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Liquid−Liquid Phase Equilibrium of Triblock Copolymer F68, Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide), with Sulfate Salts

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Phase diagrams of aqueous two-phase systems (ATPS) composed of triblock copolymer (F68) 8460 g·mol^{-1}, sodium sulfate, lithium sulfate, zinc sulfate, or ammonium sulfate were determined at (278.2, 288.2, and 298.2) K. The temperature effect on the biphasic area is very small, indicating a strong entropic contribution associating to the phase separation. The ability of these four salts in inducing the formation of the ATPS with F68 followed the order: sodium sulfate > zinc sulfate > ammonium sulfate > lithium sulfate.

Introduction

An aqueous two-phase system (ATPS) consists of a mixture of two structurally different polymers (or polymer + salt) that separate into two phases above a critical concentration and temperature. The ATPS offers advantages of high capacity,[1] lower excess interfacial properties,[2] and facile scale-up.[3] These systems can be used for determination, purification, or separation of chemical compounds such as phenol,[4] natural dye,[5] metal ions,[6,7] amino acids,[8] or biological structures[9,10] such as viruses, antibodies, or cells. The partitioning behavior of a chemical compound depends on properties such as net charge, size, and hydrophobicity. It can also be affected by including various salts in the system and changing the pH,[11] temperature,[12] type of polymer,[13] or polymer molecular mass.[14]

Many ATPS have been found utilizing hydrophilic or hydrophobic polymers in aqueous solution. The most commonly used system is formed by the macromolecule poly(ethylene glycol) (PEG, $HO{-}(CH_2{-}CH_2{-}O)_n{-}H$) and an inorganic salt. However, the low hydrophobicity of ATPS formed by PEG + salt mixtures hinders separation of insoluble water molecules.

An alternative, to use ATPS to purify or separate hydrophobic compounds, is to exchange the PEG for a triblock copolymer. This type of macromolecule builds self-organized structures (micelles), which allow the application of ATPS to separate solutes with a small affinity to a water microenvironment.[15] Another advantage is the possibility of obtaining a triblock copolymer with different EO (ethylene oxide unit)/PO (polypropylene oxide unit) rates that may change hydrophobicity of the ATPS. The equilibrium data for ATPS formed by triblock copolymers of different EO/PO ratios (and/or different average molar mass) and inorganic salts[16−20] or Dextran + triblock copolymer[21] are very limited. This lack of data limits the application of ATPS formed by auto-organized structures for purifying hydrophobic compounds.

Table 1. Equilibrium Data for the F68 (w_{F68}) + Sodium Sulfate (w_S) + Water (w_W) System from (278.15 to 298.15) K

		overall			top phase			bottom phase		
system	tie line length	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W
$T = 278.2$ K										
1	20.13	10.91	7.06	82.03	20.43	3.77	75.80	1.40	10.34	88.26
2	27.82	12.94	7.68	79.38	25.88	2.57	71.55	0.01	12.79	87.20
3	33.80	15.83	7.94	76.23	31.66	2.01	66.33	0.01	13.86	86.13
4	35.98	17.41	7.96	74.63	34.24	1.61	64.15	0.58	14.31	85.11
5	41.11	18.82	9.69	71.49	37.62	1.40	60.98	0.01	17.99	82.00
$T = 288.2$ K										
1	23.65	12.52	6.62	80.86	23.81	3.10	73.09	1.23	10.14	88.63
2	29.09	15.19	6.88	77.93	29.04	2.43	68.53	1.34	11.33	87.33
3	34.54	17.58	7.51	74.91	33.94	1.97	64.09	1.23	13.05	85.72
4	38.81	18.93	8.45	72.62	37.12	1.70	61.18	0.73	15.20	84.07
5	42.33	21.13	8.93	69.94	40.93	1.45	57.61	1.33	16.42	82.25
$T = 298.2$ K										
1	24.67	12.39	5.81	81.80	24.28	2.52	73.20	0.51	9.11	90.38
2	26.53	12.90	6.34	80.76	25.65	2.68	72.67	0.15	10.00	89.85
3	31.94	15.35	6.97	77.68	30.69	2.54	66.77	0.01	11.41	88.58
4	37.42	17.85	7.70	74.45	35.56	1.65	62.79	0.15	13.75	86.10
5	39.89	18.70	8.69	72.61	37.40	1.74	60.86	0.01	15.63	84.36

