Partition Coefficients of Proteins in Poly(ethylene glycol) + Dextran + Water at 298 K

Ljudmila Fele and Maurizio Fermeglia*†‡

National Institute of Chemistry, Hajdrihova 19, P.O. Box 30, 61115 Ljubljana, Slovenia and DICAMP, Department of Chemical, Environmental and Raw Material Engineering, University of Trieste, Piazzale Europa 1, I-34127 Trieste, Italy

The partition coefficients of lysozyme and bovine serum albumin in poly(ethylene glycol) + dextran + water at 298.15 K are reported. The investigation focuses on the variation of protein coefficients with pH and total polymer concentration at constant temperature and constant polymer molecular weight. In addition, liquid–liquid equilibrium for the water polymer systems at different total polymer concentration as well as the difference in density between the top and bottom phases has been measured.

Introduction

Aqueous two-phase extraction for the purification of proteins and other biopolymers is a method which is being increasingly used and whose applications are becoming numerous (Walter et al., 1985). The method is based on the uneven partitioning that occurs when a protein is added to a phase-separated aqueous solution of incompatible polymers. An example of such a system is poly(ethylene glycol) (PEG) + dextran (Dx) + water (Albertsson, 1986), but many other systems of incompatible polymers show the same behavior. In particular there is a strong impetus to replace dextran (and PEG) with another polymer that has the same separation characteristics but is less expensive and more easily available in large quantities.

Some examples of possible alternative polymers are amylose, amylopectin, and agarose. Medin and Jonson (1993) have investigated agarose + gelatin, agarose + PEG, agarose + poly(1-vinyl-2-pyrrolidone), agarose + poly(vinyl alcohol), agarose + polysaccharide, and agarose + poly[1–6-glucan] systems.

Experimental data on protein partitioning in the two-phase aqueous systems and on phase equilibrium data in polymers are essential for the prediction of biomolecule distribution coefficients as a function of system parameters such as protein concentration, molecular weight and type of polymer, pH, and temperature. Also, liquid–liquid equilibrium (LLE) data for the water + polymer systems are very interesting for the development of models for describing how the phase behavior is modified by the polymer molecular weight, system temperature, and total polymer concentration. Many results have been published since the pioneering work of Albertsson, allowing us to establish a complete data base for PEG + Dx + water. In particular, the papers of Fordini et al. (1991, 1992) are very good examples of a complete and reliable phase equilibrium investigation for the above-mentioned system; the effect of temperature, molecular weight, and total polymer concentration on the phase equilibria and on protein partitioning is described. Density and viscosity data for concentrated aqueous solutions of PEG have been recently measured by Gonzalez et al. (1994).

The present investigation is a preliminary study of a wide project that has the final goal of measuring, correlating, and predicting partition coefficients of proteins in novel aqueous two-phase systems. In this respect our main goal in this paper is to tune the experimental procedure and apparatus and to contribute to the establishment of a data base of partition coefficients of proteins in the PEG + Dx + water system at 298.15 K. The peculiar aspect of this investigation is to provide a complete set of measurements (densities of the two liquid phases, phase equilibrium, and partition coefficients) which can be used in the development of a model for the description of phase equilibria of the aqueous two-phase system and the protein distribution coefficient. The importance of the complete set of data is related to the fact that one of the primary driving forces for protein partitioning in aqueous two-phase systems is the polymer concentration difference between the top and the bottom phases which is strictly related to the size and shape of the binodal curve in the isothermal liquid–liquid phase equilibria. The difference in PEG and Dx concentration in the top and bottom phases (Δ[PEG] and Δ[Dx]) is related to the partition coefficient as follows:

\[
\ln K_p \propto A_{Dx-Protein} \Delta[Dx] - A_{PEG-Protein} \Delta[PEG] \quad (1)
\]

where \(K_p\) is the protein partition coefficient and \(A_{Dx-Protein}\) and \(A_{PEG-Protein}\) are interaction coefficients between the protein and Dx and the protein and PEG, respectively (Edmonds and Ogston, 1968).

Experimental Section

Materials. Poly(ethylene glycol) (nominal \(M_w = 20,000\); number average \(M_w = 19,081\); mass average \(M_w = 21,043\)) and dextran (nominal \(M_w = 50,000\); number average \(M_w = 88,255\); mass average \(M_w = 216,810\)) were both supplied by Fluka. Stock aqueous solutions of PEG (mass fraction of ~50%) and Dx (mass fraction of ~30%) were prepared using deionized water. The PEG concentration in the stock solution was checked by the freeze-drying method (dry mass), and the Dx concentration in the stock solution was checked both by polarimetry and by the freeze-drying method. The proteins albumin from bovine serum (BSA) and lysozyme from hen egg white were obtained from Fluka.

