J. Chem. Eng. Data **2005,** *50,* 1457−1461

Equilibrium Phase Behavior of Triblock Copolymer + Salt + Water Two-Phase Systems at Different Temperatures and pH

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Data on phase equilibrium of aqueous two-phase systems formed by mixture of triblock copolymer, potassium phosphate, and water were measured as a function of three temperatures (283.15 K, 298.15 K, and 313.15 K), two pH values (7 and 12), and two copolymer molar masses (L35 of 1900 g mol^{-1} and F68 of 8400 g mol^{-1}). The effect of the temperature was more pronounced on the behavior of the ATPS formed by the L35 copolymer as compared with that induced by the F68 copolymer. Temperature variation affected more intensively the tie line slope than the binodal position. The pH increase shifted the binodal curve to higher copolymer and phosphate concentrations corresponding to a decrease in the region of two-phase coexistence. The effect of the molar masses on the behavior of the phase diagrams for both copolymers was offset by the effects associated with the hydrophobicity of the macromolecules.

Introduction

Aqueous two-phase systems (ATPS) are capable of promoting solute partitioning between the two phases of a system formed by mixtures of either two polymers or one polymer and a salt.[1] The poly(ethylene oxide) (PEO) + dextran + water system is the main example for the polymer + polymer ATPS,[2] and the PEO + potassium phosphate ATPS is the most studied in the class of polymer + salt systems.[3] Despite the advantages presented by polymer + salt systems such as low cost, low viscosity, and a short time for phase splitting, it presents difficulties in isolating target compounds from the polymeric solutions.[4−6] An alternative type of ATPS has been studied aiming to improve such separation and also the cost of polymer recovery. These systems are composed by a thermosensitive polymer, which undergoes phase separation through a moderate change in temperature. These polymers are named thermo-separating polymers.[6−8] PEO is also a thermo-separating polymer, but its cloud point (i.e, the temperature at the start of the phase splitting) is too high (above 100 °C) for biomolecule separation.[9,10]

The triblock copolymer formed by arrays of ethylene oxide and propylene oxide units (symbolized as $(EO)_n(PO)_m(EO)_n$) are thermo-separating materials and have lower cloud points. ATPS composed of triblock copolymers have been applied in the partitioning of hydrophilic and hydrophobic biomolecules.[9,11−13] Protein separation using block copolymers was accomplished to investigate the influence of copolymer hydrophobicity on the partitioning and relationship between copolymer hydrophobicity and surface hydrophobicity of proteins.[14] These studies have shown the applicability of triblock copolymers ATPS and the need for their liquid−liquid equilibrium data in both the design of aqueous two-phase extraction process and the development of models to predict phase separation.

In this work, biphasic systems composed of $(PEO)_{11}(PPO)_{16}(PEO)_{11}$, notation L35 (50 % EO) + salt + water and $(PEO)_{80}(PPO)_{30}(PEO)_{80}$, notation F68 (80 % EO) + salt + water were prepared, and phase compositions were measured. Liquid−liquid equilibrium data at 283.15 K, 298.15 K, and 313.15 K were determined for the (i) L35 (1900 g mol^{-1}) + salt + water and F68 (8400 g mol^{-1}) + salt + water systems, at pH 7 and (ii) L35 + salt + water system at pH 12. The salts used were mixtures of K_2HPO_4 + KH_2PO_4 or K_2HPO_4 + KOH. The influence of copolymer molecular weight, temperature, and hydrophilic/hydrophobic balance on the phase diagram formation was also evaluated.

Experimental Section

Materials. The triblock copolymers L35, $(PEO)_{11}(PPO)_{16}(PEO)_{11}$, and F68, $(PEO)_{80}(PPO)_{30}(PEO)_{80}$, were purchased from Aldrich (USA). The copolymers were analyzed by gel permeation chromatography (GPC) (AKTA, purifier, 10×, Pharmacya) using the following conditions: for L35 tetrahydrofuran as mobile phase at a rate of 0.8 μL/min, injection temperature 30 °C, and for F68 toluene as mobile phase at a rate of 0.8 μL/min, injection temperature 45 °C, refraction index detector, sample injection of 100 μL. In both cases the standard polymer was polystyrene. This methodology was used in order to obtain the average molecular weight (M_w) and polydispersity index (M_w/M_n) copolymers. For L35, copolymer M_w and M_w/M_n was 1945 g mol^{-1} and 1.14, respectively; and for F68, copolymer M_w and M_w/M_n was 8460 g mol^{-1} and 1.42, respectively. GPC traces of homopolymers or diblock copolymer were not observed. The analytical grade reagents K_2HPO_4, KH_2PO_4, and KOH were purchased from Merck (USA) and used without further purification. Deionized water (Millipore, Bedford, MA) was used in all experiments.

