

# LIQUID-LIQUID EQUILIBRIUM OF WATER + PEG 8000 + MAGNESIUM SULFATE OR SODIUM SULFATE AQUEOUS TWO-PHASE SYSTEMS AT 35°C: EXPERIMENTAL DETERMINATION AND THERMODYNAMIC MODELING

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**Abstract** - Liquid-liquid extraction using aqueous two-phase systems is a highly efficient technique for separation and purification of biomolecules due to the mild properties of both liquid phases. Reliable data on the phase behavior of these systems are essential for the design and operation of new separation processes; several authors reported phase diagrams for polymer-polymer systems, but data on polymer-salt systems are still relatively scarce. In this work, experimental liquid-liquid equilibrium data on water + polyethylene glycol 8000 + magnesium sulfate and water + polyethylene glycol 8000 + sodium sulfate aqueous two-phase systems were obtained at 35°C. Both equilibrium phases were analyzed by lyophilization and ashing. Experimental results were correlated with a mass-fraction-based NRTL activity coefficient model. New interaction parameters were estimated with the Simplex method. The mean deviations between the experimental and calculated compositions in both equilibrium phases is about 2%.

**Keywords:** Liquid-liquid equilibrium; Aqueous two-phase systems; Data; poly(ethylene glycol); Salt; NRTL model.

## INTRODUCTION

When two different polymers [e.g. dextran and poly(ethylene glycol), PEG] or one polymer and one salt (e.g. PEG and sodium sulfate) are mixed at certain concentrations in an aqueous solution, the solution separates into two immiscible phases, one rich in one polymer and the other rich in the other polymer (or salt), with water as solvent in both phases. This statement, made by Albertsson in the mid-1950's (Albertsson, 1986), is the basis for separation processes using aqueous two-phase

systems (ATPS). Liquid-liquid extraction using aqueous two-phase systems is widely recognized today as a highly efficient separation technique (Zaslavsky, 1995), particularly in partitioning and purification of biomolecules, since these systems form a mild environment for enzymes and other biologically active proteins. This extraction technology offers the advantages of high capacity, high activity yields and easy scale-up.

Reliable data on the composition and properties of aqueous two-phase systems are necessary for the design of extraction processes and for the

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development of both thermodynamics and mass transfer models. Phase diagrams have been reported for a large number of polymer-polymer systems (Albertsson, 1986; Zaslavsky, 1995); however, experimental liquid-liquid equilibrium (LLE) data for aqueous polymer-salt mixtures are still relatively scarce. Lei et al. (1990) reported liquid-liquid equilibrium data for the PEG 400, PEG 600, PEG 1000, PEG 1500, PEG 3400, PEG 8000 and PEG 20000 with potassium phosphate systems at 4°C. Gao et al. (1991) obtained experimental data for systems containing ammonium sulfate and PEG 1000, PEG 1540, PEG 2000 and PEG 4000. Snyder et al. (1992) studied systems containing PEG 1000, PEG 3350 and PEG 8000 and magnesium sulfate, sodium sulfate, sodium carbonate and potassium phosphate as salts at 25°C. Voros et al. (1993) used PEG 1000 and PEG 2000 with ammonium sulfate and sodium carbonate at 15, 25, 35 and 45°C. Hammer et al. (1994) analyzed systems with PEG 1550, PEG 3000 and PEG 6000, using sodium sulfate at 20, 30 and 40°C. Peng et al. (1994) reported data for systems composed of PEG 1000, PEG 2000, PEG 4000 and PEG 6000 with a mixture of potassium hydrogen phosphate and potassium dihydrogen phosphate at 25°C. Mishima et al. (1995) measured liquid-liquid equilibrium for systems containing PEG 7500 and potassium phosphate at 15, 30 and 40°C. Silva et al. (1997) studied the phase behavior of systems containing PEG 1000 and PEG 8000 at 4, 25 and 40°C and pH 6, 7 and 9. Mishima et al. (1998) published data for systems containing PEG 4000 and PEG 20000 with potassium phosphate at 25°C. Sé and Aznar (2002a) measured liquid-liquid equilibrium data for systems containing PEG 4000 and potassium phosphate at 10, 15, 20 and 30°C. In this work, new experimental liquid-liquid equilibrium data for the water + PEG 8000 + magnesium sulfate and water + PEG 8000 + sodium sulfate aqueous two-phase systems were determined at 35°C.

