*J. Chem. Eng. Data* **2008,** *53,* 2441–2443

# Equilibrium Phase Behavior for Ternary Mixtures of Poly(ethylene) Glycol 6000 + Water + Sulfate Salts at Different Temperatures

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Phase diagrams of aqueous two-phase systems composed of poly(ethylene) glycol (PEG) 6000 $g \cdot mol^{-1}$, lithium sulfate, sodium sulfate, and magnesium sulfate or zinc sulfate were determined at (283.15, 298.15, and 313.15) K. The effect of temperature on binodal position was not significant, indicating a small enthalpic contribution associating to the phase segregation. The ability of four sulfate salts to induce the formation of the biphasic system with PEG 6000 is as follows: $ZnSO_4 > MgSO_4 > Na_2SO_4 > Li_2SO_4$.

## Introduction

Separation processes are applied in various types of industries including chemical, pharmaceutical, and food. Classic methods involving liquid−liquid extraction use an organic solvent and an aqueous solution as the two immiscible phases for the fractionation and purification of molecules. However, these systems are very toxic and present risks to human health.

An alternative to substitute organic two-phase systems, based on the beginning of green chemistry, is aqueous biphasic systems (ABS). They are formed by the mixture of a polymer + water + salt or two water-soluble polymers differing in chemical structure.[1,2] This methodology has been widely used to separate metallic ions[3,4] and biological materials,[1,2,5,6] such as viruses, nucleic acids, and proteins. These characteristics are important because an organic solvent is not used in the separation processes.

The ABS typically applied in separation or purification processes are constituted of poly(ethylene) glycol (PEG) + dextran + water or PEG + inorganic salt + water. The literature mentions several equilibrium data for different ABS formed of PEGs of different average molar masses and inorganic salts.[1,2,7−12] However, this type of data for systems containing PEG6000 + sulfate salts ($Li_2SO_4$, $Na_2SO_4$, $MgSO_4$, $ZnSO_4$) at different temperatures are scarce.

In this work, aqueous two-phase systems composed of PEG6000 + sulfate salt + water were determined, and the phase compositions were measured. Equilibrium data at (283.15, 298.15, and 313.15) K were determined for the systems consisting of PEG6000 + lithium sulfate + water, PEG6000 + sodium sulfate + water, PEG6000 + magnesium sulfate + water, and PEG6000 + zinc sulfate + water. The influences of the electrolyte nature and temperature on the phase diagram were also investigated.

## Experimental Section

***Materials.*** The following reagents were used: poly(ethylene) glycol with an average molar mass of 6000 $g \cdot mol^{-1}$ (Sigma, USA) and the sulfate salts $Na_2SO_4$, $Li_2SO_4$, $ZnSO_4$, and $MgSO_4$ (Vetec, Brazil), all of analytical grade. Millipore (USA) water was used in all experiments ($R \geq 18.2\ M\Omega \cdot cm^{-1}$).

***Aqueous Two-Phase Systems (ATPS).*** Ternary phase diagrams were prepared by weighing appropriate quantities of PEG6000, sulfate salts (lithium, sodium, zinc, or magnesium), and water on an analytical balance (Gehaka, AG200, Brazil) accurate to ± 0.0001 g. Liquid−liquid glass tubes were used to carry out phase equilibrium determinations. Typically, 15 g of the system was prepared. After vigorously stirring the system until becoming turbid, it was placed in a temperature-controlled bath (Microquímica, MQBTC 99-20), accurate to ± 0.1 K, for 48 h at (283.15, 298.15, or 313.15) K. The equilibrium state was characterized by the absence of turbidity in both the top and bottom phases. Samples from the top and bottom phases were collected for analysis.

***Quantitative Analysis.*** Salt concentration ($Na_2SO_4$, $Li_2SO_4$, $MgSO_4$, and $ZnSO_4$) was determined by conductivity (Schott CG853, Germany) of the electrolyte in the range of ($10^{-4}$ to $10^{-3}$) M. The salt solutions showed the same conductivity in water or the diluted polymer solution [(0.01 to 0.001) %]. The standard deviation of the salt mass percent from this method was ± 0.10 %. A refractometer (Analytic Jena AG Abbe refractometer 09-2001, Germany) was used to measure PEG6000 quantity at 298.15 K. Since the refractive index of the phase depends on the polymer and salt concentrations, PEG6000 content was obtained by subtracting the salt concentration obtained by conductivity from the total solution composition (refractive index). The standard deviation of the PEG6000 mass percent was on the order of 0.03 %. Analytical curves were obtained for the salt and PEG measurements, and water content was determined by mass balance. All analytical measurements were performed in triplicate.

