

# Liquid-Liquid Equilibrium of Aqueous Mixtures of Poly(ethylene glycol) with Na<sub>2</sub>SO<sub>4</sub> or NaCl

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Experimental liquid-liquid equilibrium phase diagrams, tie lines, and plait points were obtained for ternary systems consisting of water, sodium sulfate, and poly(ethylene glycol) 1000 at 301 K, poly(ethylene glycol) 3350 at 301 and 308 K, and poly(ethylene glycol) 8000 at 301 K. The equilibrium phase diagram of water, sodium chloride, and poly(ethylene glycol) 8000 at 333 K was also determined.

## Introduction

The aqueous two-phase partitioning method of liquid-liquid extraction is useful for separating materials of biological origin (1). In this technique, two liquid phases are created from a single homogeneous aqueous phase by the addition of one or more compounds. The additives are usually either two structurally different hydrophilic polymers, such as dextran and poly(ethylene glycol) (PEG), or a polymer and a salt, such as PEG and potassium phosphate. Many solutes of large molecular weight, including biomolecules such as proteins and enzymes, are unevenly distributed in such systems, with different components being preferentially retained in one of the phases (1-4), providing a basis for separation.

In these systems, both phases consist mainly of water, an environment suitable for biomolecules which are denatured in organic solvents. The degree of partitioning of all species in the system can be varied by changing the temperature, polymer or salt type, molecular weight and concentration of solutes. In addition to their use as phase-forming solvents for separating large molecules, biphasic salt systems provide a method of separating phase components from each other.

Ananthapadmanabhan and Goddard (5) determined the location of part of the binodal curve for an aqueous mixture of PEG 3350 and Na<sub>2</sub>SO<sub>4</sub>. The same researchers (6) measured the effect of temperature ( $T = 298, 313, \text{ and } 333 \text{ K}$ ) and polymer molecular weight (PEG 1450, 3350, and 20 000) on phase equilibrium in this system. Increasing the temperature or polymer molecular weight increased the size of the two-phase region. Pathak et al. (7) measured the location of the binodal curve and tie lines for aqueous mixtures of PEG 4000 and Na<sub>2</sub>SO<sub>4</sub> at 293, 303, and 313 K. Snyder et al. (8) determined phase compositions, viscosities, and densities for aqueous mixtures of PEG 1000, PEG 3350, and PEG 8000 at 298 K. There are few published liquid-liquid equilibrium studies for mixtures of water, PEG, and NaCl since these systems do not form two liquid phases at ambient temperatures. Florin et al. (9) measured the location of a small portion of the phase boundary and a single tie line for an aqueous mixture of PEG  $4 \times 10^6$  and NaCl at 356 K.

This study presents data for ternary systems consisting of water, Na<sub>2</sub>SO<sub>4</sub>, and PEG 1000 at 301 K, PEG 3350 at 301 and 308 K, and PEG 8000 at 301 K. The equilibrium phase diagram of water, sodium chloride, and PEG 8000 at 333 K was also determined.

## Experimental Section

**Materials.** The PEG 1000 and PEG 8000 were obtained from the Aldrich Co., Milwaukee, WI. The PEG 3350 and

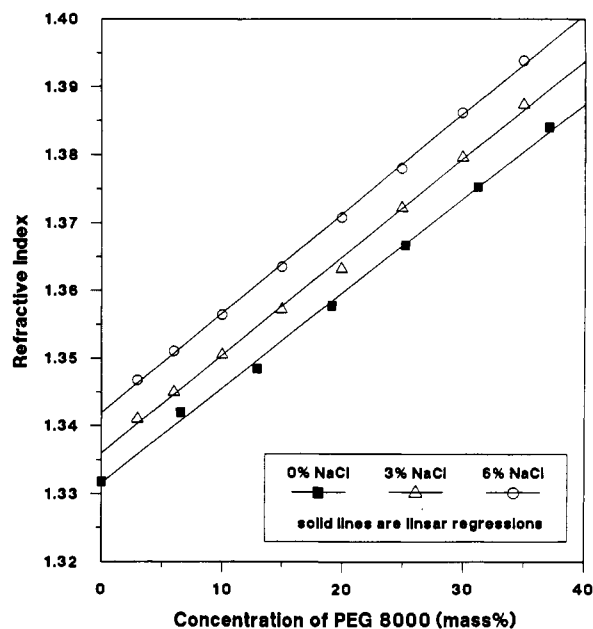


Figure 1. Refractive index calibration curves for PEG 8000 + NaCl + water.