Procedure. For each measurement a tube containing the aqueous two-phase system was prepared by adding a buffer solution to the stock solutions of PEG and Dx. The order in which the stock solutions were added was Dx, PEG, 0.2 g of chloroaceticamide (preservative), 0.8 g of buffer
Results and Discussion

All the results reported refer to the PEG + Dextran + Water system at 298.15 K and at atmospheric pressure. The pH of the system was controlled by the buffer solution. Table 1 reports a comparison between LLE results measured in this work and literature values (Forciniti et al., 1991). The agreement is fair, considering the relative difficulty associated with exactly reproducing the experimental conditions in terms of the purity of the compounds and feed concentration. With a view toward investigating the relationship between the tie line length of the LLE and the difference in density between the top and the bottom phases, density measurements for the two binary systems PEG + Water and Dextran + Water were performed and reported in Table 2 and plotted in Figure 1. The tie line length (TLL) is defined by the following expression:

\[
TLL = \left| \left( D_{\text{PEG}} - D_{\text{Dx}} \right)^2 + \left( P_{\text{PEG}} - P_{\text{Dx}} \right)^2 \right|^{1/2}
\]

where \( D_{\text{PEG}} \), \( D_{\text{Dx}} \), \( P_{\text{PEG}} \), and \( P_{\text{Dx}} \) are the Dextran and PEG mass fractions and densities in the top and the bottom phases, respectively. The TLL, expressed in mass fraction, is thus directly related both to the total polymer mass fractions and to the interactions between the two polymers.

Table 3 shows the small effect of pH on the LLE. In contrast, the effect of pH on the protein partition coefficients is not negligible. Forciniti et al. (1991) considered the effect of temperature and polymer molecular weight on the LLE in terms of the shape of the binodal curves and slope of the tie lines. Their conclusion is that the effect of temperature on the LLE is negligible, especially when the temperature is relatively low and the polymer has a relatively high molecular weight, while the effect of the polymer molecular weight is important only at low molecular weight. The polymer molecular weights considered in this investigation are high enough to assure constancy of the results even at different molecular weights of the polymers as well as at different temperatures.

A comparison between partition coefficients of proteins in PEG + Dextran + Water at 298.15 K measured in this work and some literature results for the same systems (Forciniti et al., 1992) is shown in Table 4. The good agreement obtained allowed us to proceed to the experimental determination of partition coefficients at different conditions.

Table 5 reports the isothermal LLE experimental data measured and the value of the partition coefficients \( K_p \) measured in this work as a function of the tie line length.
at different values for the pH. No data have been reported for BSA at pH > 9 because of protein denaturation. Figure 2 shows the partition coefficients for BSA as a function of pH at different TLLs while Figure 3 refers to lysozyme data. The values of the partition coefficients are influenced by the pH, and at fixed pH, they decrease with decreasing TLL, as expected. Furthermore, the variation of $K_p$ with pH is influenced by the TLL.

Figure 4 shows that the ratio of the partition coefficients, i.e., the selectivity of the separation, are strongly influenced by both pH and TLL. Finally, Figure 5 shows the correlation between the TLL and the difference in density between the top and the bottom phases.

### Conclusions
The experimental methods and the procedure described in this work have been proved to be valid for the determi-
nation of the volumetric and equilibrium properties that might be necessary for the investigation of the feasibility of the protein partition in aqueous two-phase systems.

The partition coefficient of the proteins considered and their selectivity are functions of the tie line length, which is related to the total polymer concentration and the pH. By modification of those variables it should be possible to achieve the desired degree of separation for the proteins.

In order to give a quantitative answer to the problem of the separation of proteins, a model for the description of the process is necessary. The LLE results of PEG + Dx + water will be very useful for modeling phase equilibria in different conditions and calculating the TLL. The protein partition data will be used to calculate parameters of a semiempirical model able to give the partition coefficient as a function of pH and the TLL.

Figure 5 shows that the difference between the density of the top and the bottom phases is linearly related to the TLL. This observation, if confirmed by further measurements on different systems, will allow us to estimate the TLL directly from density data, which are more accurate and easier to measure than composition data.

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