In this work, aqueous two-phase systems composed of triblock copolymer F68 ($(PEO)_{80}{-}(PPO)_{30}{-}(PEO)_{80}$) + sodium salt + water were constructed, and their phase compositions were observed. Equilibrium data at (278.2, 288.2, and 298.2) K were determined for the systems consisting of F68 + sodium sulfate + water, F68 + zinc sulfate + water, F68 + lithium sulfate + water, and F68 + ammonium sulfate + water. The influences of the cation electrolyte and temperature on the biphasic area were also investigated.

Experimental Section

Materials. The following reagents were used: triblock copolymer F68 (80 % EO), $(PEO)_{80}{-}(PPO)_{30}{-}(PEO)_{80}$ (Sigma, USA), sodium sulfate, zinc sulfate, ammonium sulfate, and lithium sulfate (Vetec, Brazil), all of analytical grade. Millipore (USA) water was used in all experiments ($R \geq 18.2$ MΩ·cm^{-1}). The triblock copolymer and salts were used as received. The copolymer was analyzed by gel permeation chromatography

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Table 2. Equilibrium Data for the F68 (w_{F68}) + Zinc Sulfate (w_S) + Water (w_W) System from (278.2 to 298.2) K

		overall			top phase			bottom phase		
system	tie line length	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W
$T = 278.2$ K										
1	23.12	11.09	8.51	80.40	21.77	4.10	74.13	0.40	12.93	86.67
2	27.67	12.96	8.86	78.18	25.46	2.93	71.61	0.46	14.79	84.75
3	30.44	13.74	9.51	76.75	27.35	2.70	69.95	0.13	16.32	83.55
4	33.14	16.16	9.39	74.45	31.02	2.06	66.92	1.30	16.72	81.98
5	37.99	16.62	10.82	72.56	33.23	1.60	65.17	0.01	20.04	79.95
$T = 288.2$ K										
1	16.50	8.52	8.14	83.34	16.18	5.09	78.73	0.85	11.20	87.95
2	25.55	11.63	8.74	79.63	23.21	3.36	73.43	0.04	14.13	86.32
3	28.85	13.91	8.60	77.49	27.04	2.64	70.33	0.77	14.56	84.66
4	33.67	15.24	9.43	75.33	30.43	2.18	67.38	0.04	16.68	83.39
5	38.13	17.01	10.37	72.62	33.98	1.67	64.35	0.04	19.05	81.78
$T = 298.2$ K										
1	16.69	9.31	7.12	83.57	17.03	3.96	79.01	1.58	10.28	88.14
2	22.66	10.61	7.88	81.51	21.15	3.72	75.13	0.07	12.04	87.89
3	28.74	13.43	8.04	78.53	26.84	2.89	70.27	0.01	13.19	86.80
4	33.32	15.46	8.49	76.05	30.92	2.27	66.81	0.01	14.70	85.29
5	36.92	17.06	8.98	73.96	34.10	1.89	64.01	0.01	16.07	83.92

(GPC) (AKTA, purifier, 10×, Pharmacia) using the following conditions: toluene as mobile phase at a rate of 0.8 $mL \cdot min^{-1}$, injection temperature of 318.2 K, refraction index detector, sample injection of 100 μL. The standard polymer was polystyrene. This methodology was used to obtain the average molar mass (M_w) and polydispersity index (M_w/M_n) of 8460 $g \cdot mol^{-1}$ and 1.42, respectively, for the copolymers. GPC traces of homopolymers or diblock copolymer were not observed.

