

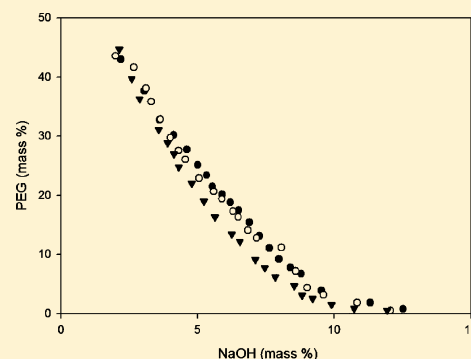
Liquid–Liquid Equilibria of Aqueous Two-Phase Systems Containing Sodium Hydroxide + Poly(ethylene glycol) of (1450, 4000, or 10 000) $\text{g}\cdot\text{mol}^{-1}$ at (288.2, 298.2, and 308.2) K

Renata S. Diniz,[†] Evaldo C. Souza, Jr.,[‡] Jane S. R. Coimbra,^{*,†,‡} Eduardo B. de Oliveira,[†] and Angélica Ribeiro da Costa[†]

[†]Departamento de Tecnologia de Alimentos (DTA), Universidade Federal de Viçosa (UFV), Campus Universitário s/n, Centro, 36570-000, Viçosa-MG, Brazil

[‡]Departamento de Tecnologia Rural e Animal (DTRA), Universidade Estadual do Sudoeste da Bahia (UESB), Praça Primavera 40, Bairro Primavera, 45700-000, Itapetinga-BA, Brazil

ABSTRACT: Liquid–liquid equilibrium data and phase diagrams of new aqueous two-phase systems (ATPS's), composed of water + sodium hydroxide + poly(ethylene glycol) (PEG) of molar masses (1450, 4000, or 10 000) $\text{g}\cdot\text{mol}^{-1}$, were determined at (288.2, 298.2, and 308.2) K. The effects of temperature and PEG molar mass on the equilibrium data were studied. It was found that an increase in temperature caused the expansion of the two-phase region and increased the slope and the length of the tie line. It was also observed that binodal curves shifted toward the origin with the increase in PEG molar mass.



■ INTRODUCTION

Systems containing two liquid phases at certain critical concentrations are very useful to extract and recover many kinds of molecules of biotechnological interest, such as proteins, peptides, and other small bioactive molecules.¹ To preserve the structure and the functionality of such compounds, biphasic systems composed of two aqueous-rich phases are more appropriate than those formed by one aqueous and an organic phase. Indeed, their high content in water implies a better biocompatibility and lower values of interfacial tension, thus minimizing the degradation of biomolecules during their transfer from one phase to the other. Usually, aqueous two-phase systems (ATPS's) can be obtained by mixing two aqueous solutions of structurally distinct hydrophilic polymers or one aqueous solution of a hydrophilic polymer and another one of an inorganic compound. In ATPS's, the compound separation is enabled by the difference of thermodynamic affinity of the molecules with regard to the two phases composing the system. The consequence of this is the migration and the consequent concentration of a given molecular entity within the phase for which it has a higher thermodynamic affinity.^{1,2}

Accurate knowledge of the binodal curves and tie lines for a given ATPS in a range of temperatures is essential to establish the correct operational conditions for an optimal separation process. The slope of a tie line indicates how the composition of the phases may vary with the change of a physical and/or chemical property, such as the system temperature, the molar mass of the polymer, or the type and concentration of polymers

and salts involved. The length of the tie line is the size of the segment which joins the composition of the global system, of the top phase and the bottom phase. A small tie line length means that the system will be closer to the critical point, and on the contrary, greater tie line lengths indicate that the system will be far from the critical point.³ With regards to the polymer molar mass influences on the phase separation for polymeric salt ATPS, in general, the higher the molar mass, the lower the polymer concentration required for phase separation.^{3,4} Also, changes in the system temperature may affect the chemical potential of species in each phase in equilibrium. Some authors have reported, for polymeric salt systems, that an increment in temperature leads to an increase in the biphasic region as well as in the length of the tie lines.^{5–7}

