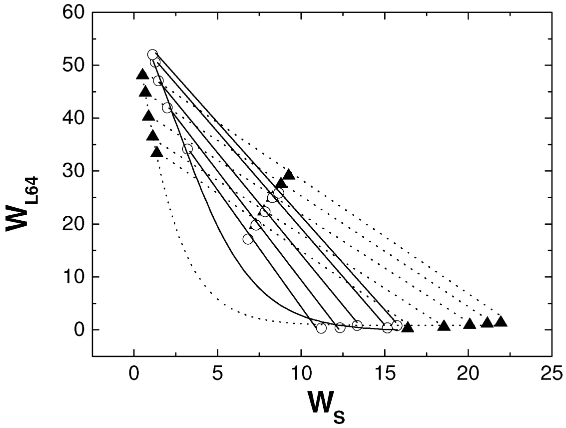

Figure 4. Temperature effect on the phase diagram for the L64 + $ZnSO_4$ system: ▲, 278.15 K; ○, 298.15 K.

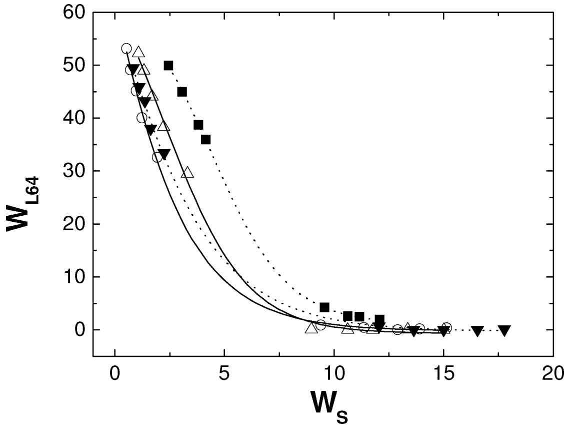

Figure 5. Infuence of cation on the phase diagram of the L64 + sulfate salt systems at 288.15 K: ■, Li_2SO_4; ○, $MgSO_4$; △, Na_2SO_4; ▼, $ZnSO_4$.

Table 5. STL Values for the L64 + Salt + Water Systems

	L64 + lithium sulfate + water		
	T/K		
system	278.15	288.15	298.15
1	−4.60	−5.84	−7.24
2	−4.70	−5.32	−7.12
3	−4.44	−5.27	−6.37
4	−4.24	−4.99	−6.12

	L64 + magnesium sulfate + water		
	T/K		
system	278.15	288.15	298.15
1	−2.59	−4.21	−9.14
2	−2.62	−3.89	−8.08
3	−2.72	−3.76	−7.40
4	−2.78	−3.70	−6.52
5	−2.82	−3.61	−5.77

	L64 + sodium sulfate + water		
	T/K		
system	278.15	288.15	298.15
1	−3.04	−5.21	−5.99
2	−2.96	−4.56	−5.56
3	−3.14	−4.38	−5.39
4	−3.14	−4.07	−4.90
5	−3.04	−3.75	−4.74
6	−2.87		

	L64 + zinc sulfate + water		
	T/K		
system	278.15	288.15	298.15
1	−2.20	−3.34	−4.21
2	−2.06	−3.17	−4.02
3	−2.05	−2.97	−3.89
4	−2.13	−3.17	−3.61
5	−2.18	−2.91	−3.50

STL = ($\Delta_{polymer}/\Delta_{salt}$), in which $\Delta_{polymer}$ and Δ_{salt} are the differences between the compound composition in the two coexisting phases, respectively. According to the results, for all ATPS, an increase in the temperature promotes an increase in the STL. A possible explanation for this change is the spontaneous diffusion of water molecules from the top phase to the bottom phase, resulting in an increase in the copolymer composition in the upper phase and a reduction in the salt composition in the lower phase.

Figure 5 presents the influence of the cation to induce phase segregation at 288.15 K. The three salts promote the formation of the ATPS in the order $ZnSO_4 = MgSO_4 > Na_2SO_4 > Li_2SO_4$. The same results were observed for L64 + M_xSO_4 + H_2O (M = Li^+, Na^+, Mg^{2+}, or Zn^{2+}) diagrams determined at (278.15 and 298.15) K. This behavior may be explained by the model proposed by da Silva and Loh.[23] Through calorimetric measurements, it was suggested that the trend in the effectiveness of the sulfate salts to induce ATPS formation is due to the cation−polymer interactions. According to this model, when the polymer and sulfate salts are mixed, cations interact with the ethylene oxide copolymer groups, releasing some water molecules that solvate EO groups 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.[24]

Remarkably, the ATPS composed of L64 + $ZnSO_4$ and L64 + $MgSO_4$ show a larger biphasic area as compared with the system L64 + Li_2SO_4. This behavior may be explained by the fact that a lower amount of cations Mg^{2+} and Zn^{2+} can energetically saturate the copolymer chain as compared with the Li^+ cation.

Conclusions

Liquid−liquid equilibrium data for the systems L64 + Li_2SO_4 + water, L64 + $MgSO_4$ + water, L64 + Na_2SO_4 + water, and L64 + $ZnSO_4$ + water were obtained from (278.15 to 298.15) K. For the ATPS L64 + Li_2SO_4, the rise in the temperature causes an increase in the two-phase area. Surprisingly, the phase separation process for the systems L64 + $MgSO_4$, L64 + Na_2SO_4, and L64 + $MgSO_4$ is exothermic. It is the first work in the literature that verified this effect of temperature on the phase diagram of ATPS. An increase in the STL with an increase in the temperature was also observed. The efficacy of the salts to induce phase segregation follows the order $ZnSO_4 = MgSO_4 > Na_2SO_4 > Li_2SO_4$.

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