Aqueous Two-Phase Systems. Feed samples were prepared by mixing appropriate amounts of stock solutions of F68, sulfate salts (sodium, zinc, ammonium, or lithium), and water on an analytical balance (Gehaka, AG200, Brazil), with a given uncertainty ± 0.0001 g. Glass tubes were used to carry out phase equilibrium determinations. Typically, 10 g of each system was prepared. After sufficient mixing, the turbid solution was placed in a temperature-controlled bath (Microquímica, MQBTC 99-20, with an uncertainly of ± 0.1 K) for 72 h at (278.2, 288.2, or 298.2) K. The equilibrium state was characterized by the absence of turbidity in both top and bottom phases. Aliquots of solutions in both phases were carefully removed for analysis.

Quantitative Analysis. The salt concentration (sodium sulfate, zinc sulfate, ammonium sulfate, lithium sulfate) 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 [(0.5 to 0.001) %]. 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 F68 quantity at 298.2 K. Since the refractive index of the phase depends on the polymer and salt concentrations, F68 content was obtained by subtracting the salt concentration obtained by conductivity from the total solution composition (refractive index). The standard deviation of the F68 mass percent was on the order of 0.05 %. Analytical curves were obtained for the salt and F68 measurements. The water content was determined by difference of mass (mass, wt) of each component ($w_{H2O} = w_{total} - w_S - w_{F68}$), where w_{total} = 100 % (w/w). All analytical measurements were performed in triplicate.

Table 3. Equilibrium Data for the F68 (w_{F68}) + Ammonium Sulfate (w_S) + Water (w_W) System from (278.2 to 298.2) K

		overall			top phase			bottom phase		
system	tie line length	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W
$T = 278.2$ K										
1	18.70	10.29	9.36	80.35	18.97	5.89	75.14	1.61	12.83	85.56
2	23.96	11.85	9.63	78.52	22.96	5.13	71.91	0.75	14.12	85.13
3	28.26	13.65	9.79	76.56	26.72	4.42	68.86	0.58	15.15	84.27
4	33.29	16.58	10.17	73.25	31.91	3.68	64.41	1.25	16.66	82.09
5	38.02	17.77	11.38	70.85	35.01	3.37	61.62	0.53	19.38	80.09
$T = 288.2$ K										
1	19.51	9.25	9.65	81.10	18.20	5.77	76.03	0.30	13.53	86.17
2	26.03	12.34	9.19	78.47	24.54	4.66	70.80	0.14	13.72	86.14
3	29.58	13.88	9.70	76.42	27.65	4.31	68.04	0.10	15.09	84.81
4	32.32	14.52	11.3	74.18	29.04	4.21	66.75	0.00	18.39	81.61
5	41.60	19.16	11.12	69.72	38.32	3.02	58.66	0.00	19.21	80.79
$T = 298.2$ K										
1	17.05	9.51	8.02	82.47	17.73	5.75	76.52	1.30	10.30	88.40
2	23.66	13.06	7.95	78.99	24.49	4.90	70.61	1.63	11.01	87.36
3	32.93	15.78	8.72	75.50	31.46	3.71	64.83	0.09	13.73	86.18
4	36.25	16.91	10.19	72.90	33.79	3.58	62.63	0.04	16.80	83.16
5	40.04	18.64	11.07	70.29	37.23	3.65	59.12	0.04	18.50	81.46

Table 4. Equilibrium Data for the F68 (w_{F68}) + Lithium Sulfate (w_S) + Water (w_W) System from (278.2 to 298.2) K