Aqueous Two-Phase Systems. The biphasic systems were prepared by weighing appropriate quantities of L35

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10.1021/je050104v CCC: \$30.25

Table 1. Mass Fraction of the L35 (1) + Phosphate Potassium/Potassium Hydroxide (2) + Water (3) Systems, at pH 12

	overall composition			upper phase			lower phase		
tie line	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$
				283.15 K					
1	28.69	25.14	46.17	56.99	0.55	42.46	5.96	42.91	51.13
2	34.02	28.62	37.36	66.01	0.13	33.86	3.65	55.98	40.37
3	38.95	32.60	28.45	70.60	0.27	29.13	2.86	65.09	32.05
4	42.65	36.76	20.59	79.30	0.34	20.36	1.88	78.51	19.61
				298.15 K					
1	24.04	16.34	59.65	63.22	0.24	36.54	4.43	24.44	71.13
2	28.81	20.28	50.91	71.15	0.26	28.59	4.17	32.78	63.05
3	34.14	22.86	43.00	80.10	0.09	19.81	2.39	38.53	59.08
4	38.97	25.94	35.09	85.86	0.03	14.11	0.88	46.70	52.42
				313.15 K					
1	28.89	14.03	57.08	68.62	0.35	31.03	2.52	23.25	74.23
2	32.05	16.92	51.03	72.69	0.25	27.06	1.10	29.93	68.97
3	37.38	18.79	43.83	78.60	0.17	21.23	2.55	35.53	61.92
4	42.65	20.92	36.43	86.72	0.04	13.24	1.31	40.23	58.46

Table 2. Mass Fraction of the L35 (1) + Potassium Phosphate (2) + Water (3) Systems, pH 7

	overall composition			upper phase			lower phase		
tie line	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$	$100w_1$	$100w_2$	$100w_3$
				283.15 K					
1	13.11	9.99	76.90	30.41	0.46	69.13	5.04	12.79	82.17
2	18.36	14.00	67.64	40.10	0.01	59.89	4.35	21.51	74.14
3	23.00	16.99	60.01	52.14	0.01	47.85	4.55	28.93	66.52
4	28.22	21.00	50.78	64.24	0.01	35.75	4.77	35.46	59.77
				298.15K					
1	12.78	8.83	78.39	34.97	0.03	65.00	2.60	11.77	85.63
2	17.15	11.98	70.87	48.54	0.02	51.44	2.74	15.66	81.60
3	20.07	14.92	65.01	56.65	0.01	43.34	2.71	21.98	75.31
4	24.01	16.96	59.03	63.00	0.01	36.99	3.04	24.10	72.86
				313.15K					
1	21.07	4.99	73.94	32.25	0.17	67.58	3.12	8.86	88.02
2	30.15	6.99	62.86	53.10	0.01	46.89	1.64	15.35	83.01
3	38.03	8.77	53.20	63.82	0.02	36.16	2.10	20.47	77.43
4	47.06	10.99	41.95	72.77	0.02	27.21	2.71	29.61	67.68

(50 % EO) or F68 (80 % EO), potassium phosphate (pH 7 or 12), and water on an analytical balance (Denver Instruments, M-310, USA), with a given uncertainty of ±0.0001 g. Stock solutions of L35 (85 mass %) were only used to prepare the system at 283.15 K on account of the dissolution difficulties at this temperature. Typically, 30 g of the system was prepared and fed into the liquid−liquid equilibrium cells.[15] The mixture was vigorously stirred for 2 min and then allowed to settle for 24 h at the desired temperature to attain equilibrium state characterized by the absence of turbidity in both top and bottom phases. After equilibrium, the two phases became clear and transparent, and the interface was well-defined. Any changes in the phase compositions were not observable in longer settling periods.[16] For the experiments at pH 12, the mixture was centrifuged at 2900*g* (Centrifuge Eppendorf 5804, Germany) for 40 min after vigorously mixing, aiming to accelerate the phase separation. The temperature in the cell was controlled at ± 0.1 K using a thermostatic bath (TECNAL, TE-184, Brazil). The Henderson−Hasselbach[17] equation was used to determinate the ratio, in mass, of mono- and dibasic salts necessary to obtain pH = 7 and dibasic potassium phosphate and potassium hydroxide to obtain pH =12. Both pH values were confirmed using a pH meter (Digimed, DM20, Brazil). Samples in triplicate from the top and bottom phases were collected to determine the composition of each phase, using syringes and needles. The top phase was carefully sampled, with care being taken to leave a layer of solution at least 0.5 cm thick above the interface. Samples of the bottom phase were withdrawn using a syringe with a long needle. A tiny bubble of air was retained on the needle tip and expelled once in the bottom phase to prevent contamination from top phase, with special attention not to disturb the equilibrium systems in the subsequent analysis.

