Liquid–Liquid Equilibrium Phase Diagrams of Polyethyleneglycol + Sodium Tartrate + Water Two-Phase Systems

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The phase diagrams of aqueous two-phase systems formed by water, sodium tartrate, and polyethyleneglycol of different molecular weights (600, 1000, 2000, 4000, 6000, and 8000) were determined at 298 K. The effect of NaCl on the liquid–liquid equilibrium was also analyzed. The binodal curves were satisfactorily described using a four-parameter sigmoidal equation. The tie lines were determined by combining a colorimetric method and density measurements. The reliability of the measured tie line compositions was ascertained by the Othmer−Tobias and Bancroft correlation equations.

Introduction

Aqueous two-phase systems (ATPSs) are formed when two mutually incompatible water-soluble polymers (e.g., dextran and polyethyleneglycol, PEG) or one polymer and one inorganic salt (e.g., PEG and potassium phosphate) are dissolved in water above a critical concentration. The unique feature of these biphasic systems is that both phases have mass fractions of almost (90 to 95) % water. This biaqueous nature provides a gentle and protective environment for biological materials and makes it possible to use aqueous two-phase systems for partitioning and separation of cells, organelles, enzymes, etc. Although ATPSs were reported for the first time by Beijernick[1] as early as 1896, almost 60 years later, Albertsson[2] began employing ATPSs for bioseparation purposes. Since then, many research groups have been devoted to the study of these systems and their applications. Among ATPSs suitable for bioseparation, those formed by polymer and salt are the most widely used for large-scale extraction due to their low cost and rapid phase disengagement. PEG has been predominantly combined with either phosphate or sulfate salts.[3] However, high concentrations of these salts are not desirable in the effluent streams due to environmental problems. Recently, several authors[4,5] have used a citrate salt as a substitute due to its biodegradability and nontoxicity. Later, Tubio et al.[6] demonstrated that PEG + sodium citrate systems had partitioning properties similar to the traditional PEG + phosphate ATPSs and could be satisfactorily applied for separating pancreatic enzymes. Ananthapadmanabhan and Goddard[7] have reported that tartrate salts could also form ATPS with PEG of molecular weight 3350. The tartrate anion is known to be readily and 100 % biodegradable.[8] It is used to enhance flavors in foods, confectionery, and beverages. No pollution problems are known to be caused by it. Therefore, PEG + tartrate salts + water could form environmentally safe aqueous two-phase systems which might constitute a suitable alternative for extraction of biological materials. Before applying these systems for bioseparation purposes, detailed information on their phase composition and physicochemical properties is required. However, at present, these ternary PEG + tartrate + water systems have not been explored enough, and the available experimental data of their liquid–liquid equilibrium are limited.

The goal of this work is to obtain a complete set of measurements on the phase equilibrium for PEGs of increasing molecular weights and sodium tartrate solutions with pH 4.90 at 298 K. In addition to the equilibrium measurements, the

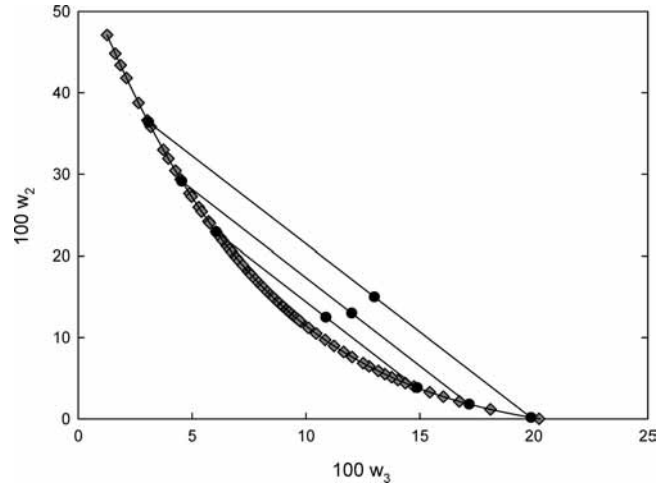

Figure 1. Binodal curve and tie lines for water (1) + PEG2000 (2) + sodium tartrate (3) systems at 298 K and pH 4.90.