		overall			top phase			bottom phase		
system	tie line length	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W	w_{F68}	w_S	w_W
$T = 278.2$ K										
1	31.46	17.51	8.22	74.27	32.58	3.72	63.70	2.43	12.72	84.85
2	35.95	18.75	8.56	72.69	35.80	3.50	60.70	1.70	13.61	84.69
3	40.87	20.70	8.45	70.85	39.95	2.84	57.21	1.44	14.07	84.49
4	42.34	21.05	9.12	69.83	41.01	3.05	55.94	0.99	15.20	83.81
5	42.12	21.59	9.53	68.88	42.44	2.90	54.66	0.75	16.16	83.09
$T = 288.2$ K										
1	21.82	11.77	7.91	80.32	22.31	5.09	72.61	1.23	10.72	88.04
2	27.13	14.03	7.93	78.04	27.11	4.35	68.53	0.94	11.51	87.55
3	30.64	15.41	8.33	76.26	30.12	4.04	65.84	0.71	12.63	86.67
4	35.01	17.35	8.37	74.28	34.19	3.59	62.23	0.51	13.16	86.33
5	37.36	18.33	8.69	72.98	36.27	3.48	60.25	0.40	13.91	85.69
$T = 298.2$ K										
1	30.71	15.27	8.84	75.89	30.03	4.60	65.37	0.51	13.08	86.41
2	38.15	18.41	10.68	70.91	36.45	4.49	59.06	0.37	16.88	82.75
3	41.65	19.76	11.47	68.77	39.33	4.33	56.34	0.20	18.60	97.94
4	45.74	21.83	12.29	65.88	43.20	4.14	52.66	0.47	20.45	79.08
5	48.80	22.63	13.29	64.08	45.23	4.08	50.69	0.04	22.50	77.49

Results and Discussion

The experimental equilibrium compositions for the systems formed by F68 + salt (Li_2SO_4, Na_2SO_4, $(NH_4)_2SO_4$, or $ZnSO_4$) + water were obtained as listed in Tables 1 to 4. The compositions are given in mass percent [% (w/w)]. As expected, the top phase is rich in copolymer and poor in salt, while the bottom phase contained most of the salt and little copolymer. All ATPS formed by various types of polymers and salts present this behavior.[22] However, the systems formed by PEG or L35 + sodium nitrate[23,24] present a high concentration of a salt, (25 to 30) % (w/w), in the top phase.

The coexisting phases are close in composition. A mass balance check was made between the initial mass composition (global composition) of each component and the amounts of each component in the bottom and top phases on the basis of equilibrium composition.

The tie line length (TLL) is a thermodynamic parameter that expressed the difference of intensive properties of each phase. The tie line is determined by connecting each corresponding set of total, top, and bottom phase compositions. The TLL can be calculated by the equation

$$\mathrm{TLL} = [(w_{\mathrm{F68}}^{\mathrm{T}} - w_{\mathrm{F68}}^{\mathrm{B}})^2 + (w_{\mathrm{S}}^{\mathrm{T}} - w_{\mathrm{S}}^{\mathrm{B}})^2]^{1/2} \qquad (1)$$

where w_{F68} and w_{S} are the copolymer and salt compositions, and the superscripts T and B designate the top and bottom phases, respectively.

The TLL was calculated for all studied compositions (Tables 1 to 4). We observe that an increase of overall composition causes an increase of TLL. The ATPS formed by PEG6000[23] or PEG1500[24] + sulfate salts and L35 + sulfate salts[17] present the same behavior as compared with the systems studied in the present work.

The effects of temperature on the splitting phase ability were investigated (Figures 1 to 4). All phase diagrams obtained show a small dependence on the temperature change. An increase in temperature causes a small expansion of the biphasic area, indicating a strong entropic contribution associated with the phase segregation event. The small contribution of temperature on the biphasic area is observed for the systems formed by PEG6000,[25] PEG1500,[26] L35, or F68[17] + salt.

The slope of the tie line (STL) is a relevant parameter in ATPS. This parameter can indicate a relationship between overall composition and volume of phases. The STL can be calculated by the equation

$$\mathrm{STL} = \frac{w_{\mathrm{F68}}^{\mathrm{T}} - w_{\mathrm{F68}}^{\mathrm{B}}}{w_{\mathrm{S}}^{\mathrm{T}} - w_{\mathrm{S}}^{\mathrm{B}}} \qquad (2)$$

where w_{F68} and w_{S} are the triblock copolymer and salt compositions, and the superscripts T and B designate the top and bottom phases, respectively.