Phase Diagram Determination. The potassium phosphate used in the constitution of the systems (i) at pH 7 was quantified using spectrophotometry (Cary 50, Varian, Australia) according to Silva and Queiroz[18] and Fiske and Subbarow.[19] The standard deviation in the salt mass percent by this method was ± 0.08 % (ii) at pH 12 and was quantified by flame photometry (Photometer CELM FC-180, Brazil), as described by Zafarani-Moattar and Sadeghi.[20,21] The standard deviation in the salt mass percent this method was ± 0.12 %. The concentration of triblock copolymers (L35 and F68) was determined by measurements at 298.15 K using a refractometer (Analytic Jena AG Abbe refractometer 09-2001 model, Germany).[22,23] Since the refractive index of the phase samples depends on the total concentration (copolymer and salt concentrations) and is an additive property, copolymer concentration was obtained by subtracting from the total solution composition (refractive index) the salt concentration measured by spectrophotometry. The standard deviation in the block copolymer mass percent was of ± 0.03 %. The concentration of water was determined by freeze-drying (Speed Vacuum Concentration HETO, model VR-1, Denmark) at −20 °C for 24 h.[24] The standard deviation of the water mass percent was ± 0.06 %. All analytical measurements were performed in triplicate.

Table 3. Mass Fraction of the F68 (1) + Potassium Phosphate (2) + Water (3) Systems, pH 7

	overall composition			upper phase			lower phase		
tie line	100w_1	100w_2	100w_3	100w_1	100w_2	100w_3	100w_1	100w_2	100w_3
				283.15 K					
1	14.25	7.98	77.77	22.78	1.77	75.45	1.64	12.86	85.50
2	17.02	9.98	73.00	32.69	0.72	66.59	2.57	16.98	80.45
3	20.12	10.99	68.89	37.82	0.80	61.38	3.04	18.61	78.35
4	21.89	12.79	65.32	41.76	1.06	57.18	4.10	21.60	74.30
				298.15 K					
1	14.02	7.00	78.98	28.48	0.29	71.23	3.53	9.20	87.27
2	17.02	7.99	74.99	34.08	0.17	65.75	2.33	12.51	85.16
3	18.99	8.98	72.03	37.30	0.49	62.21	2.60	15.29	82.11
4	22.07	10.99	66.94	41.03	1.57	57.40	2.54	18.99	78.47
				313.15 K					
1	12.99	6.00	81.01	25.11	0.54	74.35	3.06	8.40	88.54
2	15.99	7.99	76.02	35.53	0.04	64.43	2.11	11.99	85.90
3	19.00	8.99	72.01	39.04	0.06	60.90	1.92	13.44	84.64
4	20.93	9.97	69.10	42.83	0.05	57.12	2.37	16.39	81.24

Results and Discussion

The phase compositions for the aqueous two-phase systems formed by different triblock copolymers are shown in Tables 1 to 3. All concentrations are expressed in mass percent. For each copolymer/salt combination, four tie lines were determined. The tie lines were obtained by linear regression of the corresponding set of overall bottom phase and top phase concentrations. This new aqueous two-phase system has a higher polymer concentration at the upper phase when compared to the well-known poly(ethylene oxide) + phosphate + water systems most frequently used. This behavior could be attributed to the hydrophobic character of the triblock copolymer that decreases water−polymer interactions.[25]

The effect of the temperature increase on the phase diagram of the L35 + potassium phosphate + water system is illustrated in Figures 1 and 2. A significant alteration in the biphasic area is not observed for the temperature range from 283.15 K to 313.15 K (Figure 1). However, at 283.15 K in the inferior part of the curve, the points shift to higher L35 concentrations, leading to a very small reduction of the two-phase area. A considerable change was also observed in the tie line slope (STL) that was less pronounced for the temperature range from 298.15 K to 313.15 (Figure 2).