Table 1. Values of Fit Parameters *i*, *j*, x_0, and y_0 of Equation 3 for Water (1) + PEG (2) + Sodium Tartrate (3) at 298 K and pH 4.90

	parameters				
PEG	*i*	*j*	x_0	y_0	σ^a
600	1797.0	−13.34	−45.184	−6.00	0.39
1000	63.7	−5.03	5.90	−1.90	0.25
2000	245.2	−6.14	−7.11	−2.80	0.13
4000	55.4	−3.09	4.88	−1.56	0.49
6000	81.1	−3.21	2.13	−1.09	0.69
8000	92.7	−3.27	1.43	−0.86	0.55
	in NaCl 5 wt %/wt presence				
1000	1303.0	−7.59	−22.80	−3.40	0.69
4000	78.1	−3.21	1.43	−1.65	0.81
8000	74.8	−2.32	1.62	−0.49	0.65

[a] σ, Standard deviations.

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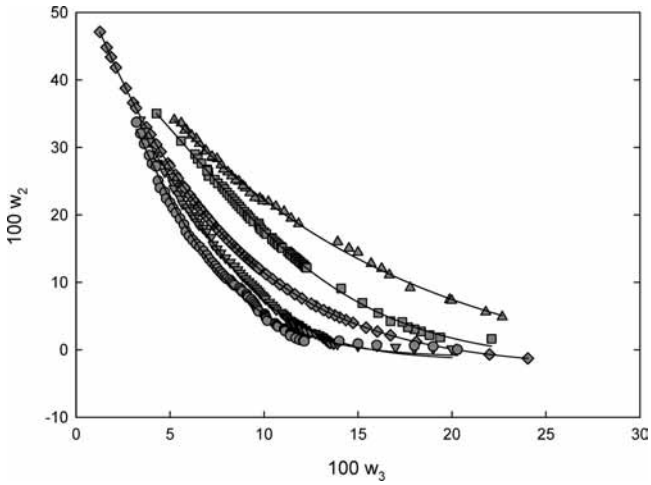

Figure 2. Binodal curves for water (1) + PEG of varying molecular weight (2) + sodium tartrate (3) systems at 298 K and pH 4.90. Experimental data: ▲, PEG600; ■, PEG1000; ◆, PEG2000; ▼, PEG4000; ●, PEG6000. Calculated data using eq 3 (—).

empirical description of the phase equilibrium data with an adequate equation will be performed to provide the basis for interpolating and extrapolating experimental data and predicting phase compositions when such data are not available.

Experimental Section

Chemicals. Polyethyleneglycols of the following average molecular masses, 600 (PEG600), 1000 (PEG1000), 2000 (PEG-2000), 4000 (PEG4000), 6000 (PEG6000), and 8000 (PEG8000), were purchased from Sigma Chemical Co. and used without further purification. Tartaric acid was obtained from Sigma Chemical Co. and used without further purification. All the other reagents were of analytical grade with a minimum purity of 99 %.

Procedures. A phase diagram includes the binodal curve and the tie lines. The determination of the binodal curve was carried out by a turbidimetric titration method.[9] Stock aqueous solutions of PEGs of different molecular weights with a mass fraction of 40 % and sodium tartrate with a mass fraction of 25 % at pH 4.90 were employed. Sodium tartrate solution was prepared from a tartaric solution whose pH was adjusted to 4.90 by the addition of the appropriate quantities of sodium hydroxide. Small aliquots of approximately (0.01 to 0.05) g of the polymer stock solution were added to 1 g of the sodium tartrate stock solution that was placed in a glass tube. After each aliquot addition, the system was thoroughly mixed. The first appearance of turbidity (the cloud point) indicated that the system was about to enter the two-phase region. With knowledge of the composition of the starting polymer and salt solutions and of the added masses, the total system composition, just prior to the two-phase formation, was calculated and provided a point on the binodal curve. The starting and added solution masses were measured on an analytical balance with a precision of ± 0.0001 g. Additional binodal points were obtained by adding a small amount of water to clear the system and then enough drops of the PEG stock solution to produce turbidity again. To obtain the binodal points corresponding to higher polymer mass fractions, the above-mentioned procedure was inverted, thus titrating the stock polymer solution with the stock salt solution. The system temperature was maintained constant and controlled to within ± 0.1 °C by immersing the glass tube and the stock solutions in a thermostatic bath.