## EXPERIMENTAL

PEG, with a mass average 8000, magnesium sulfate and sodium sulfate were of analytical grade (Merck) and were used without further purification. Experiments were carried out in equilibrium cells similar to those suggested by Stragevitch (1997) and described elsewhere (Aznar et al., 2000; Sé, 2000).

Cell temperature was regulated by a controlled thermostatic bath (Tecnal TE-184, with a precision of  $\pm 0.01$  °C). The overall mixture was prepared directly inside the cell, and the components were weighed on an analytical balance (Ohaus AS200, with a precision of  $\pm 0.0001$  g). The mixture was vigorously stirred with a magnetic stirrer (Tecnal TE-085) for 3 h, in order to provide direct contact between the phases, and equilibrium was achieved by leaving the mixture to rest for 12 h. Preliminary tests (Duarte et al., 2000) showed that these times are long enough to achieve equilibrium. The system separated into two liquid phases, that become clear and transparent with a well-defined interface. Separate samples of both phases were collected and analyzed. The samples were collected with the aid of syringes, through lateral sample orifices, sealed with rubber septa. The phase compositions were determined gravimetrically using lyophilization (freeze drying) and ashing, as described below.

The samples were weighed, lyophilized (Telstar Lioalfa 6) and weighed again until a constant mass was achieved so that the water content could be determined directly. The lyophilized samples were thus heated in a high-temperature oven (Quimis) for five days, evaporating the PEG until mass was constant. In this manner, the PEG content could also be determined directly. The salt content was then determined as the difference. This technique was used with success by Stewart and Todd (1992) and by Snyder et al. (1992).

## EXPERIMENTAL RESULTS AND DISCUSSION

The experimental liquid-liquid equilibrium data for the ternary water + PEG 8000 + magnesium sulfate and sodium sulfate aqueous two-phase systems at 35°C are shown in Tables 1 and 2, respectively, as mass percentages (% w/w). These quantities are based on Snyder et al. (1992), which studied the same systems at 25°C.

These results are also presented in Figures 1 and 2 together with the correlation results. It can be seen that both ATPS show a large two-phase region suitable for the separation and purification of biomolecules. It can also be seen that sodium sulfate provides a larger two-phase region, showing a wider range of application than magnesium sulfate. These results are in agreement with the phase

behavior of similar systems presented by Snyder et al. (1992).

From these figures it can be observed that the compositions of the polymer-rich phase (and hence the tie-line length) behave in a slightly erratic manner.

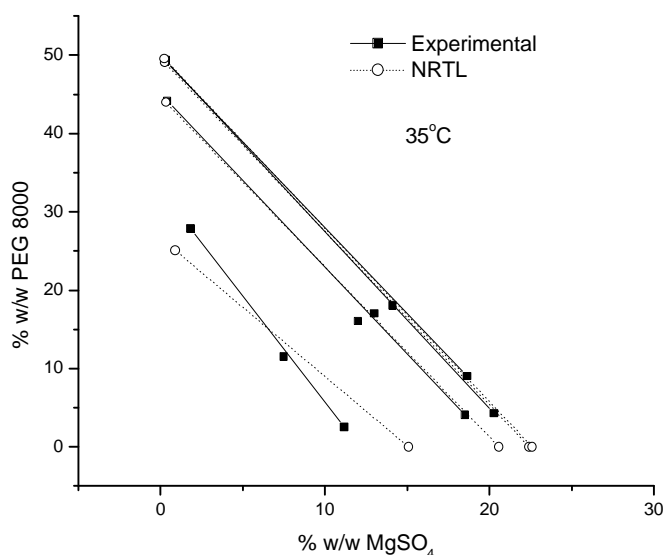
This is due to analytical difficulties encountered in the ashing procedure; however, the composition of the mixing point, also shown in the figures, appears to be aligned with the tie-line ends, indicating that the mass balance is satisfied.