## Results and Discussion

The phase compositions for the aqueous two-phase systems formed of PEG6000 and sulfate salts are shown in Tables 1 to

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10.1021/je800443p CCC: $40.75

**Table 1. Equilibrium Data (Mass %) for the PEG6000 ($w_{PEG}$) + Lithium Sulfate ($w_S$) + Water ($w_W$) System from (283.15 to 313.15) K**

| | overall | | | top phase | | | bottom phase | | |
|---|---|---|---|---|---|---|---|---|---|
| tie line | $w_{PEG}$ | $w_{Salt}$ | $w_W$ | $w_{PEG}$ | $w_{Salt}$ | $w_W$ | $w_{PEG}$ | $w_{Salt}$ | $w_W$ |
| 283.15 K | | | | | | | | | |
| 1 | 21.36 | 8.62 | 70.02 | 35.52 | 3.22 | 61.26 | 3.64 | 15.51 | 80.85 |
| 2 | 22.91 | 9.05 | 68.04 | 39.63 | 2.69 | 57.68 | 3.96 | 16.31 | 79.73 |
| 3 | 25.10 | 9.53 | 65.37 | 43.40 | 2.29 | 54.31 | 4.01 | 17.97 | 78.02 |
| 4 | 26.82 | 9.98 | 63.20 | 46.65 | 1.96 | 51.39 | 4.47 | 18.92 | 76.61 |
| 5 | 29.45 | 10.58 | 59.97 | 50.93 | 1.62 | 47.45 | 4.55 | 21.07 | 74.38 |
| 298.15 K | | | | | | | | | |
| 1 | 22.54 | 8.36 | 69.10 | 37.81 | 2.89 | 59.30 | 3.10 | 15.31 | 81.59 |
| 2 | 24.14 | 8.71 | 67.15 | 41.41 | 2.50 | 56.09 | 4.02 | 15.95 | 80.03 |
| 3 | 26.04 | 9.21 | 64.75 | 45.06 | 1.97 | 52.97 | 4.14 | 17.53 | 78.33 |
| 4 | 27.94 | 9.73 | 62.33 | 47.96 | 1.95 | 50.09 | 3.86 | 19.14 | 77.00 |
| 5 | 30.33 | 10.34 | 59.33 | 52.91 | 1.42 | 45.67 | 4.42 | 20.59 | 74.99 |
| 313.15 K | | | | | | | | | |
| 1 | 23.65 | 8.26 | 68.09 | 41.29 | 2.26 | 56.45 | 3.17 | 15.33 | 81.50 |
| 2 | 25.30 | 8.71 | 65.99 | 45.70 | 1.74 | 52.56 | 3.40 | 16.14 | 80.46 |
| 3 | 27.25 | 9.12 | 63.63 | 46.51 | 1.70 | 51.79 | 3.38 | 18.10 | 78.52 |
| 4 | 29.83 | 9.70 | 60.47 | 52.40 | 1.45 | 46.15 | 3.48 | 19.30 | 77.22 |
| 5 | 31.81 | 10.17 | 58.02 | 55.76 | 1.13 | 43.11 | 4.36 | 20.46 | 75.18 |

**Table 2. Equilibrium Data (Mass %) for the PEG6000 ($w_{PEG}$) + Sodium Sulfate ($w_S$) + Water ($w_W$) System from (283.15 to 313.15) K**