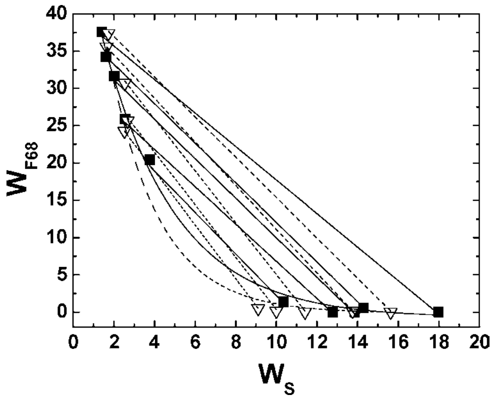

Figure 1. Temperature effects on the phase diagram for the F68 + sodium sulfate system: ▽, 278.2 K; ■, 298.2 K.

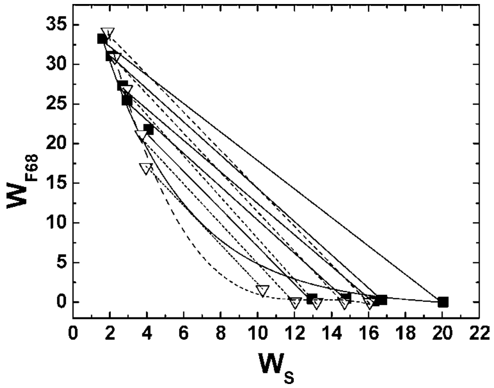

Figure 2. Temperature effects on the phase diagram for the F68 + zinc sulfate system: ▽, 278.2 K; ■, 298.2 K.

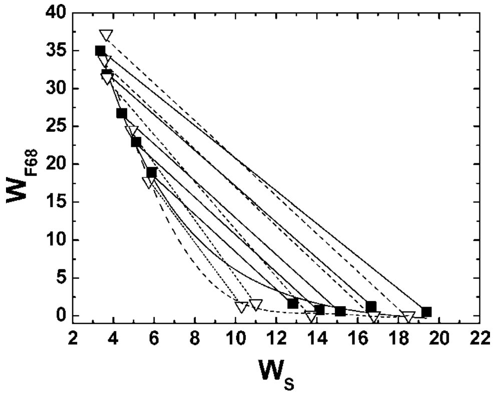

Figure 3. Temperature effects on the phase diagram for the F68 + ammonium sulfate system: ▽, 278.2 K; ■, 298.2 K.

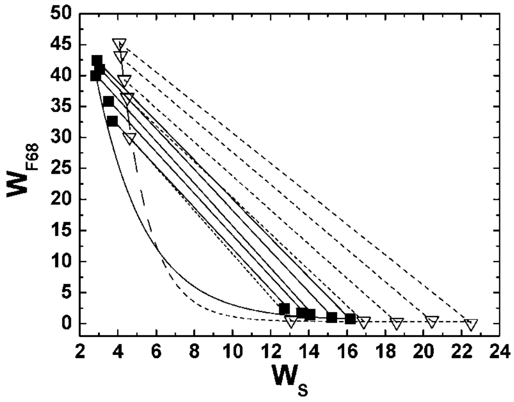

Figure 4. Temperature effects on the phase diagram for the F68 + lithium sulfate system: ▽, 278.2 K; ■, 298.2 K.

Table 5. STL Values for F68 + Salt + Water Systems

	T/K		
system	278.2	288.2	298.2
	F68 + sodium sulfate + water		
1	−2.90	−3.21	−3.61
2	−2.53	−3.11	−3.48
3	−2.67	−2.95	−3.46
4	−2.65	−2.70	−2.93
5	−2.27	−2.64	−2.69
	F68 + zinc sulfate + water		
1	−2.42	−2.51	−2.44
2	−2.11	−2.15	−2.53
3	−2.00	−2.20	−2.60
4	−2.03	−2.10	−2.49
5	−1.80	−1.95	−2.41
	F68 + ammonium sulfate + water		
1	−2.47	−2.30	−3.61
2	−2.46	−2.68	−3.74
3	−2.43	−2.54	−3.13
4	−2.36	−2.04	−2.55
5	−2.15	−2.37	−2.50
	F68 + lithium sulfate + water		
1	−3.35	−3.74	−3.48
2	−3.25	−3.66	−2.91
3	−3.15	−3.42	−2.74
4	−3.07	−3.52	−2.62
5	−3.80	−3.44	−2.45