**Table 1: Experimental LLE data for water + PEG 8000 + MgSO<sub>4</sub> at 35°C**

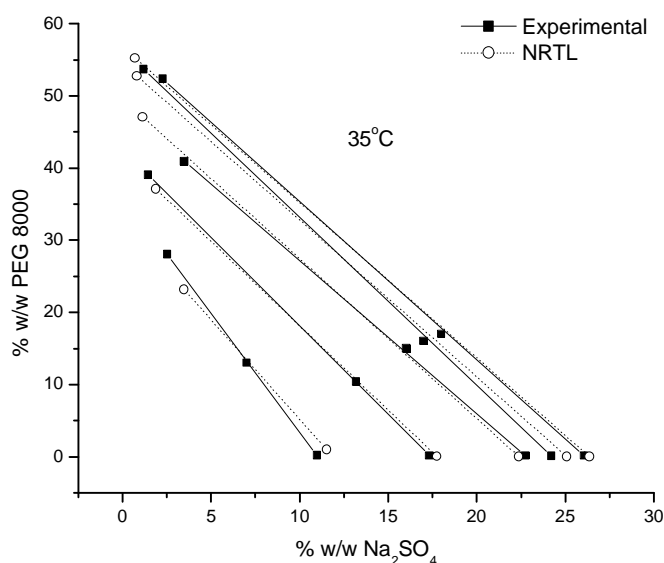
Overall		Lower phase		Upper phase	
PEG %w/w	MgSO <sub>4</sub> %w/w	PEG %w/w	MgSO <sub>4</sub> %w/w	PEG %w/w	MgSO <sub>4</sub> %w/w
11.51	7.50	2.50	11.17	27.83	1.83
16.03	12.01	4.07	18.52	44.15	0.42
17.02	13.00	4.27	20.28	49.32	0.34
18.02	14.12	9.02	18.64	49.18	0.30

**Table 2: Experimental LLE data for water + PEG 8000 + Na<sub>2</sub>SO<sub>4</sub> at 35°C**

Overall		Lower phase		Upper phase	
PEG %w/w	Na <sub>2</sub> SO <sub>4</sub> %w/w	PEG %w/w	Na <sub>2</sub> SO <sub>4</sub> %w/w	PEG %w/w	Na <sub>2</sub> SO <sub>4</sub> %w/w
10.37	13.20	0.13	17.33	39.04	1.44
13.00	7.01	0.20	10.98	28.04	2.53
14.99	16.03	0.15	22.75	40.92	3.48
16.01	17.01	0.12	24.22	53.69	1.20
17.00	18.00	0.14	26.06	52.34	2.28



**Figure 1: Experimental and calculated liquid-liquid equilibrium for the water + PEG 8000 + magnesium sulfate system at 35°C**



**Figure 2:** Experimental and calculated liquid-liquid equilibrium for the water + PEG 8000 + sodium sulfate system at 35°C

### THERMODYNAMIC MODEL

The concept of local composition, introduced by Wilson (1964), basically establishes that the composition of the system in the neighborhood of a given molecule is not the same as “bulk” composition because of the intermolecular forces. The NRTL activity coefficient model – non random, two-liquid – (Renon and Prausnitz, 1968) is based on the local composition concept, and is applicable to partially miscible systems. Chen et al. (1982) and Chen and Evans (1986) extended the original NRTL model to electrolyte systems containing small molecules, while Wu et al. (1998) proposed a modified NRTL model for multicomponent salt systems, successfully applied to polymer-polymer and polymer-salt ATPS. In this work, the original NRTL model (Renon and Prausnitz, 1968) was used to describe the LLE of PEG 4000 + potassium phosphate ATPS. Previous

work (Santos et al., 2000, 2001; Sé and Aznar, 2002a, 2002b) has shown that the original NRTL model is able to represent electrolyte systems. In this case, all the electrostatic contribution to the activity coefficient is built into the binary interaction parameters, which become purely empirical.