Several published studies have reported equilibrium data for ATPS formed by PEG with different molar mass and inorganic compounds, mainly sulfate or phosphate salts.^{4–7} However, to the best of our knowledge, there are only a few reports of equilibrium data for biphasic systems formed by water + sodium hydroxide (NaOH) + PEG.^{8–10} Thus, given the need and importance of equilibrium data for new ATPS, the aim of this study was to determine experimental data of liquid–liquid phase equilibrium for three ATPS, composed by NaOH + PEG (1450, 4000, or 10 000) $\text{g}\cdot\text{mol}^{-1}$. For each of these systems, equilibrium data were determined at three temperatures:

Received: July 19, 2011

Accepted: December 14, 2011

Published: January 4, 2012

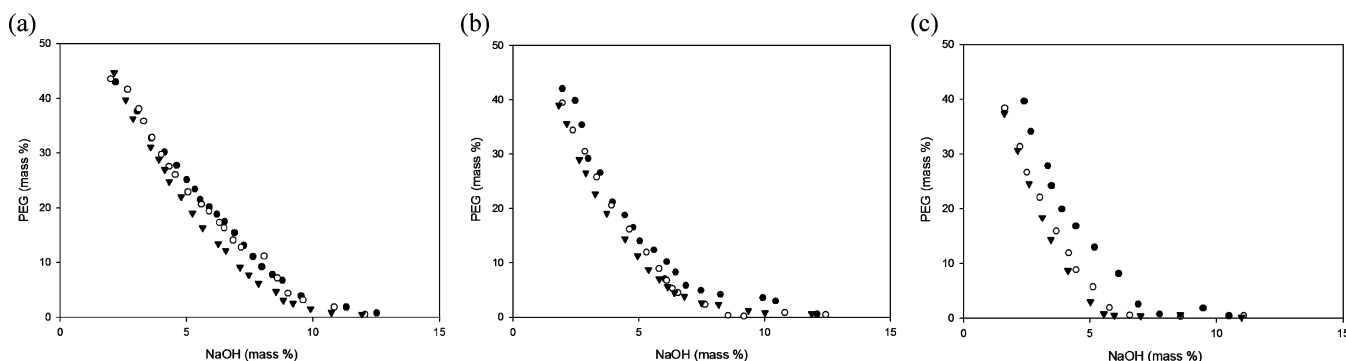


Figure 1. Binodal curves for the systems (a) PEG 1450 + NaOH + H₂O, (b) PEG 4000 + NaOH + H₂O, and (c) PEG 10000 + NaOH + H₂O. Temperatures: ●, 288.2 K; ○, 298.2 K; and ▼, 308.2 K.

Table 1. Phase Equilibrium Compositions for the Studied ATPS PEG (1) + NaOH (2) at (288.2, 298.2, and 308.2) K

288.2 K				298.2 K				308.2 K			
100W ₁ ^{top}	100W ₂ ^{top}	100W ₁ ^{bot}	100W ₂ ^{bot}	100W ₁ ^{top}	100W ₂ ^{top}	100W ₁ ^{bot}	100W ₂ ^{bot}	100W ₁ ^{top}	100W ₂ ^{top}	100W ₁ ^{bot}	100W ₂ ^{bot}
PEG1450 (1) + NaOH (2)											
29.47	4.00	2.87	9.41	29.05	3.85	1.65	8.71	30.18	3.61	1.29	10.21
34.56	3.15	2.56	11.02	32.45	4.19	0.84	9.60	29.61	4.27	1.03	11.47
32.83	4.45	0.12	12.56	33.38	4.66	1.34	10.60	32.79	3.97	3.95	12.54
PEG4000 (1) + NaOH (2)											
28.10	3.89	1.93	7.92	36.80	2.13	0.99	7.04	27.96	1.84	0.39	9.86
31.65	3.72	2.03	9.93	30.19	3.31	0.22	8.88	31.75	1.72	0.46	10.86
35.55	4.65	6.84	12.45	32.31	3.70	1.15	9.81	29.99	3.23	2.61	11.57
43.24	1.63	1.24	6.99	39.38	2.99	2.08	10.81	38.45	1.95	1.29	10.83
40.06	2.86	1.65	11.31	42.08	3.72	2.91	12.91	35.97	3.12	2.98	11.90
38.99	4.97	4.17	13.90	38.96	3.84	2.39	11.88	34.95	3.81	3.93	12.81
PEG10000 (1) + NaOH (2)											
35.36	1.56	2.97	9.64	29.61	1.94	0.43	9.46	30.70	1.52	1.26	7.83
37.67	2.03	1.05	10.95	32.05	2.87	1.07	10.81	32.62	2.65	2.04	8.95
35.75	3.32	0.13	13.04	35.84	2.61	3.65	11.15	35.76	2.94	2.80	10.90

