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Solvatochromic relationship: Prediction of distribution of ionic solutes in aqueous two-phase systems

Pedro P. Madeira[a,*], Ana Bessa[a], Dragana P.C. de Barros[c,d], Miguel A. Teixeira[a], Luís Álvares-Ribeiro[b], M. Raquel Aires-Barros[c,d], Alírio E. Rodrigues[a], Arnon Chait[e], Boris Y. Zaslavsky[e]

[a] Laboratory of Separation and Reaction Engineering, Dpt. de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, s/n 4200-465 Porto, Portugal
[b] Requimte, Dep. Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre 687, 4169-007 Porto, Portugal
[c] IBB – Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
[d] Department of Bioengineering, Instituto Superior Técnico, Lisboa, Portugal
[e] Analiza, Inc., 3615 Superior Ave., Suite 4407b, Cleveland, OH 44114, USA

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ABSTRACT

Partition ratios of several ionic compounds in 20 different polymer/polymer aqueous two-phase systems (ATPS) containing 0.15 M NaCl in 0.01 M phosphate buffer, pH 7.4, were determined. The differences between the electrostatic properties of the phases in all the ATPS were estimated from partitioning of the homologous series of dinitrophenylated-amino acids. Also the solvatochromic solvent parameters characterizing the solvent dipolarity/polarizability (π^*), solvent hydrogen-bond donor acidity (α), and solvent hydrogen-bond acceptor basicity (β) of aqueous media were measured in the coexisting phases of the ATPS. The solute-specific coefficients for the compounds examined were determined by the multiple linear regression analysis using the modified linear solvation energy relationship equation. The minimal number of ATPS necessary for determination of the coefficients was established and 10 ATPS were selected as a reference ATPS set. The solute-specific coefficients values obtained with this reference set of ATPS were used to predict the partition ratios for the compounds in 10 ATPS not included in the reference set. The predicted partition ratios values were compared to those determined experimentally and found to be in good agreement. It is concluded that the presented model of solute–solvent interactions as the driving force for solute partitioning in polymer/polymer ATPS describes experimental observations with 90–95% accuracy.

1. Introduction

Aqueous two-phase systems (ATPS) are aqueous solutions of incompatible polymers or of one polymer and certain salts that phase separate when the polymer concentration exceeds a specific threshold value. Thereby two immiscible liquids are formed, largely consisting of water. In the 1950s, Albertsson [1] utilized such two-phase systems to separate different biological materials. The separation results from the different affinities of molecules or cells to the two phases. The differential affinity of a protein for the phases depends on several factors such as its surface hydrophobicity and charge, thereby enabling selective partitioning and purification of target proteins [1,2]. In contrast to many other systems of immiscible liquids, such as organic solvent–water systems, ATPS form a biocompatible environment. The samples can be recovered after partitioning, further used and analyzed.

Extraction in ATPS has been clearly demonstrated as an efficient method for large-scale recovery, purification and analysis of biomolecules and particles [1–5]. The lack of understanding of fundamental principles of partition behavior of solutes in ATPS is the main hurdle to the wide application of the method on industrial scale. Better understanding of the properties of ATPS and factors governing partition behavior of solutes and particles would be important for development of cheap and efficient biopurification processes [6].

We reported previously [7] that partitioning of proteins in polymer/polymer aqueous two-phase systems (ATPS) at fixed ionic composition may be described within the linear solvation parameter model as:

$$\log \quad K = S_s \Delta\pi^* + B_s \Delta\alpha + A_s \Delta\beta + C_s c \qquad (1)$$

where K is the solute partition ratio; $\Delta\pi^*$ is the difference between the solvent dipolarity/polarizability in the phases, $\Delta\alpha$ and $\Delta\beta$ are

* Corresponding author. Tel.: +351 220414824; fax: +351 225081674.
E-mail address: ppalma@fe.up.pt (P.P. Madeira).

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the difference between the solvent hydrogen bond donor acidity and the solvent hydrogen bond acceptor basicity in the phases, respectively; c is the difference between the solvent electrostatic properties, i.e. the solvent ability to participate in ion–dipole interactions in the phases; S_s, A_s, B_s, and C_s are solute-specific coefficients representing the relative susceptibility of the particular solute–solvent interactions to the complementary solvent properties. It should be noted that for the sake of simplicity, the terminology is different from the previously reported [8]: B_s and A_s now stand for solute hydrogen bond basicity and acidity, respectively.

The difference between the solvent electrostatic properties may be determined from partitioning of a homologous series of DNP-amino acids with different lengths of alkyl side-chains as described in [3,7,9].

In this study, we explored the minimal number of ATPS necessary for determination of four coefficients S_s, A_s, B_s, and C_s in Eq. (1) for ionic solutes, and the possibility to use these coefficients to predict partitioning of ionic solutes in ATPS with known solvent properties. Solutes with one, two and three ionized groups were examined.

