

## Relative hydrophobicity of (PEG or Ucon)-salt ATPSs

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### Abstract

Aqueous Two-Phase Systems (ATPSs) are biphasic systems composed mainly by water. ATPSs are obtained upon mixing of two aqueous solutions of certain polymers or a polymer and a salt (above certain critical conditions, e.g. concentration, temperature). These systems are commonly indicated for the extraction of biomolecules. In this work, the partition coefficients for a series of five dinitrophenylated amino-acids (ranging from glycine to amino-caprylic acid) were determined experimentally in five different polymer-salt ATPSs (polymers: PEG or Ucon; salts: Na<sub>2</sub>SO<sub>4</sub>, Li<sub>2</sub>SO<sub>4</sub> or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) at 23°C. Values of the free energy of transfer of a methylene group,  $\Delta G(CH_2)$ , for the five ATPSs were obtained from the partition coefficients and compared with  $\Delta G(CH_2)$  previously obtained for PEG-Na<sub>2</sub>SO<sub>4</sub> (Rodríguez et al., 2007). Ucon-salt ATPSs presented higher values of  $\Delta G(CH_2)$  than the corresponding PEG-salt systems, which indicates that the Ucon-rich phase is more hydrophobic than the PEG-rich phase.

### 1 Introduction

Aqueous Two-Phase Systems (ATPSs) are biphasic systems composed mainly by water. ATPSs are obtained upon mixing of two aqueous solutions of certain polymers or a polymer and a salt (above certain critical conditions, e.g. concentration, temperature). These systems are regarded as a powerful extraction technique for biotechnology because each phase is predominantly composed of water (> 80%) and provide a gentle environment for the partitioning of sensitive biomaterials. For over 40 years ATPSs have been used for the purification and recovery of biological solutes such as proteins, amino acids, lipids, nucleic acids, viruses, plant and animal cells, etc. In the last 10 years ATPSs formed by thermo-separating polymers have been successfully used for the separation and purification of different types of biomolecules with considerable advantages in the recycling of the polymers (Pereira et al. 2003). Thermo-separating polymers, such as Ucon, are polymers whose solubility in water changes drastically with temperature and thus, can be easily recovered from the aqueous solution (and re-used).

Although the potential of ATPSs is well known, the wide use of this technology is partially limited by the lack of a clear comprehension of the factors and mechanisms that govern the distribution of solutes in ATPSs. Therefore, all attempts to obtain more information that allows understanding and predicting the behaviour of a solute are extremely important.

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Among the studied solvent properties of ATPSs, the free energy of transfer of a methylene group between the coexisting phases,  $\Delta G(CH_2)$ , has been shown (Zaslavsky, 1995; Willauer et al., 2002) to be useful for the characterization of ATPSs.  $\Delta G(CH_2)$  represents the affinities of the phases for a  $CH_2$  group and is considered as a measure of the relative hydrophobicity of the phases. Hydrophobic interactions are critical for protein stability and thus, may play an important role in the partitioning of proteins in ATPSs.

In this work,  $\Delta G(CH_2)$  for five different polymer-salt ATPSs are obtained from the experimental partition coefficients (K) of a series of dinitrophenylated amino-acids (DNP-amino-acids) at 23 °C.

## 2 Experimental

**Preparation of ATPSs:** Biphasic systems were prepared at 23 °C mixing suitable amounts of water and stock solutions of the polymers (PEG or Ucon) and the salts ( $Na_2SO_4$ ,  $Li_2SO_4$  or  $(NH_4)_2SO_4$ ) in eppendorf tubes. The total weight of all systems was approximately 1.2 g.

**Partition coefficients:** The partition coefficients of the five DNP-amino-acids (*N*-(2,4-dinitrophenyl)glycine, *N*-(2,4-dinitrophenyl)-L-alanine, *N*-(2,4-dinitrophenyl)-DL-*n*-valine, *N*-(2,4-dinitrophenyl)-DL-*n*-leucine and *N*-(2,4-dinitrophenyl)-DL- $\alpha$ -amino-*n*-caprylic acid) were obtained adding different amounts of a given DNP-amino-acid stock solution (from 0 to 100 mg) to six replicates of the ATPSs with the same feed composition (table 1). The corresponding amount of water (ranging from 100 to 0 mg) was also added to keep all compositions constant except the solutes. The components were vortex mixed for 2 minutes and then centrifuged at  $10^4$  r.p.m. for 15 minutes to achieve phase separation. Samples of each phase were withdrawn, conveniently diluted, and their absorbance at 362 nm was measured. Partition coefficients were calculated as the slope of the straight line obtained when comparing the concentration (mg/mL) in the polymer-rich phase against that in the salt-rich phase, corrected with the corresponding dilution factor (DF):

$$K = \frac{Abs(polymer) \times DF_{polymer}}{Abs(salt) \times DF_{salt}} \quad (1)$$

Table 1: Tie-line feed compositions for partitioning experiments, in mass fraction

System	Salt	Polymer
PEG- $Na_2SO_4$	0.0727	0.1186
PEG- $Li_2SO_4$	0.1000	0.1400
PEG- $(NH_4)_2SO_4$	0.1100	0.1250
Ucon- $Na_2SO_4$	0.0413	0.1676
Ucon- $Li_2SO_4$	0.0570	0.1200
Ucon- $(NH_4)_2SO_4$	0.0650	0.1260

## 3 Results and Conclusions

The phase diagrams of the biphasic systems used in this work were previously obtained experimentally at 23°C: PEG-  $Na_2SO_4$  (Rodríguez et al., 2007), PEG- $Li_2SO_4$ , Ucon- $Na_2SO_4$ , Ucon- $Li_2SO_4$  (Silvério et al., 2008) and Ucon- $(NH_4)_2SO_4$  (Pereira et al., 2004). The phase diagram for the system PEG- $(NH_4)_2SO_4$  was obtained from the literature (Zaslavsky, 1995).