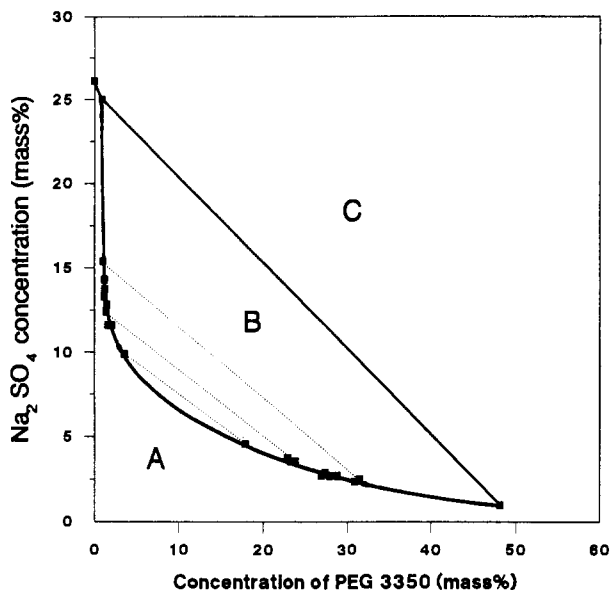
Table 1. Refractive Index Calibration Constants for Eq 1

$a_0$	$a_1$	$a_2$
1.3325	Na <sub>2</sub> SO <sub>4</sub> , 0.1422	PEG 1000, 0.1455
	NaCl, 0.1770	PEG 3350, 0.1450
		PEG 8000, 0.1455

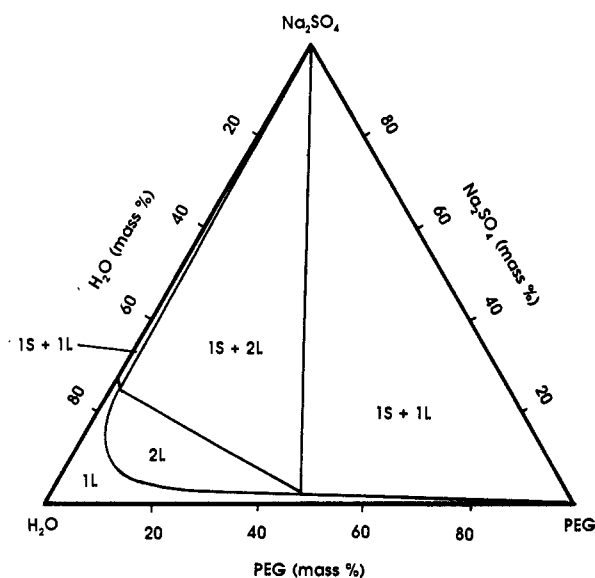
Na<sub>2</sub>SO<sub>4</sub> were obtained from American Chemicals Ltd., Montreal, Quebec. The NaCl was obtained from Anachemia, Montreal. The salts, which were reagent grade, were dried in an oven for 48 h at 393 K before use. The polymers were in the form of solid powders, and the polydispersity index reported by the suppliers was 1.05. Oven drying for a week at 330 K showed the polymers to contain about 0.5 mass % water. Distilled water of resistivity greater than 0.3 M $\Omega$ -cm was used in all experiments.

**Apparatus and Procedures.** Sodium sulfate (2 M) and sodium chloride (5 M) stock solutions were prepared by dissolving appropriate amounts of solid in distilled water. For the Na<sub>2</sub>SO<sub>4</sub> solutions, slight heating (303 K) was necessary to ensure complete dissolution. For the polymer, stock solutions containing between 30 and 60 mass % polymer were prepared and stored at 277 K.