For the determination of the tie lines, a series of ATPSs of at least three different known total compositions were prepared in 5 mL graduated glass tubes (uncertainty ± 0.05 mL) and placed in a thermostatic bath. Each mixture was prepared in triplicate. After reaching the phase equilibrium, visual estimates of top and bottom volumes (V_T and V_B, respectively) were made. When phases were separated, they were properly diluted to determine the polymer and salt equilibrium concentrations.

The concentrations of tartrate were determined according to a colorimetric assay.[10] It was based on the reaction of tartaric acid with ammonium metavanadate in dilute aqueous acetic acid solution to form a red dye that was determined spectrophotometrically at 530 nm. The uncertainty in the measurement of the mass fraction of the salt was estimated to be ± 0.002. The analysis showed that the presence of PEG did not interfere with the tartaric determination.

The mass fraction of PEG was determined from density measurements at 25 °C using an Anton Paar model DMA 35N densimeter. All density measurements were done with a ± 0.0001 g·cm^{-3} uncertainty. Each mixture was prepared and analyzed in triplicate. Temperature was maintained within ± 0.1 °C. Since the density of the phase samples depends on the PEG and salt compositions, calibration plots of density versus both polymer and sodium tartrate concentrations were obtained.

The calibration was given by the following relation between density (ρ) and the tartrate (w_3) and PEG (w_2) mass fractions

$$\rho = a + bw_3 + cw_2 \tag{1}$$

The estimated values of the coefficients a, b, and c (in g·cm^{-3} units) for the present system were 0.9980, 0.9215, and 0.1776, respectively. We found that these coefficients were practically independent of the molecular mass of the PEG and comparable to those coefficients obtained by Taboada et al.[11] for Na_2CO_3 and PEG mixtures. The uncertainty in the measurement of the mass fraction of PEG was estimated to be 0.003. A mass balance check was made between the initial mass of each component and the amounts in the bottom and top phases on the basis of equilibrium compositions. The relative error in the mass balance was < 2 %. The tie line lengths (TLL) for the different compositions were calculated according to

$$\mathrm{TLL} = \left[\left(w_2^{\mathrm{T}} - w_2^{\mathrm{B}}\right)^2 + \left(w_3^{\mathrm{T}} - w_3^{\mathrm{B}}\right)^2\right]^{1/2} \tag{2}$$

where w_2^{T}, w_3^{T}, w_2^{B}, and w_3^{B} are the top (T) and bottom (B) equilibrium mass fractions of PEG (2) and sodium tartrate (3). The tie line lengths are expressed in mass fractions.

Results and Discussion

Figure 1 shows the phase diagram corresponding to the water (1) + PEG2000 (2) + sodium tartrate (3) at pH 4.90 ATPS at 298 K. It includes the binodal curve and the tie lines. ATPSs with total initial compositions above the binodal curve separate into two equilibrium immiscible phases: a PEG-poor, tartrate-rich bottom layer and a PEG-rich, tartrate-poor upper phase. A similar behavior was observed for the traditional PEG + phosphate ATPSs.[2,3]

Binodal data were fitted with different expressions, previously used by other authors;[5,12,13] however, better results were obtained with the following empirical equation[14]

$$w_2 = y_o + \frac{i}{1 + e^{\frac{w_3 - x_o}{j}}} \tag{3}$$

where w_2 and w_3 (PEG and Tart mass fraction, respectively) are the coordinates of the binodal points; and y_o, x_o, i, and j

