### **Accepted Manuscript**

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PII: S0378-3812(16)30339-9

DOI: 10.1016/j.fluid.2016.07.009

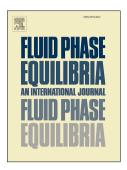
Reference: FLUID 11181

To appear in: Fluid Phase Equilibria

Received Date: 4 April 2016 Revised Date: 6 July 2016 Accepted Date: 7 July 2016

Please cite this article as: K. Wysoczanska, E.A. Macedo, Effect of molecular weight of polyethylene glycol on the partitioning of DNP-amino acids: PEG (4000, 6000) with sodium citrate at 298.15 K, *Fluid Phase Equilibria* (2016), doi: 10.1016/j.fluid.2016.07.009.

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#### ACCEPTED MANUSCRIPT

# Effect of Molecular Weight of Polyethylene Glycol on the partitioning of DNP-amino acids: PEG (4000, 6000) with sodium citrate at 298.15 K

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Keywords: ATPS, phase diagrams, DNP-amino acids, citrate salts, PEG

#### **Abstract**

Two different polyethylene glycol (PEG) – salt aqueous two-phase systems (ATPSs) were studied, at T=298.15 K. Different polymer molecular weights, 4000 and 6000, were used to form the biphasic systems. The binodal curves and the tie-lines were determined using analytical methods commonly studied in the literature, such as cloud point method, conductivity and gravimetric analysis. The partition coefficients of four DNP-amino acids, in six different tie-lines, were determined spectrophotometrically. Studies on the behaviour of (liquid-liquid) phase equilibrium and partitioning showed that, in the range of the studied polymer's molecular weight, the system formed by PEG with higher molecular weight presents higher efficiency. The influence of PEG's molecular weight on the partitioning process was considered in terms of the relative hydrophobicity of the phases, using the  $\Delta G^*(CH_2)$  calculations.

#### 1. Introduction

Aqueous two-phase systems (ATPS) are of particular interest for the biotechnological industry as it is a promising and still developing method for the separation of biomolecules, in a gentle and environmentally friendly condition [1,2,3]. Polyethylene glycol, being a biodegradable, low-toxicity and relatively inexpensive component, is a widely chosen polymer to form ATPS. It also presents interesting properties such as low melting point and very low volatility, when compared to other, commonly used, toxic organic solvents [4,5].

In order to specify the range of possible system applications, (liquid-liquid) equilibrium data should be provided. There are some studies in the literature on the influence of the polymer molecular weight on the phase compositions and partitioning in polymer-salt ATPSs [6,7,8].

It has been reported that PEG combined with salts can form very adequate ATPS, for the separation of various compounds – amino acids [9,10], proteins [8,11], enzymes [7,12], antibiotics [13,14,15], among others. Widely studied salts for ATPSs with PEG are phosphates [8,11,12] and sulfates [9,10,13,14]. However at an industrial level, they can cause environmental pollution.

In order to characterize the ATPS and compare different ATPSs, the solvent feature, which is the relative hydrophobicity of the equilibrium phases  $\Delta G^*(CH_2)$  can be investigated [16]. The free energy

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