(288.2, 298.2 and 308.2) K. The effects of temperature and PEG molar mass on the behavior of the equilibrium data are discussed.

EXPERIMENTAL SECTION

Materials. The poly(ethylene glycol), (1450, 4000, and 10 000) g·mol⁻¹, and the sodium hydroxide (minimum purity of 99 %) were obtained from Vetec (São Paulo, Brazil). The polymers and the NaOH were both used without further purification. Double-distilled and deionized water (electrical resistivity equal to 18.2 MΩ·cm) was used in the experiments (Millipore Inc., Milli-Q, Billerica Headquarters, MA).

Procedure. Aiming to locate the two-phase region in the phase diagrams, the binodal curves were experimentally obtained by applying a turbidimetric method, using glass tubes of 50 mL previously weighed at temperatures of (288.2, 298.2 and 308.2) K. In each experiment, a predefined amount (5 g) of the stock aqueous solution of NaOH with known concentration (30 mass %) was weighted (analytical balance Shimadzu AY220, Japan; accuracy of $\pm 1 \cdot 10^{-7}$ kg) within a glass tube, which was immersed in a thermostatic bath (Tecnal, TE-184, Brazil) calibrated at the desired temperature (± 0.1 K). Next, the NaOH solution was titrated under agitation with the polymeric aqueous solution (50 mass %) until the clouding was visually detected. After that, the mixture was kept for 3 min at controlled temperature, and then, the final mass of the systems was measured. By the difference between this value and the

predefined amount (5 g) of the NaOH solution, the polymer mass added during the titration was calculated, and thus, the composition of the binodal point was determined. Additional binodal points were obtained by adding small amounts of distilled water until cleaning the system, and then, enough drops of the stock polymer solution to produce turbidity again.

The tie-line data were obtained by adding appropriate amounts of polymer, NaOH, and water in a glass tube, as described in detail elsewhere.^{11,12} Briefly, water and aqueous stock solutions of PEG (50 mass %) and NaOH (30 mass %) were mixed and then stirred using a vortex equipment (Phoenix Luferto, AP-56, Brazil) for 2 min, centrifuged at 4600g (Eppendorf, 5430, Germany) and then maintained at rest, at a controlled temperature, for 24 h. When the phases were visually clear and separated, with a well-defined interface, it was assumed that the equilibrium condition was reached. Then, the NaOH concentrations in both the top and bottom phases were determined by the conductometric method (Thermo Electron Corporation, Orion 011510, USA). For this purpose, an analytical curve was previously obtained by measuring the electrical conductivity of NaOH aqueous solutions with concentrations varying in the range of (0.0001 mass to 0.001 mass). Three samples of the top and bottom phases were withdrawn using 20 mL syringes with needles of 5 cm length. To be within the concentration range of the analytical curve, dilution factors of 97.5 % for the top phase and another of 98.0 % for bottom phase were used to calculate the concentration of