| | overall | | | top phase | | | bottom phase | | |
|---|---|---|---|---|---|---|---|---|---|
| tie line | $w_{PEG}$ | $w_S$ | $w_W$ | $w_{PEG}$ | $w_S$ | $w_W$ | $w_{PEG}$ | $w_S$ | $w_W$ |
| 283.15 K | | | | | | | | | |
| 1 | 16.35 | 7.81 | 75.84 | 29.28 | 2.28 | 68.44 | 1.72 | 13.99 | 84.29 |
| 2 | 18.31 | 8.36 | 73.33 | 32.28 | 1.95 | 65.77 | 1.37 | 16.22 | 82.41 |
| 3 | 19.64 | 8.82 | 71.54 | 35.00 | 1.56 | 63.44 | 1.18 | 17.51 | 81.31 |
| 4 | 21.53 | 9.41 | 69.06 | 37.41 | 1.37 | 61.22 | 0.91 | 19.93 | 79.16 |
| 5 | 22.94 | 9.85 | 67.21 | 39.93 | 1.21 | 58.86 | 0.90 | 21.10 | 78.00 |
| 298.15 K | | | | | | | | | |
| 1 | 16.82 | 8.08 | 75.10 | 31.53 | 2.37 | 66.10 | 0.72 | 14.42 | 84.86 |
| 2 | 18.17 | 8.45 | 73.38 | 34.22 | 1.96 | 63.82 | 0.72 | 15.49 | 83.79 |
| 3 | 20.14 | 8.94 | 70.92 | 37.19 | 1.65 | 61.16 | 0.67 | 17.49 | 81.84 |
| 4 | 21.64 | 9.34 | 69.02 | 40.07 | 1.36 | 58.57 | 0.69 | 18.23 | 81.08 |
| 5 | 23.94 | 9.82 | 66.24 | 41.96 | 1.41 | 56.63 | 0.88 | 19.89 | 79.23 |
| 313.15 K | | | | | | | | | |
| 1 | 17.18 | 7.97 | 74.85 | 34.09 | 1.92 | 63.99 | 0.58 | 14.00 | 85.42 |
| 2 | 19.00 | 8.45 | 72.55 | 36.71 | 1.65 | 61.64 | 0.50 | 15.40 | 84.10 |
| 3 | 20.55 | 8.89 | 70.56 | 39.42 | 1.44 | 59.14 | 0.54 | 16.83 | 82.63 |
| 4 | 22.46 | 9.39 | 68.15 | 42.77 | 1.24 | 55.99 | 0.44 | 18.39 | 81.17 |
| 5 | 24.18 | 9.84 | 65.98 | 44.54 | 1.09 | 54.37 | 0.25 | 20.25 | 79.50 |

4. All concentrations are expressed in mass percent. It was observed that polymer and salt segregation rose with the increase in overall composition. This behavior is in agreement with reported results describing the system formed by macromolecules and electrolytes.[4,13]

The influence of temperature was not relevant for all systems, indicating only a small change in $\Delta Cp$ and $\Delta H$ associated with the phase separation process.[14] However, temperature affected the concentration and volume of both phases for all systems studied.

Table 5 shows the slope of the tie line (STL) values for the systems in all temperatures. The STL is defined as the ratio of the difference in the polymer and the salt concentration in the top and bottom phases, as presented by eq 1

$$\mathrm{STL} = \frac{w_{PEG}^{T} - w_{PEG}^{B}}{w_{S}^{T} - w_{S}^{B}} \qquad (1)$$

where $w_{PEG}$ and $w_S$ are the polymer and salt concentrations and the superscripts T and B designate the top and bottom phases, respectively.

**Table 3. Equilibrium Data (Mass %) for the PEG6000 ($w_{PEG}$) + Magnesium Sulfate ($w_S$) + Water ($w_W$) System from (283.15 to 313.15) K**

| | overall | | | top phase | | | bottom phase | | |
|---|---|---|---|---|---|---|---|---|---|
| tie line | $w_{PEG}$ | $w_S$ | $w_W$ | $w_{PEG}$ | $w_S$ | $w_W$ | $w_{PEG}$ | $w_S$ | $w_W$ |
| 283.15 K | | | | | | | | | |
| 1 | 18.15 | 7.78 | 74.07 | 31.57 | 1.58 | 66.85 | 2.91 | 14.85 | 82.24 |
| 2 | 20.15 | 8.29 | 71.56 | 34.84 | 1.15 | 64.01 | 2.57 | 16.72 | 80.71 |
| 3 | 21.88 | 8.77 | 69.35 | 37.82 | 0.83 | 61.35 | 3.97 | 17.71 | 78.32 |
| 4 | 23.88 | 9.30 | 66.82 | 41.38 | 0.62 | 58.00 | 4.81 | 18.82 | 76.37 |
| 5 | 26.13 | 9.90 | 63.97 | 44.35 | 0.40 | 55.25 | 4.55 | 21.21 | 74.24 |
| 298.15 K | | | | | | | | | |
| 1 | 18.52 | 7.63 | 73.85 | 32.94 | 1.43 | 65.63 | 2.50 | 14.52 | 82.98 |
| 2 | 20.09 | 8.15 | 71.76 | 35.90 | 1.06 | 63.04 | 2.79 | 15.89 | 81.32 |
| 3 | 21.99 | 8.75 | 69.26 | 39.05 | 0.77 | 60.18 | 3.45 | 17.38 | 79.17 |
| 4 | 24.05 | 9.45 | 66.50 | 42.46 | 0.57 | 56.97 | 3.19 | 19.81 | 77.00 |
| 5 | 26.20 | 10.15 | 63.65 | 46.00 | 0.37 | 53.63 | 3.90 | 21.22 | 74.88 |
| 313.15 K | | | | | | | | | |
| 1 | 19.26 | 7.50 | 73.24 | 34.58 | 1.05 | 64.37 | 1.53 | 15.01 | 83.46 |
| 2 | 21.43 | 8.06 | 70.51 | 38.13 | 0.77 | 61.10 | 1.81 | 16.73 | 81.46 |
| 3 | 23.19 | 8.47 | 68.34 | 40.94 | 0.63 | 58.43 | 2.80 | 17.56 | 79.64 |
| 4 | 25.10 | 8.91 | 65.99 | 43.77 | 0.42 | 55.81 | 4.33 | 18.21 | 77.46 |
| 5 | 27.10 | 9.49 | 63.41 | 46.52 | 0.25 | 53.23 | 4.23 | 20.37 | 75.40 |