The concentrations of Na<sub>2</sub>SO<sub>4</sub> and NaCl were determined by sodium analysis using atomic absorption spectroscopy



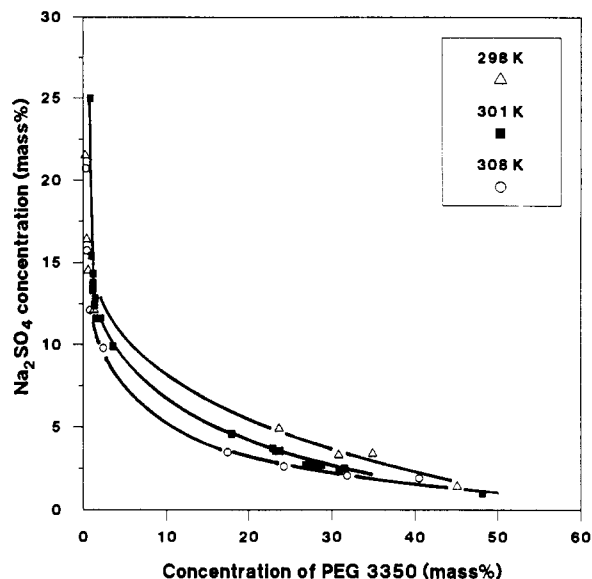
**Figure 2.** Binodal curve of PEG 3350 + Na<sub>2</sub>SO<sub>4</sub> + water at 301 K: (A) one liquid phase, (B) two liquid phases, and (C) liquid and solid phases.



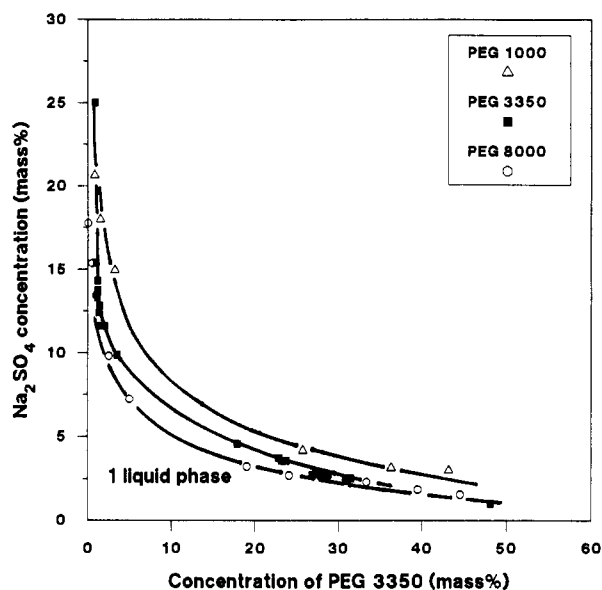
**Figure 3.** Complete phase diagram of PEG 3350 + Na<sub>2</sub>SO<sub>4</sub> + water at 301 K in triangular coordinates.

(AAS). The AAS measurements were performed using a Thermo Jarell Ash Corp. Model Smith-Hieftje II instrument at a wavelength of 330.2 nm. The concentration of PEG was determined from refractive index measurements at 298 K using a Carl Zeiss Model 41433 refractometer. Temperatures were maintained within  $\pm 1$  K of their set points using water baths supplied by Neslab Instruments.

Before each analysis of unknown (diluted) samples by AAS, a calibration plot of absorbance versus concentration was prepared. Generally, these calibrations were nonlinear for the range 0–120 ppm NaCl by mass, and for each run the sodium concentration was correlated with the absorbance using a quadratic polynomial. Since the refractive index of phase samples depends on the PEG and salt concentrations, calibration plots of refractive index versus polymer concentration were prepared for different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub>. The calibration plot for PEG 8000 + NaCl is shown in Figure 1. For both Na<sub>2</sub>SO<sub>4</sub> and NaCl the calibrations were described by the following relationship between the



**Figure 4.** Effect of temperature on the binodal curve of PEG 3350 + Na<sub>2</sub>SO<sub>4</sub> + water [data for 298 K were obtained by Snyder et al. (8)].



**Figure 5.** Effect of molecular weight on the binodal curve of PEG + Na<sub>2</sub>SO<sub>4</sub> + water at 301 K.

refractive index ( $n$ ) and the salt ( $w_s$ ) and PEG ( $w_p$ ) mass fractions:

$$n = a_0 + a_1 w_s + a_2 w_p \quad (1)$$

The parameters for the different salts and polymer molecular weights are listed in Table 1. The values of  $a_0$  and  $a_1$  were obtained from refractive index data for binary aqueous salt solutions, i.e., the intercept of Figure 1. The parameter  $a_2$  was essentially independent of the polymer molecular weight.