2. Materials and methods

2.1. Materials

2.1.1. Polymers

Dextran 75 (lot 126567), weight-average molecular weight (M_w) ≅ 75,000 was purchased from USB (Cleveland, OH, USA). Polyethylene glycol 10000 (lot BCBB0795), M_w = 10,000; polyethylene glycol 8000 (lot 050M0215V), M_w = 8000; polyethylene glycol 6000 (lot BCBC7560), M_w = 6000; polyethylene glycol 4000 (lot BCBD2874), M_w = 4000; polyethylene glycol 1000 (lot 0001452731), M_w = 1000 and polyethylene glycol 600 (lot BCBD8607V), M_w = 600 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ucon 50-HB-5100 (lot SJ1955S3D2), M_w = 3930 was purchased from Dow-Chemical (Midland, MI, USA). Ficoll 70 (lot 10022579), M_w ≅ 70,000 was purchased from GE Healthcare Biosciences AB (Sweden). All polymers were used without further purification.

2.1.2. Solvatochromic dyes

The solvatochromic probes 4-nitrophenol (reagent grade, >98%), and 4-nitroanisole (GC, >97%) were supplied by Aldrich, Milwaukee, WI, USA. Reichardt's carboxylated betaine dye was kindly provided by Professor C. Reichardt (Philipps University, Marburg, Germany).

2.1.3. Dinitrophenylated amino acids

Dinitrophenylated (DNP) amino acids – DNP-glycine, DNP-alanine, DNP-norvaline, DNP-norleucine, DNP-DL-α-amino-n-octanoic acid (DNP-AO), were purchased from Sigma.

2.1.4. Other chemicals

O-phthaldialdehyde (OPA) reagent solution (complete) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All salts and other chemicals used were of analytical-reagent grade.

2.2. Methods

2.2.1. Solvatochromic studies

The ATPSs of the compositions shown below (see in Table 1) were prepared as previously described [7,10]. The phases were separated and used for solvatochromic analysis. The solvatochromic probes 4-nitroanisole, 4-nitrophenol and Reichardt's carboxylated betaine (the carboxylated form of the dye of 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridinio)phenolate) were used to measure the dipolarity/polarizability π^*, H-bond acceptor (HBA) basicity β, and H-bond donor (HBD) acidity α in both phases of each particular ATPS using the technique previously described [8,9].

The results of the solvatochromic studies were used to calculate π^*, β and α as described by Marcus [11].

a) Determination of the solvent dipolarity/polarizability π^*

π^* was determined from the wave number ($\nu_{(1)}$) of the longest wavelength absorption band of the 4-nitroanisole dye using the relationship:

$$\pi* = 0.427(34.12 - \nu_{(1)}) \quad (2)$$

b) Determination of the solvent hydrogen-bond acceptor basicity β

β values were determined from the wave number ($\nu_{(2)}$) of the longest wavelength absorption band of the 4-nitrophenol dye using the relationship:

$$\beta = 0.346(35.045 - \nu_{(2)}) - 0.57\pi* \quad (3)$$

c) Determination of the solvent hydrogen-bond donor acidity α

α values were determined from the longest wavelength absorption band of Reichardt's betaine dye using the relationship:

$$\alpha = 0.0649E_T(30) - 2.03 - 0.72\pi* \quad (4)$$

The $E_T(30)$ values are based on the solvatochromic pyridinium N-phenolate betaine dye as probe, and are obtained directly from the wavelength (λ, nm) of the absorption band of the carboxylated form, as

$$E_T(30) = \frac{1}{0.932(28,591/\lambda - 3.335)} \quad (5)$$

2.2.2. Partitioning

Solutions of each compound were prepared in water at concentrations of 1–5 mg/mL. Varied amounts (e.g. 0, 10, 20, 30, 40, and 50 μL) of a given compound solution and the complementary amounts (e.g. 100, 90, 80, 70, 60 and 50 μL) of water were added to a set of the same polymer/buffer/salt mixtures using a Multipette Xstream pipette (Eppendorf, Hamburg, Germany). The ATPS prepared had the compositions as used for the solvatochromic studies with the final polymer compositions given in Table 1 in the presence of NaCl 0.15 M and phosphate buffer, 0.01 M, pH 7.4. Systems were vortexed and centrifuged for 30–60 min at 10,000 × *g* in a minispin centrifuge (Eppendorf) to accelerate phase settling. Aliquots of 20–70 μL from the upper and lower phases were withdrawn with a Multipette Xstream pipette in duplicate for analysis. Two aliquots from both phases were diluted with water up to 250 μL in microplate wells (in the case of free amino acids the aliquots were combined with 250 μL of OPA solution). Following moderate shaking at room temperature (23 °C), a synergy-2 UV–vis plate reader (fluorescence plate reader with a 360 nm excitation filter and a 460 nm emission filter for the free amino acids) (Bio-Tek Instruments) was used to measure optical absorbance at maximum wavelength of each compound. Phases of blank systems at corresponding dilutions were measured for comparison. Calibration of OPA assay for each compound in both phases was performed in a similar manner.

The partition ratio, K, is defined as the ratio of the compound concentration in the upper phase to the compound concentration in the lower phase. The partition ratio value for each solute was determined as the slope of the plot of the solute concentration in the upper phase as a function of the solute concentration in the bottom phase obtained from six partition experiments carried out at different concentrations of the solute and at the fixed composition