Mole fractions are traditionally used in the original NRTL model, but they are not suitable for polymeric systems because, due its large molecular mass, the mole fraction of a polymer is an extremely small quantity. Instead, mass fraction, as originally proposed by Oishi and Prausnitz (1978) can be used for the calculation of the activity coefficient of a solvent in polymeric solutions with the UNIQUAC and the UNIFAC methods. Stragevitch (1997), Velezmoro-Sánchez (1999), Batista et al. (1999), Lintomen et al. (2000) and Sé and Aznar (2002a, 2002b) used this approach with the NRTL model. When mass fractions are used, the model is

$$\ln \gamma_i = \frac{\sum_j \frac{\tau_{ji} G_{ji} w_j}{M_j}}{\sum_j \frac{G_{ji} w_j}{M_j}} + \sum_j \left[ \frac{w_j G_{ji}}{M_j \sum_k \frac{G_{kj} w_k}{M_k}} \left( \tau_{ij} - \frac{\sum_k \frac{\tau_{kj} G_{kj} w_k}{M_k}}{\sum_k \frac{G_{kj} w_k}{M_k}} \right) \right] \quad (1)$$

where

$$\tau_{ij} = \frac{A_{ij}}{T} \quad (2)$$

$$G_{ij} = \exp(-\alpha_{ij}\tau_{ij}) \quad (3)$$

where  $A_{ij}$  and  $A_{ji}$  are characteristic parameters of the energy interactions between  $i$  and  $j$ , and the parameter  $\alpha_{ij}$  is related to the non randomness of the mixture. This means that the components are distributed through a pattern dictated by the local composition.

$$S = \sum_k^D \sum_j^{N_k} \left[ \left( \frac{T_{jk}^{calc} - T_{jk}^{exp}}{\sigma_{T_{jk}}} \right)^2 + \sum_i^{C_k-1} \left[ \left( \frac{w_{ijk}^{I,calc} - w_{ijk}^{I,exp}}{\sigma_{w_{ijk}^I}} \right)^2 + \left( \frac{w_{ijk}^{II,calc} - w_{ijk}^{II,exp}}{\sigma_{w_{ijk}^{II}}} \right)^2 \right] \right] \quad (4)$$

where  $D$  is the number of data sets,  $N_k$  and  $C_k$  are the number of data points and components in the data set  $k$ ,  $w_{ijk}^I$  and  $w_{ijk}^{II}$  are the mass fractions in both liquid phases and  $\sigma_{T_{jk}}$  (set equal to 0.1 K) is the standard deviation in temperature, while  $\sigma_{w_{ijk}^I}$  and  $\sigma_{w_{ijk}^{II}}$  (set equal to 0.0005) are the standard deviations in the composition of both liquid phases at equilibrium. In this equation, "calc" stands for "calculated" and "exp" stands for "experimental." The stability of the phases is tested by calculating the Hessian matrix of the Gibbs free energy of the mixture [second

The experimental liquid-liquid equilibrium data were used to estimate the molecular interaction and the non randomness parameters of the NRTL model by liquid-liquid equilibrium flash calculations.

The Fortran code WTML-LLE (weight-temperature-maximum likelihood – liquid-liquid equilibrium) (Stragevitch, 1997; Sé, 2000) was used for estimation; the procedure is based on the Simplex method (Nelder and Mead, 1965) and consists in minimization of the objective function,  $S$ .

derivative in respect to the  $n - 1$  independent compositions (Sørensen et al., 1979)], which must be positive-defined for a stable homogeneous phase.