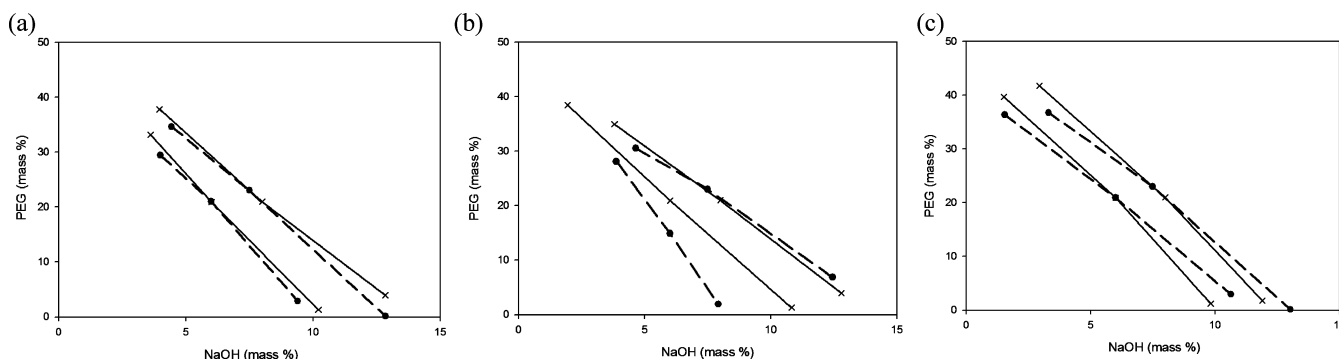


Figure 2. Effect of temperature on the equilibrium phase compositions for the PEG (a) 1450, (b) 4000, and (c) 10 000 + NaOH + H₂O system. Observed data at ●, 288.2 K and ×, 308.2 K; obtained tie lines at —, 288.2 K and - - -, 308.2 K.

Table 2. Coefficients a and b of the Regression Linear Models ($y = a + bx$) Obtained for Tie Lines of Each Studied PEG + NaOH ATPS at (288.2, 298.2, and 308.2) K

PEG 1450 + NaOH			PEG 4000 + NaOH			PEG 10000 + NaOH		
a	b	R^2	a	b	R^2	a	b	R^2
				288.2 K				
49.8761	-4.9616	0.9983	59.0375	-3.9092	0.9984	48.0579	-3.6658	0.9999
48.4547	-4.0725	0.9939	53.8648	-3.7130	0.9940	46.4709	-4.0890	0.9985
52.3899	-4.0564	0.9873	52.6464	-4.5457	0.9999	42.0415	-3.9976	0.9987
			49.8889	-4.8033	0.9995			
			54.1251	-6.5660	0.9983			
			55.7621	-7.8345	0.9998			
			298.2 K					
58.1976	-5.3720	0.9999	58.4481	-4.1436	0.9909	46.2302	-3.7673	0.9981
57.6266	-5.8898	0.9993	56.9338	-4.5194	0.9975	43.7655	-3.9210	0.9991
52.1003	-5.7344	0.9960	52.5956	-4.7443	0.9967	38.5022	-3.9308	0.9922
			50.8812	-5.0827	0.9998			
			47.3405	-5.3322	0.9992			
			50.8106	-7.1898	0.9950			
			308.2 K					
32.1100	-2.4272	0.9987	48.7744	-3.4131	0.9941	48.2583	-4.1374	0.9993
34.0225	-2.9787	0.9939	46.9914	-3.4453	0.9996	46.5721	-4.8325	0.9906
46.0425	-3.3551	0.9992	45.0962	-3.5252	0.9998	45.8385	-5.5754	0.9929
			41.1852	-3.3148	0.9990			
			38.2999	-3.4523	0.9986			
			35.0344	-3.4875	0.9984			

PEG and NaOH in both phases. The final concentration of NaOH in each phase was obtained with a standard deviation ≤ 0.2 %. The water contents were determined by freeze-drying (Terroni, LS 3000, Brazil) of samples. The PEG amounts were obtained by a simple difference. All of these quantifications were performed in triplicate for both phases.