The STL was calculated for each system (Table 5). We observe that an increase in temperature causes an increase in STL. This dependence (*T* × STL) is observed in ATPS formed by polymer and salt, for example, the systems formed by PEG6000 or PEG1500 + sulfate salts.[25,26] However, the dependence (*T* × STL) is more pronounced for the systems formed by triblock copolymer + salt, especially for the systems formed by L35 (50 % of PPO units) + sulfate salts.[17] This fact

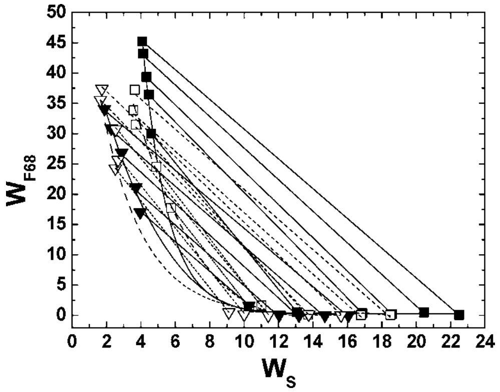

Figure 5. Cation effects on the phase diagram for the F68 + salt + water system at 298.2 K: ■, lithium sulfate; □, ammonium sulfate; ▼, zinc sulfate; ▽, sodium sulfate.

can be understood because the PPO (the triblock copolymer, F68, has 20 % of PPO units in the structure) becomes more hydrophobic with an increase in temperature.[27,28] By increasing temperature, water molecules are driven from the top phase to the bottom phase. Thus, the F68 concentration at the F68-rich phase increases, while the salt-rich phase will become somewhat more diluted.

The effects of electrolyte nature were also studied (Figure 5). The preference (a major biphasic area) of four salts in inducing the phase separation in ATPS follows the order: $Na_2SO_4 > ZnSO_4 > (NH_4)_2SO_4 > Li_2SO_4$. This order is the same at all temperatures. The phase separation process was studied by da Silva and Loh.[29] They utilized calorimetric measurements to identify the driving forces responsible by the phase separation in ATPS. The enthalpic ($\Delta_{Sep}H > 0$) contribution for phase separation in ATPS is not relevant, and the driving force responsible for phase separation has an entropic origin. A model, based on calorimetric results, attributed the different behavior of lithium and sodium sulfates in inducing ATPS formation to the cation−polymer interactions. In this model, when the macromolecule [poly(ethylene glycol)] and salts (sulfate) are mixed, the cations interact with the EO groups of polymer, releasing some water molecules, which were solvating the polymer in a process that is driven by an increase in entropy. 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. This model can be expanded to understand the phase separation in triblock copolymers and salts. The same order was observed for the system formed by PEG6000,[25] PEG1500,[26] or PEG 2000[30] + sulfate salts. However, the systems formed by L35 + sulfate salts present the same biphasic region and are independent of the electrolyte cation type. The difference in behavior among PEG, F68 (80 % EO unit), and L35 (50 % EO unit) can be understood based on different hydrophobicity because a decrease of EO groups in the triblock copolymer promotes an increase of hydrophobicity of the entire polymer. Consequently, phase separation with a lower salt concentration is more favored.

Conclusions

Equilibrium data for the systems formed by F68 + sodium sulfate + water, F68 + zinc sulfate + water, F68 + lithium sulfate + water, and F68 + ammonium sulfate + water were determined at different temperatures from (278.2 to 298.2) K. The temperature effect on the biphasic area was observed, but the cation type has a major effect on the phase diagram of these ATPS. The efficacy of the salts in inducing phase segregation follows the order sodium sulfate > zinc sulfate > ammonium sulfate > lithium sulfate. In general, the different interactions of the EO or PO groups with cations promote a different biphasic area.

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