The approximate location of the binodal curve was determined by mixing known amounts of the components until two phases were observed. Feed compositions yielding roughly equal volumes of the top and the bottom phases were then calculated. Feed samples of 60–95 mL were prepared by mixing appropriate amounts of stock solutions of salt, PEG, and water in 100-mL graduated Pyrex cylinders. These cylinders were capped using either paraffin wax film or, in the case of experiments conducted at 333 K, rubber membranes secured with rubber bands. The membranes were

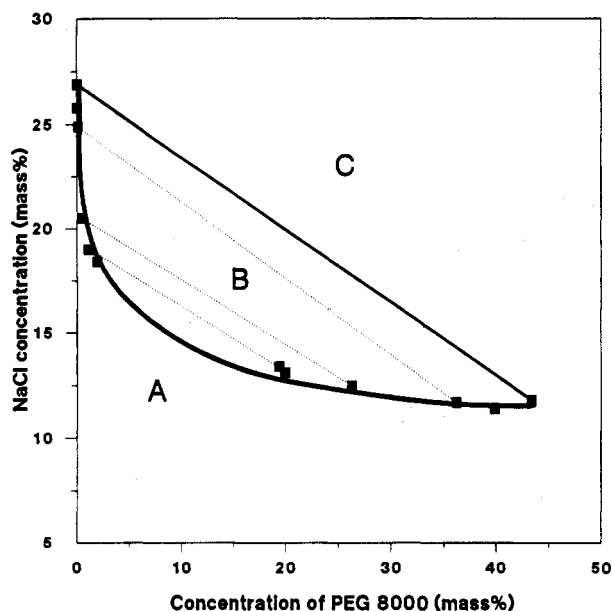


Figure 6. Binodal curve of PEG 8000 + NaCl + water at 333 K: (A) one liquid phase, (B) two liquid phases, and (C) liquid and solid phases.

Table 2. Tie Line Data for PEG 1000 + Na<sub>2</sub>SO<sub>4</sub> + Water at 301 K

top phase		bottom phase	
[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)	[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)
25.72	4.17	3.28	14.94
36.35	3.14	1.52	17.99
43.16	2.98	0.79	20.64

Table 3. Tie Line Data for PEG 3350 + Na<sub>2</sub>SO<sub>4</sub> + Water at 301 K

top phase		bottom phase	
[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)	[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)
48.14	0.980	0.85	25.00
31.51	2.510	0.985	15.38
30.87	2.35	1.19	14.31
28.75	2.70	1.21	13.74
27.84	2.70	1.17	13.68
27.31	2.87	1.15	13.29
26.87	2.71	1.425	12.82
23.74	3.56	1.36	12.40
23.20	3.56	1.55	11.60
22.85	3.73	2.06	11.60
17.88	4.58	3.57	9.88

Table 4. Tie Line Data for PEG 8000 + Na<sub>2</sub>SO<sub>4</sub> + Water at 301 K

top phase		bottom phase	
[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)	[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)
19.018	3.21	5.04	7.24
24.11	2.69	2.54	9.81
33.45	2.29	0.985	13.42
39.51	1.84	0.50	15.36
44.51	1.53	0.001	17.76

used because the high temperature softened the wax film and allowed escape of water vapor. The cylinders were placed in a water bath at the desired temperature for 24 h. Since equilibrium compositions depend on the temperature, it was necessary to ensure that phase separation did not occur before the sample attained the temperature of the bath. Hence, the samples were withdrawn and shaken thoroughly at least twice within 1 h of being immersed in the bath.

Table 5. Tie Line Data for PEG 3350 + Na<sub>2</sub>SO<sub>4</sub> + Water at 301 K

top phase		bottom phase	
[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)	[PEG] (mass %)	[Na <sub>2</sub> SO <sub>4</sub> ] (mass %)
17.407	3.48	0.27	20.71
24.19	2.625	0.42	15.72
31.82	2.08	0.80	12.10
40.48	1.92	2.4	9.78

Table 6. Tie Line Data for PEG 8000 + NaCl + Water at 333 K

top phase		bottom phase	
[PEG] (mass %)	[NaCl] (mass %)	[PEG] (mass %)	[NaCl] (mass %)
19.4	13.4	1.1	19.0
20.0	13.1	1.9	18.4
26.3	12.5	0.5	20.5
36.2	11.7	0.1	24.9
39.9	11.4	0.05	25.8
43.4	11.8	0.01	26.9