The tie line slope (STL) is defined as the ratio: STL = (Δpolymer/Δsalt) in which Δpolymer and Δsalt are, respectively, the difference of compound concentration in the two coexisting phases. Table 4 presents the increase of the STL (in absolute value) for L35 + phosphate systems with the temperature increase. A possible cause for this STL change is the transfer of water from the upper to the lower phase, increasing the polymer concentration at the top phase and decreasing the salt concentration at the bottom phase. A small variation in STL, at different temperatures, was also observed for F68 + dextran T500 systems.[26]

Table 4. Tie Line Slope Values for L35 + Phosphate Systems at 283.15 K, 298.15 K and 313.15 K

	L35		
tie line	283.15 K	298.15 K	313.15 K
1	2.06	2.76	3.35
2	1.66	2.10	3.36
3	1.65	2.36	3.02
4	1.68	2.49	2.37

Figures 3 and 4 show a very small influence of temperature on phase diagrams of F68-phosphate (pH 7) systems at 283.15 K, 298.15 K, and 313.15 K. However, temperature increase promoted a change in both volume and composition of the phases. Thus, the higher the temperature, the lower the copolymer and salt concentrations needed for the two-phase formation. Table 4 shows STL values for L35 + phosphate systems. STL was found to increase with temperature rise. A similar behavior was presented by the F68 + phosphate systems (Table 5) and by the same PEO + phosphate system reported in the literature.[2,15] Generally, the increase of the polymer molecular weight cause the binodal curve shifts to lower copolymer and salt concentrations to promote the phase separation.[2,22,27] According to the model of Flory−Huggins, the larger the macromolecule size, the larger the molar mass and the lower the variation in the entropy of the mixture. In this model, the change in

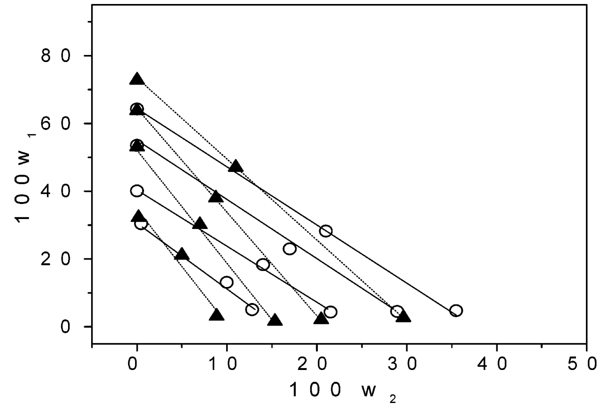

Figure 1. Temperature effect on the phase diagram for the L35 (1) + potassium phosphate (2) + water (3) system at pH 7. ○, 283.15 K; ▲, 313.15 K.

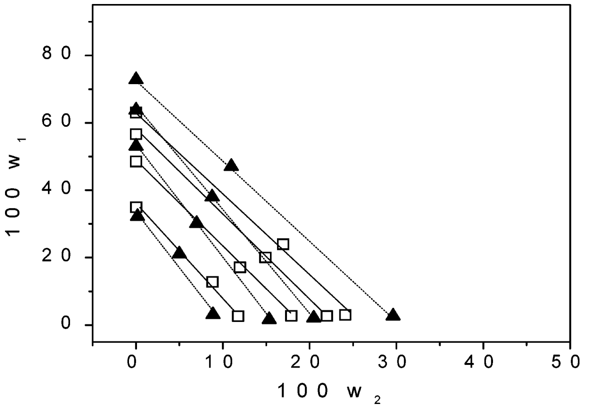

Figure 2. Temperature effect on the phase diagram for the L35 (1) + potassium phosphate (2) + water (3) system at pH 7. □, 298.15 K; ▲, 313.15 K.