**Table 4. Equilibrium Data (Mass %) for the PEG6000 ($w_{PEG}$) + Zinc Sulfate ($w_S$) + Water ($w_W$) System from (283.15 to 313.15) K**

| | overall | | | top phase | | | bottom phase | | |
|---|---|---|---|---|---|---|---|---|---|
| tie line | $w_{PEG}$ | $w_S$ | $w_W$ | $w_{PEG}$ | $w_S$ | $w_W$ | $w_{PEG}$ | $w_S$ | $w_W$ |
| 283.15 K | | | | | | | | | |
| 1 | 18.02 | 8.64 | 73.34 | 28.31 | 2.40 | 69.29 | 0.31 | 19.24 | 80.45 |
| 2 | 19.64 | 9.21 | 71.15 | 32.15 | 1.88 | 65.97 | 0.90 | 19.92 | 79.18 |
| 3 | 21.37 | 9.77 | 68.86 | 34.79 | 1.35 | 63.86 | 0.70 | 22.94 | 76.36 |
| 4 | 23.41 | 10.40 | 66.19 | 38.33 | 1.15 | 60.52 | 1.39 | 24.23 | 74.38 |
| 5 | 24.74 | 10.83 | 64.43 | 40.95 | 0.81 | 58.24 | 1.94 | 24.76 | 73.30 |
| 298.15 K | | | | | | | | | |
| 1 | 16.96 | 8.96 | 74.08 | 29.31 | 2.19 | 68.50 | 0.29 | 18.20 | 81.51 |
| 2 | 18.87 | 9.48 | 71.65 | 32.14 | 1.71 | 66.15 | 0.76 | 19.93 | 79.31 |
| 3 | 20.95 | 10.05 | 69.00 | 36.43 | 1.24 | 62.33 | 1.29 | 21.70 | 77.01 |
| 4 | 22.51 | 10.50 | 66.99 | 39.63 | 0.96 | 59.41 | 0.35 | 22.94 | 76.71 |
| 5 | 25.02 | 11.25 | 63.73 | 42.99 | 0.74 | 56.27 | 2.28 | 24.50 | 73.22 |
| 313.15 K | | | | | | | | | |
| 1 | 17.21 | 9.10 | 73.69 | 31.50 | 1.83 | 66.67 | 0.62 | 17.71 | 81.67 |
| 2 | 19.11 | 9.64 | 71.25 | 34.66 | 1.41 | 63.93 | 1.42 | 18.98 | 79.60 |
| 3 | 20.94 | 10.13 | 68.93 | 36.69 | 1.06 | 62.25 | 1.45 | 21.34 | 77.21 |
| 4 | 22.84 | 10.63 | 66.53 | 40.49 | 0.78 | 58.73 | 1.89 | 22.38 | 75.73 |
| 5 | 24.75 | 11.12 | 64.13 | 44.29 | 0.61 | 55.10 | 2.65 | 23.10 | 74.25 |

A temperature increase promoted the amplification of the STL, verifying results reported for other aqueous two-phase systems.[7] It is possible that the STL change from the PEG + sulfate salt system was due to the transfer of water from the top to the bottom phase.[14] Thus, the polymer concentration increased in the upper phase, and the salt content decreased in the lower phase.