Table 7. Plait Points

system	T (K)	plait point	
		[PEG] (mass %)	[salt] (mass %)
PEG 1000 + Na <sub>2</sub> SO <sub>4</sub> + H <sub>2</sub> O	301	10.9	8.2
PEG 3350 + Na <sub>2</sub> SO <sub>4</sub> + H <sub>2</sub> O	301	9.3	6.8
PEG 3350 + Na <sub>2</sub> SO <sub>4</sub> + H <sub>2</sub> O	308	7.7	5.3
PEG 8000 + Na <sub>2</sub> SO <sub>4</sub> + H <sub>2</sub> O	301	8.5	5.1
PEG 8000 + NaCl + H <sub>2</sub> O	333	9.0	15.0

After equilibrium was achieved, the cylinders were withdrawn and the phase volumes recorded. Aliquots of 15 mL were withdrawn using syringes. The top phase was sampled first, with care being taken to leave a layer of solution at least 0.5 cm thick above the interface. The bottom phase was 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 upper-phase material. In cases where precipitated solid salt was present, care was taken to ensure that the sample was withdrawn gently, with the needle tip well away from salt crystals. For samples at 333 K, these operations were performed rapidly, as the temperature of the cylinders fell quickly after removal from the bath. This was particularly important for the measurement of density which was carried out immediately after sample withdrawal.

The density of both phases was determined using a 10-mL pycnometer and a balance reading  $\pm 0.1$  mg, following which a 5-mL sample of each phase was pipetted and diluted by a volumetric factor of 2000 for sodium analysis. Another 5 mL pipet sample was withdrawn for refractive index measurement and equilibrated in a second water bath, maintained at 298 K. For refractive index measurements, the samples were diluted by a factor of 2–4. Three replicates of phase samples gave a sample standard deviation of 0.48 mass % corresponding to a 95% confidence interval of  $\pm 0.54$  mass %.

## Results and Discussion

Separation into two clear phases was achieved within 1 h of shaking. The top phases were rich in PEG and low in salt, while the bottom phases contained most of the salt and little polymer. Although the polymer may fractionate by molecular weight between the phases, we did not attempt to determine this fractionation. Since  $a_2$  in eq 1 was independent of the PEG molecular weight, the mass concentrations here are unaffected by molecular weight fractionation.

For the PEG + Na<sub>2</sub>SO<sub>4</sub> systems, the density of the top phases was close to unity while that of the bottom phases ranged from 1.2 to 1.4 g/mL. For the PEG + NaCl systems, the density of the top phases ranged from 0.95 to 0.98 g/mL while those for the bottom phases ranged from 1.04 to 1.07 g/mL. The density difference between the phases increased with the tie line length.

The binodal curve of the PEG 3350 + Na<sub>2</sub>SO<sub>4</sub> + water system at 301 K is given in Figure 2. Tie line compositions were verified by checking mass balances on Na<sub>2</sub>SO<sub>4</sub> and PEG. The mass balances closed to within 7%. Figure 3 shows the complete phase diagram for this system plotted on triangular coordinates. The letters L and S denote liquid and solid phases, respectively. Most of the phase diagram consists of either a solid-liquid-liquid or solid-solid-liquid region. The two-liquid region occupies a small portion of the total phase diagram, although the coexisting phases are generally quite different in composition. There are two single liquid-solid regions, one with a liquid rich in water and another with a liquid rich in PEG. The effect of temperature on the binodal curve of the PEG 3350 + Na<sub>2</sub>SO<sub>4</sub> + water system is illustrated in Figure 4. The effect of increasing the temperature is to increase slightly the area of two-phase coexistence. Similarly, the effect of increasing the molecular weight of the polymer, shown in Figure 5, is to increase the size of the two-phase region. The binodal curve for the PEG 8000 + NaCl + water system at 333 K is shown in Figure 6. The shape is similar

to that for Na<sub>2</sub>SO<sub>4</sub> systems, but the top phases contain more salt. The equilibrium data are listed in Tables 2-6. The compositions of the plait points, which were estimated by extrapolation from the midpoints of the tie lines, are listed in Table 7.

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