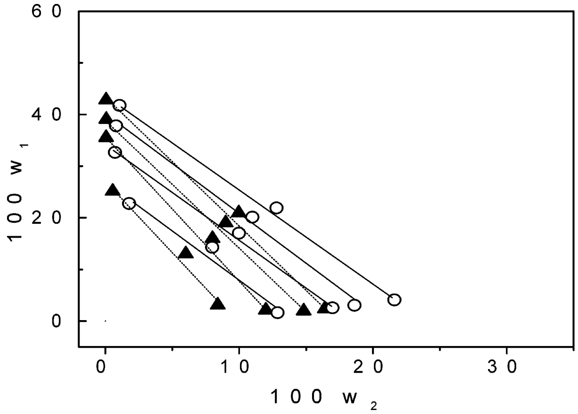

Figure 3. Temperature effect on the phase diagram for the F68 (1) + potassium phosphate (2) + water (3) system at pH 7. ○, 283.15 K; ▲, 313.15 K.

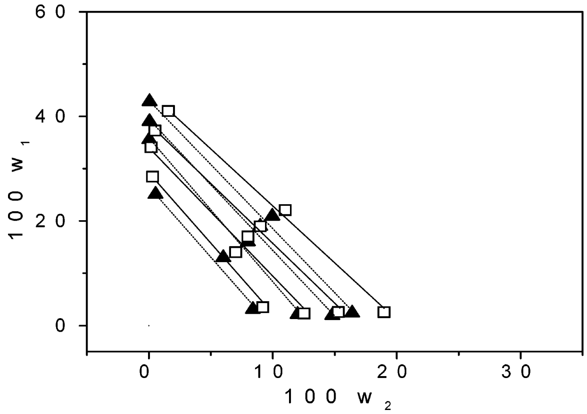

Figure 4. Temperature effect on the phase diagram for the F68 (1) + potassium phosphate (2) + water (3) system at pH 7. □, 298.15 K; ▲, 313.15 K.

Table 5. Tie Line Slope Values for F68 + Phosphate Systems at 283.15 K, 298.15 K, and 313.15 K

	F68		
tie line	283.15 K	298.15 K	313.15 K
1	1.91	2.80	2.81
2	1.85	2.57	2.80
3	1.95	2.35	2.77
4	1.83	2.21	2.48

molecular size has small influence on enthalpy mixtures values. This mixture entropy is related to the number of different spatial distributions the macromolecule presents in solution. In this sense, the copolymer F68 (8400 g mol^{-1}) should induce the separation of phases more easily than L35 (1900 g mol^{-1}). Such behavior, however, was not verified in the present work as it can be seen in phase diagram of Figure 5, at 298.15 K. The overlap of the phase diagrams is verified, regardless of the temperature and molecular size. This absence of molar mass influence can be attributed to compensation due to the hydrophobicity effect caused by the weak molecular interaction between PO segments and water molecules. Then, an increase in macromolecular percentage of poly(propylene oxide) promotes a decrease in its water solubility. So, in terms of only molecular size (i.e., entropic effect), the system formed by F68 (20 % PO) would present a larger two-phase area. However, taking the hydrophobic influence into account, L35, containing 50 % of hydrophobic poly(propylene oxide) group, would be the largest inductor of the separation of phases.

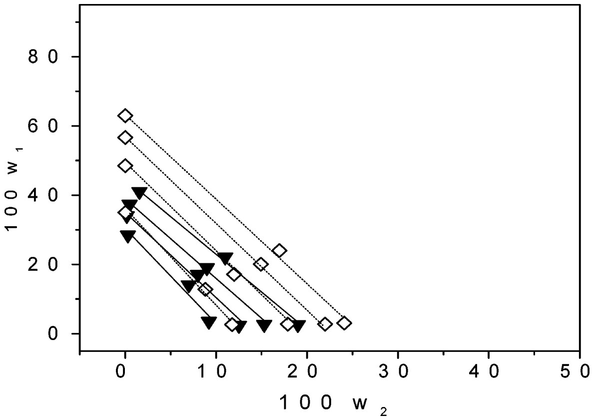

Figure 5. Influence of copolymer molecular weight on phase diagram of the copolymer (1) + potassium phosphate (2) system at 298.15 K and pH 7. ◇, L35 (1900 g mol^{-1}); ▼, F68 (8400 g mol^{-1}).