RESULTS AND DISCUSSION

Binodal Curves. The diagrams in Figure 1 represent the binodal curve for the ATPS's composed by PEG (1450, 4000, or 10 000) g·mol⁻¹ + NaOH, obtained by the turbidimetric method, at each of the three studied temperatures, that is, (288.2, 298.2, and 308.2) K. In these diagrams, an expansion of the two-phase region is observed when increasing the temperature. A similar trend has been reported by several authors for other ATPS studied at different temperatures, as for instance Sadeghi and Golabiazar,⁵ for PEG6000 + sodium tungstate systems; Zafarani-Moattar and Sadeghi,⁸ for PEG6000 + sodium hydroxide; Oliveira et al.,¹¹ for PEG4000 + zinc sulfate; Carvalho et al.,¹² for PEG 4000 + sodium sulfate,

PEG4000 + lithium sulfate, and PEG4000 + potassium phosphate; Ananthapadmanabham and Goddard,¹³ for (PEG3350 + sodium sulfate); Hammer et al.,¹⁴ for (PEG3000 + sodium sulfate); Da Silva et al.,¹⁵ for (PEG1000 + dibasic potassium phosphate).

The analysis of the Figure 1 also allows inferring the influence of PEG molar mass in the formation of the binodal curves. This is likely to be due to the fact that the larger the PEG molar mass, the less soluble is the polymer and, thence, the lower the amount of NaOH needed to induce the splitting of the phases. This same effect of the PEG molar mass was observed by Oliveira et al.,⁴ for PEG1500 + sodium citrate and PEG4000 + sodium citrate systems, and also by Graber et al.,¹⁶ for PEG2000 + sodium nitrate, PEG6000 + sodium nitrate, and PEG10000 + sodium nitrate systems.

Equilibrium Data. The equilibrium compositions at (288.2, 298.2, and 308.2) K are given in Table 1, for the ATPS composed by PEG, (1450, 4000 or 10 000) g·mol⁻¹, + NaOH. In this table, the superscript "top" refers to the top phase, and "bot" refers to the bottom one. The corresponding tie lines for

the lower and the higher studied temperatures, (288.2 and 308.2) K, are plotted in Figure 2, in which it can be observed that their slopes and lengths increase for higher temperatures. This can be explained by the fact that the compositions of phases in equilibrium vary with temperature. In PEG + NaOH ATPS, an increase in the temperature engenders an increase in the concentration of PEG in top phase and a decrease in the NaOH concentration in the lower phase. Linear regression models ($y = a + bx$) were well-fitted to these equilibrium data, as indicated by $R^2 \geq 0.98$ in all cases (Table 2). Some literature reports have shown similar equilibrium behavior for other PEG + salt ATPS, such as the works of Zafarani-Moattar and Sadeghi¹⁷ with PEG6000 + sodium citrate systems, Azimaie et al.⁶ with PEG6000 + magnesium sulfate, Sé and Aznar¹⁸ with PEG4000 + potassium phosphate, and Carvalho et al.¹⁹ with PEG1500 + potassium phosphate.

It must be emphasized that the effect of temperature in ATPS is quite complex, varying differently for different systems. In general, an increment in the temperature leads to a decrease in the solubility of the polymer, leading to an increase of the biphasic region.^{12,17} Indeed, it has been shown that ATPS formation is an endothermic process.²⁰

CONCLUSIONS

Liquid–liquid equilibrium of systems PEG1450 + NaOH + H₂O, PEG4000 + NaOH + H₂O, and PEG10000 + NaOH + H₂O at (288.2, 298.2, and 308.2) K was studied, and new equilibrium data were generated. The increase in temperature triggered a shifting of the binodal curves toward the axis origin and an increase in both the slope and the length of tie lines. Considering systems at the same temperature, the observed biphasic regions in the diagrams followed the sequence: PEG10000 > PEG 4000 > PEG1450. This behavior was expected because the higher the polymer molar mass, the lower the amount of NaOH necessary to induce the phase splitting. These new systems are now being tested in our laboratory for the partition of bioactive food-derived compounds.

AUTHOR INFORMATION

Corresponding Author

*E-mail: jcoimbra@ufv.br. Phone: +55-31-3899-1618. Fax: +55-31-3899-2208.

Funding

The authors would like to thank CNPq, FAPEMIG, FAPESB, and CAPES for the financial support to this research work and for the scholarship of Ms. Diniz and Mr. Cardoso, Jr.

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