The logarithms of the partition coefficients ( $\ln K$ ) as a function of the average number of equivalent methylene groups,  $n(\text{CH}_2)$ , in the aliphatic side-chain of the homologous DNP-amino-acids are shown in figure 1.

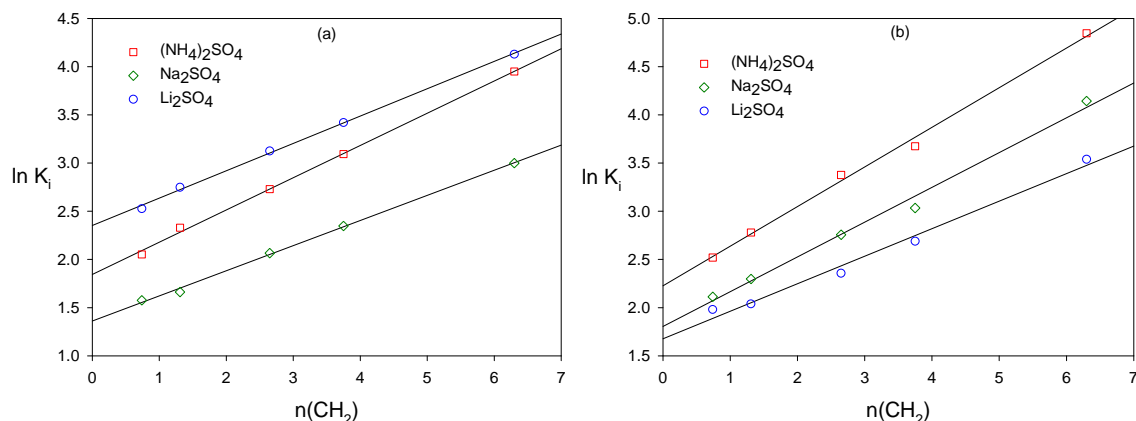


Figure 1: Logarithm of partition coefficients for a series of DNP-amino-acids in (a) PEG-salt and (b) Ucon-salt ATPSs at 23 °C. Lines are linear regressions of experimental data.

The linearity observed in figure 1 is described as:

$$\ln K = C + E \times n(\text{CH}_2) \quad (2)$$

where  $C$  and  $E$  are constants which have been previously described in detail (Zaslavsky, 1995). Parameter  $E$  is a measure of the difference between the different affinities of the two phases for methylene groups, i.e., a measure of the relative hydrophobicity of the phases. Both parameters  $C$  and  $E$  were obtained by linear regression. Parameter  $E$  is related to the free energy of transfer of a methylene group between the phases:

$$\Delta G(\text{CH}_2) = -R \times T \times E \quad (3)$$

with  $R$  being the universal gas constant and  $T$  the absolute temperature.

$\Delta G(\text{CH}_2)$  values calculated from the partition coefficients of the five DNP-amino-acids in the five polymer-salt ATPSs using equations (2) and (3) (Zaslavsky, 1995) are presented in Table 2 together with values from literature (Rodríguez et al., 2007) for comparison purposes.

Table 2: Comparison of  $\Delta G(\text{CH}_2)$  for six ATPSs at 23°C

System	$-\Delta G(\text{CH}_2)$ , kcal/mol
PEG- $\text{Na}_2\text{SO}_4$	0.153*
PEG- $\text{Li}_2\text{SO}_4$	0.167
PEG- $(\text{NH}_4)_2\text{SO}_4$	0.196
Ucon- $\text{Na}_2\text{SO}_4$	0.212
Ucon- $\text{Li}_2\text{SO}_4$	0.179
Ucon- $(\text{NH}_4)_2\text{SO}_4$	0.242

\* from Rodríguez et al. (2007).

Ucon-salt ATPSs provide higher values of  $\Delta G(CH_2)$  than the corresponding PEG-salt systems. This fact indicates that the Ucon-rich phase is more hydrophobic than the PEG-rich phase. The same conclusion can be obtained from the direct inspection of the PEG and Ucon chemical structure: PEG is an ethylene oxide homopolymer while Ucon is a random copolymer of ethylene oxide and propylene oxide monomers (larger alkyl chain).

The higher relative hydrophobicity of the Ucon-rich ATPSs suggests a more efficient biphasic separation system, with lower cross-contamination between the equilibrium phases. The use of thermo-separating polymers, like Ucon, provides thermal phase separation and simplifies the recycling of polymers. When heated above the critical temperature (about 50°C) Ucon suffers phase separation into one separate polymer enriched phase and one aqueous solution (Persson et al., 2000). This results in a more suitable ATPSs for use in large scale, continuous processes: Ucon can be recovered and recycled, with a reduction in the production costs.

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