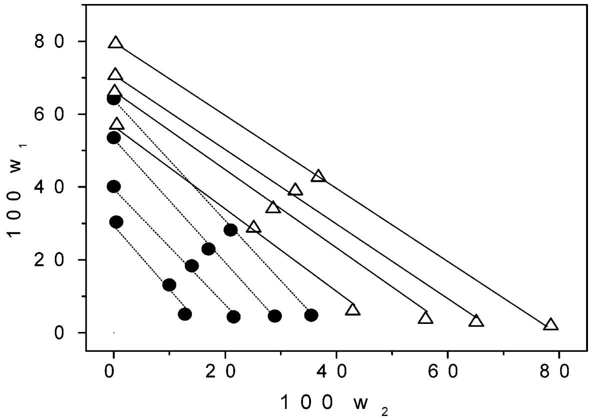

Figure 6. Phase diagram for the L35 (1) + salt (2) + water (3) system at 283.15 K. ●, pH 7; △, pH 12.

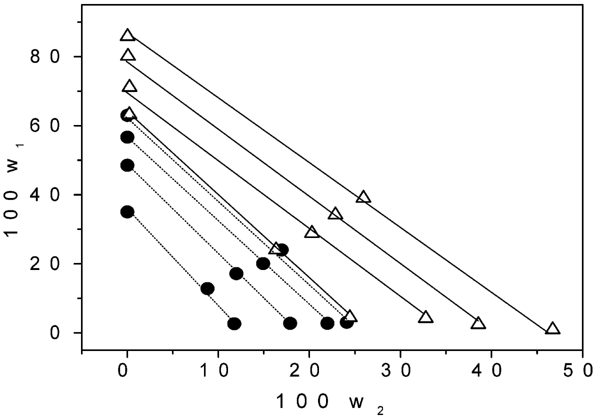

Figure 7. Phase diagram for the L35 (1) + salt (2) + water (3) system at 298.15 K. ●, pH 7; △, pH 12.

The phase diagrams in Figures 6 to 8 show the pH effects on phase separation at the studied temperature. As the system becomes more basic, the binodal shifts to higher copolymer and salt concentrations, thus decreasing the phase separation region. This behavior is probably due to the higher content of OH$^-$ ions in the solution with pH 12. The buffer used to reach pH 12 was a K_2HPO_4/KOH solution in which the ion phosphate was substituted for hydroxide. In the literature[28] it is evident that the higher the valence of the anion, the lower the concentration required to form two-phase systems. In other words, the smaller the valence, the smaller the efficiency in inducing the phase separation and the smaller the two-phase areas will be.

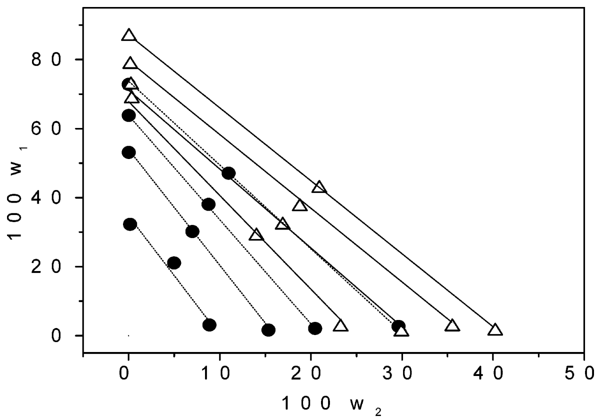

Figure 8. Phase diagram for the L35 (1) + salt (2) + water (3) system at 313.15 K. ●, pH 7; △, pH 12.

Conclusion

The effect of temperature on the equilibrium data of L35 + potassium phosphate + water, F68 + potassium phosphate + water, and L35 + potassium phosphate/potassium hydroxide + water systems was not significant. These systems present top phases with lower water content than for ATPS, constituted by PEO, with the smallest capacity of these copolymers to hold water being attributed to molecular processes of aggregation in solution, forming micelles and/or gels. The enthropic nature of the driving force that governs the phase separation should be emphasized.

It is important to observe the difference of phase diagram behavior when the pH increases from 7 to 12, using a buffer solution of potassium phosphate and potassium hydroxide, as compared with buffer solutions without hydroxide ion. An opposite behavior in the absence of anion hydroxide was observed in other works.[29] These characteristics render block copolymers ATPS a promising, versatile, and attractive system in the field of bioseparation.

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Received for review March 17, 2005. Accepted May 25, 2005. The authors thank Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support of this work and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) for a research fellowship to A.F.M. and K.S.d.N.

JE050104V