Table 2. Phase Compositions for the Water (1) + PEG (2) + Sodium Tartrate (3) Systems at 298 K and pH 4.90

total compositions		top phase		bottom phase				
100 w_3	100 w_2	100 w_3	100 w_2	100 w_3	100 w_2	STL[a]	100TLL[b]	K_p[c]
				PEG600				
12.17	19.50	7.24	28.63	19.98	7.32	−1.66	24.83	2.76
12.18	20.00	6.93	29.43	19.88	7.42	−1.70	25.53	2.87
12.85	20.62	5.78	32.53	21.80	5.61	−1.68	31.33	3.77
12.17	19.50	5.23	43.10	22.68	4.87	−1.68	42.02	4.34
				PEG1000				
12.18	20.00	8.32	25.87	15.27	7.85	−2.59	19.31	1.84
12.85	20.62	6.76	31.51	16.94	5.25	−2.58	28.16	2.50
12.93	21.20	5.36	36.93	19.00	2.72	−2.51	36.83	3.54
12.67	20.67	4.16	41.75	20.47	1.33	−2.48	43.59	4.92
				PEG2000				
10.87	12.50	6.05	22.93	14.87	3.85	−2.16	21.02	2.45
12.00	13.00	4.54	29.14	17.16	1.82	−2.16	30.09	3.78
13.00	15.00	3.08	36.39	19.87	0.18	−2.16	39.91	6.45
				PEG4000				
12.97	9.37	5.30	24.24	13.44	1.70	−2.77	23.96	2.54
13.66	9.48	4.86	26.21	14.10	1.11	−2.72	26.75	2.90
13.91	9.75	4.62	27.28	14.88	0.52	−2.61	28.66	3.22
14.57	9.95	4.22	29.07	15.68	0.07	−2.53	31.18	3.72
				PEG6000				
9.50	8.30	6.01	17.53	11.47	3.08	−2.64	15.45	1.91
10.02	10.00	4.70	24.02	13.34	1.24	−2.64	24.36	2.84
10.31	10.50	4.22	26.71	13.92	0.91	−2.66	27.56	3.30
10.65	11.45	3.51	30.86	14.68	0.48	−2.72	32.37	4.18
				PEG8000				
9.50	10.00	4.97	22.62	12.76	1.18	−2.71	22.81	2.57
8.79	14.00	4.25	26.53	13.62	0.66	−2.76	27.51	3.20
10.00	12.00	3.97	28.32	14.31	0.34	−2.71	29.83	3.60
10.00	14.00	3.48	31.46	15.21	0.03	−2.68	33.53	4.37

[a] STL, tie line slope. [b] TLL, tie line length. [c] K_p, Fraction of sodium tartrate retained in the bottom phase divided by the tartrate in the top phase.

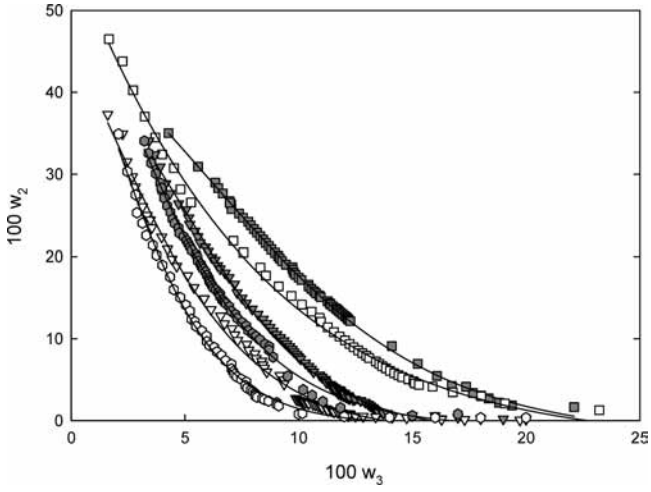

Figure 3. Effect of the presence of NaCl, mass fraction of 5 %, on the binodal curve of the water (1) + PEG (2) + sodium tartrate (3) systems at 298 K and pH 4.90. ■, PEG1000; □, PEG1000/NaCl; ▼, PEG4000; ▽, PEG4000/NaCl; ⬢, PEG8000; ⬡, PEG8000/NaCl.

Table 3. Values of the Parameters of Othmer−Tobias and Bancroft Equations, k, n, k_1, and r, for Water (1) + PEG (2) + Sodium Tartrate (3) Systems at 298 K and pH 4.90

	parameters					
PEG	k	n	σ[a]	k_1	r	σ[a]
600	0.0134	3.742	0.0110	3.072	0.2175	0.0023
1000	0.0951	1.981	0.0070	3.436	0.4153	0.0033
2000	0.1284	1.866	0.0011	3.137	0.4929	0.0011
4000	0.2444	1.369	0.0007	3.044	0.6834	0.0002
6000	0.0253	2.564	0.0118	4.378	0.3574	0.0034
8000	0.0479	2.218	0.0017	4.072	0.4346	0.0009

[a] σ, Standard deviations.

are the fit parameters. The parameters of eq 3 along with the corresponding standard deviations (σ) are summarized in Table 1.

