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

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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 in order to facilitate industrial application of this technique. Some examples of possible alternative polymers are amylose, amylopectin, and agarose. Medin and Janson (1993) have investigated agarose + gelatin, agarose + PEG, agarose + poly(1-vinyl-2-pyrrolidone), agarose + poly(vinyl alcohol), agarose + polysucrose, 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 Forciniti 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 concentrations in the top and bottom phases (Δ[PEG] and Δ[Dx]) is related to the partition coefficient as follows:

$$\ln K_p \propto A^{\mathrm{Dx-protein}}\,\Delta[\mathrm{Dx}] - A^{\mathrm{PEG-protein}}\,\Delta[\mathrm{PEG}] \quad (1)$$

where K_p is the protein partition coefficient and $A^{\mathrm{Dx-protein}}$ and $A^{\mathrm{PEG-protein}}$ 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 = 500 000; 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 chloroacetamide (preservative), 0.8 g of buffer

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Table 1. Liquid−Liquid Equilibrium Mass Fractions (w) for PEG (1) + Dx (2) + Water (3) at 298.15 K: Comparison with Literature Data (Forciniti et al., 1991) for Two Different Tie Lines

source	$100w_1$ (initial)	$100w_2$ (initial)	$100w_1$ top	$100w_1$ bottom	$100w_2$ top	$100w_2$ bottom
this work	4.31	6.79	7.9		0.22	15.32
	7.41	10.38	13.8		0.02	25.81
literature	4.37	6.75	7.2	0.4	0.31	14.63
	7.64	10.29	13.1	0.7	0.04	23.31

A [6.4 g of citric acid + 3.84 g of phosphoric acid + 3.54 g of boric acid + 343 cm^3 of sodium hydroxide solution (1 mol/L) and enough water to make up 1 L], and 0.6 g of buffer B [HCl (0.1 mol/L)], as described by Forciniti et al. (1991). A Mettler balance, with a precision of 3×10^{-5} g, was used to prepare four different total polymer concentration samples by varying the amount of PEG and Dx for each pH value. The pH values considered in this work ranged from 7.5 to 10.6. The systems were gently shaken for 20 min in a thermostated oven at 298.15 K and then were allowed to settle for 48 h to achieve phase separation. The phases were then centrifuged for 20 min at 3000 rpm. A pipet was used to remove the top phase, while the bottom phase was removed through the hole at the bottom of the tube. The reproducibility of the total polymer concentration measurements in mass fraction was 0.4%.

The analytical methods used for the determination of the concentrations in the two liquid phases were different for the two polymers: Dextran concentration was determined by polarimetry (Perkin Elmer 241) with a Hg lamp at 365 nm with a reproducibility in mass fraction of 1%, while PEG concentration was determined by dry mass (freeze-drying method) with a reproducibility in mass fraction of 5%.

Protein partitioning was performed in 10 mL tubes. The procedure was the same as described above with the addition of 1 g of protein stock (1 g/L). Special care was taken in shaking the system in order to allow distribution of the protein between the two phases, but also to prevent protein denaturation. Protein concentration was determined by UV/vis spectroscopy at 280 nm. The pH was measured in each phase after the introduction of the protein stock solution by means of a microelectrode. Ten independent measurements were done for some selected systems to determine the statistical significance of the measurements, which was found to be, with the 95% confidence interval, in the range 4−5%.

The densities of each phase were measured by means of an Anton Paar U-tube oscillator densitometer (model DMA 60 with external glass cell DMA 602H) calibrated with double-distilled water and nitrogen. Details of the experimental procedure for density determination and calibration are reported elsewhere (Fermeglia and Lapasin, 1988; Fermeglia et al., 1990). The estimated precision in the density values is greater than 3×10^{-5} g·cm^{-3}.

The estimated precision of all the temperature measurements in this work is greater than 0.05 K.

Results and Discussion

All the results reported refer to the PEG + Dx + 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 relationships 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:

$$\mathrm{TLL} = [[D'' - D']^2 + [P'' - P']^2]^{1/2} \qquad (2)$$

where D', D'', P', and P'' are the Dx and PEG mass fractions 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 + Dx + 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

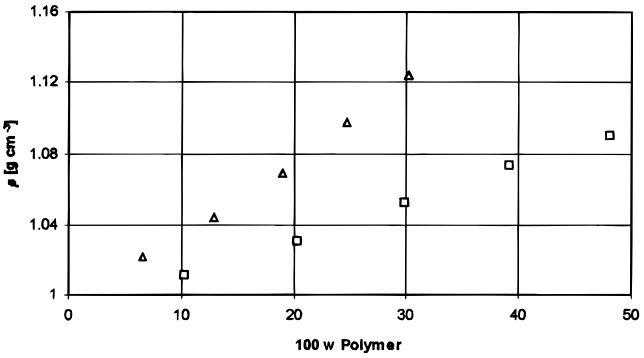

Figure 1. Density versus concentration plot for polymer + water systems at 298.15 K: (□) PEG; (△) