



Biomolecules partition in aqueous (PEG + Na₂SO₄) two-phase system

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The phase diagram for the aqueous two-phase system (PEG 8000 - Na₂SO₄) was obtained experimentally at 23°C (see Figure 1). Ends of the tie-lines were measured by analysis of samples of the conjugated phases using Atomic Absorption Spectroscopy (for Na₂SO₄ composition) and lyophilization (total solutes). Partition coefficients for several proteins and amino acids were then determined experimentally by means of UV-Visible spectroscopy in different tie-lines of the above-mentioned aqueous two-phase system (see Figure 2).

The partition coefficients of all solutes under study in the different tie-lines were analyzed applying the Collander linear equation:

$$\ln(K_i) = a_{ij} \cdot \ln(K_j) + b_{ij} \quad (1)$$

where K stands for the partition coefficient of any solute and subscripts i and j refer to a given composition or tie-line of the aqueous two-phase system. Parameters a_{ij} and b_{ij} are constants obtained by linear regression. Moreover, the aqueous two-phase system was characterized by means of the relative hydrophobicity difference of the two phases, as measured by the free-energy of transfer of a methylene group. This free-energy was obtained from the partition coefficients of a series of DNP-amino acids, from DNP-glycine up to DNP-amino-caprylic acid.

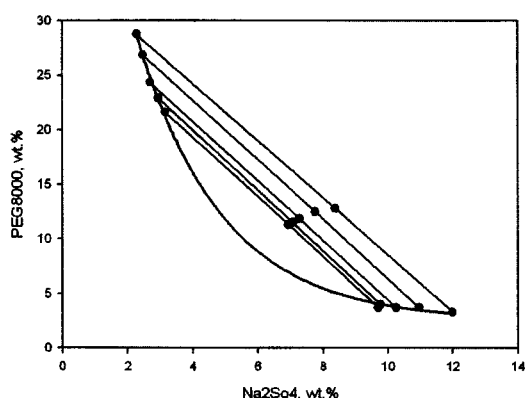


Figure 1. Phase diagram for (PEG 8000 + Na₂SO₄) aqueous two-phase system at 23°C.

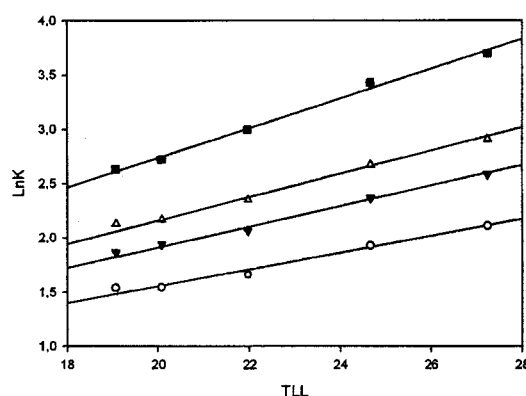


Figure 2. Partition coefficients vs. tie-line length (TLL): ■ DNP-Amino-caprylic acid; Δ DNP-Leucine; ▼ DNP-Valine; ○ DNP-Alanine.