The molecular energy interaction parameters estimated by this procedure are shown in Table 3; with these parameters, the experimental liquid-liquid equilibrium data can be correlated. Comparisons between experimental and calculated data can be made through mean deviations between experimental and calculated compositions of each component in both phases. These mean deviations are given by

$$\Delta w = 100 \sqrt{\frac{\sum_n \sum_i^{C_k} (w_{ni}^{I,exp} - w_{ni}^{I,calc})^2 + (w_{ni}^{II,exp} - w_{ni}^{II,calc})^2}{2N_k C_k}} \quad (5)$$

The results of the correlation, shown in Table 4 and Figures 1 and 2, are very satisfactory. The mean deviations are below 2%, and there is a good agreement

between experimental and calculated liquid-liquid equilibrium points. The experimental data reported in Tables 1 and 2 are also shown in Figures 1 and 2.

**Table 3: Estimated NRTL parameters**

i-j	$A_{ij}$ (K <sup>-1</sup> )	$A_{ji}$ (K <sup>-1</sup> )	$a_{ij}$
water-PEG 8000 <sup>a</sup>	-2635.1	-2937.0	0.32035
water-PEG 8000 <sup>b</sup>	-2999.9	-2907.8	0.25697
water-MgSO <sub>4</sub>	3218.2	-1500.1	0.26428
water-Na <sub>2</sub> SO <sub>4</sub>	2999.9	-1448.9	0.23398
PEG 8000-MgSO <sub>4</sub>	-0.02156	903.16	0.38573
PEG 8000-Na <sub>2</sub> SO <sub>4</sub>	2530.0	-1884.8	0.47000

<sup>a</sup>parameters estimated with data from water + PEG 8000 + MgSO<sub>4</sub>

<sup>b</sup>parameters estimated with data from water + PEG 8000 + Na<sub>2</sub>SO<sub>4</sub>

**Table 4: Mean deviation in liquid-liquid equilibrium data**

System	Dw (%)
water + PEG 8000 + MgSO <sub>4</sub> at 35°C	2.08 %
water + PEG 8000 + Na <sub>2</sub> SO <sub>4</sub> at 35°C	1.57 %

### CONCLUSION

Experimental liquid-liquid equilibrium data for the ternary water + PEG 8000 + magnesium sulfate and water + PEG 8000 + sodium sulfate aqueous two-phase systems were determined at 35°C. The systems studied show a large two-phase region suitable for the separation and purification of biomolecules; sodium sulfate provides a larger two-phase region, showing a wider range of application than magnesium sulfate. Energy interaction parameters for the original NRTL model were estimated, and the mean deviations between experimental and calculated compositions in both equilibrium phases were below 2%. This result shows that the original NRTL model is able to represent the phase behavior of polymer-salt aqueous two-phase systems.

### ACKNOWLEDGEMENTS

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### NOMENCLATURE

$A_{ij}, A_{ij}$	molecular interaction parameters in the NRTL model for the $i$ - $j$ pair
$C_k$	number of components in data set $k$
$D$	number of data sets
$G_{ij}$	energy interaction parameters in the Boltzmann form in the NRTL model for the $i$ - $j$ pair
$M_k$	molecular mass of compound $k$
$N_k$	number of data points in data set $k$
$S$	objective function to be minimized
$T$	absolute temperature
$w_k$	mass fraction of compound $k$

### Greek Letters

$\alpha_{ij}$	molecular nonrandomness parameter in the NRTL model
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$\gamma_i$	activity coefficient of compound $i$
$\sigma$	standard deviation for an independent variable, temperature or composition
$\tau_{ij}$	energy parameters in the NRTL model

### Super/Subscripts

exp	experimental
calc	calculated
I, II	liquid phases at equilibrium

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