Figure 1 shows the influence of salt in inducing phase segregation. The formation of ATPS clearly indicates the mutual exclusion of the salt and polymer and their high affinity to water. This tendency is observed in all systems constituted of PEG and inorganic salts, such as sulfates.[13] The aptitude of the four salts for inducing the phase segregation in ATPS follows the order of: $ZnSO_4 > MgSO_4 > Na_2SO_4 > Li_2SO_4$. A model proposed by da Silva and Loh[14] based on calorimetric measurements attributed the trend in efficacy of lithium and sodium sulfates for inducing ATPS formation to the cation−polymer interactions. Their proposed model states that when the PEG and sulfate salts are mixed cations interact with the ethylene oxide polymer groups, releasing some water molecules there were solvating them in a process that is driven by entropy increase. This cation binding continues as more electrolytes are

**Table 5. STL Values for PEG6000 + Salt + Water Systems**

PEG6000 + Lithium Sulfate + Water

| | *T*/K | | |
|---|---|---|---|
| tie line | 283.15 | 298.15 | 313.15 |
| 1 | −2.59 | −2.79 | −2.92 |
| 2 | −2.62 | −2.78 | −2.94 |
| 3 | −2.51 | −2.63 | −2.63 |
| 4 | −2.49 | −2.57 | −2.74 |
| 5 | −2.38 | −2.53 | −2.66 |

PEG6000 + Sodium Sulfate + Water

| | *T*/K | | |
|---|---|---|---|
| tie line | 283.15 | 298.15 | 313.15 |
| 1 | −2.35 | −2.56 | −2.77 |
| 2 | −2.17 | −2.48 | −2.63 |
| 3 | −2.12 | −2.30 | −2.53 |
| 4 | −1.97 | −2.33 | −2.48 |
| 5 | −1.96 | −2.23 | −2.31 |

PEG6000 + Magnesium Sulfate + Water

| | *T*/K | | |
|---|---|---|---|
| tie line | 283.15 | 298.15 | 313.15 |
| 1 | −2.16 | −2.32 | −2.37 |
| 2 | −2.07 | −2.23 | −2.28 |
| 3 | −2.01 | −2.14 | −2.25 |
| 4 | −2.01 | −2.04 | −2.22 |
| 5 | −1.91 | −2.02 | −2.10 |

PEG6000 + Zinc Sulfate + Water

| | *T*/K | | |
|---|---|---|---|
| tie line | 283.15 | 298.15 | 313.15 |
| 1 | −1.66 | −1.81 | −1.94 |
| 2 | −1.73 | −1.72 | −1.89 |
| 3 | −1.58 | −1.72 | −1.73 |
| 4 | −1.60 | −1.79 | −1.79 |
| 5 | −1.63 | −1.71 | −1.85 |

added until a saturation point after which no more entropy gain may be attained and phase splitting becomes more favorable.

The ATPS composed of PEG6000 + $Na_2SO_4$ present a larger biphasic area as compared to the PEG6000 + $Li_2SO_4$ system. This behavior may be explained by the fact that fewer sodium cations are required for saturating the interaction sites of the polymer chain as compared to lithium cations.

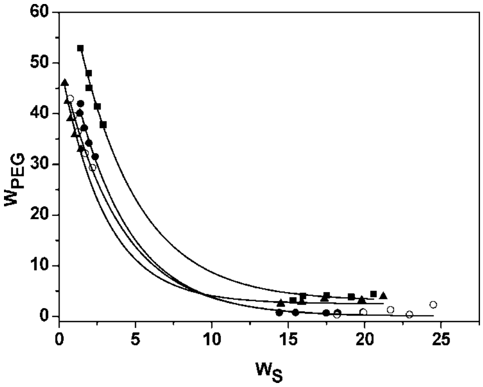

**Figure 1.** Influence of cation on the phase diagram (mass %) of the PEG6000 + salt at 298.15 K: ■, $Li_2SO_4$; ●, $Na_2SO_4$; ○, $MgSO_4$; ▲, $ZnSO_4$.

## Conclusions

Liquid−liquid equilibrium data for the systems PEG6000 + $Li_2SO_4$ + water, PEG6000 + $Na_2SO_4$ + water, PEG6000 + $MgSO_4$ + water, and PEG6000 + $ZnSO_4$ + water were determined at different temperatures ranging from (283.15 to 313.15) K. The effect of temperature on the equilibrium data of the PEG6000 + sulfate salts system was not significant. The results agreed well with those in other literatures.[8,12,13] The slope of the tie line increased with increasing temperature, and efficacy of the salts in inducing phase segregation follows the order of: $ZnSO_4 > MgSO_4 > Na_2SO_4 > Li_2SO_4$.

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Received for review June 18, 2008. Accepted August 13, 2008. The authors acknowledge the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) for their financial support.

JE800443P