The effect of PEG molecular weight on phase separation was evaluated. Figure 2 summarizes the binodal data corresponding to ATPSs formed by sodium tartrate and PEGs of different molecular weights. It can be seen in this figure that binodal curves became close to the origin with the increase in PEG molecular weight, thus requiring lower concentrations for phase separation. This may be caused by the increase in the incompatibility between the system components, due to the more hydrophobic character of PEGs of higher molecular weight. The binodal curve corresponding to systems of PEG8000 was not included in Figure 2 since it superimposed to the binodal curve of PEG6000 + sodium tartrate ATPSs.

The liquid–liquid equilibrium data of assayed water + PEG + sodium tartrate ATPSs are given in Table 2. It also lists the value of the partition coefficient (K_p) of the salt, defined as the fraction of salt retained in the bottom phase divided by the salt in the top phase. High values of K_p were obtained (from 1.84 to 6.45) being an indication of the separation obtained by adding an amount of PEG to a given brine solution.

For most of the studied systems, the slope of the tie lines (STL) is practically constant, which implies that tie lines are parallel to each other, thus allowing us to know the coexisting phase compositions for any given total polymer phase-forming composition. ATPSs formed by PEGs of molecular weights 4000, 6000, and 8000 show the higher STL absolute values. An increase in the STL magnitude indicates an increase in the difference between the polymer concentrations at a given difference in the salt concentrations. This implies a decrease in

the mutual solubility of the aqueous polymer- and salt-containing media. A similar behavior was observed for PEG + citrate ATPSs.[14]

Several authors found that the addition of NaCl to a PEG−salt system may increase the system resolution by promoting an increase in the difference between the partition coefficients of a target and its contaminant.[15] The effect of NaCl presence may affect protein partition coefficient either by modifying the interaction between the partitioned proteins with phase components or by altering the binodal compositions. Figure 3 shows that the NaCl presence does not alter the shape of binodal curves but induces a displacement of them to the left, thus expanding the biphasic area. This behavior was also observed for other polymer/salt systems[15] and is a consequence of a salting out effect due to the presence of NaCl.

Empirical equations have been proposed to ascertain the reliability of calculated tie line data in traditional liquid–liquid extraction, the most used being those of Othmer−Tobias (eq 4) and Bancroft (eq 5)[16]

$$\left(\frac{w_1^{\mathrm{B}}}{w_3^{\mathrm{B}}}\right) = k_1\left(\frac{w_1^{\mathrm{T}}}{w_2^{\mathrm{T}}}\right)^r \tag{4}$$

$$\left(\frac{1-w_2^{\mathrm{T}}}{w_2^{\mathrm{T}}}\right) = k\left(\frac{1-w_3^{\mathrm{B}}}{w_3^{\mathrm{B}}}\right)^n \tag{5}$$

where w_1^{T} and w_1^{B} are the mass fractions of water in the top and bottom phases and k, n, k_1, and r represent the parameters to be determined. Linearization of both equations produced acceptable consistency in the results. Values of the fit parameters and the corresponding coefficients of determination are given in Table 3.

Conclusions

The aqueous two-phase partitioning method of liquid–liquid extraction is useful for separating material of biological origin. Water + PEG + sodium tartrate systems seem to be attractive for protein purification because of the biodegradability of tartrate. Composition and properties of these systems are necessary for the design of an extraction process. However, this information was not available up to the present. In this work, the phase diagrams of water + PEG + sodium tartrate ATPSs were determined and reliable, and complete data were obtained. The phase formation showed to be affected by both the PEG molecular weight and the NaCl presence. These characteristics and several additional advantages such as low cost and rapid phase separation make water + PEG + sodium tartrate systems a promising, versatile, and attractive system in the field of bioseparation.

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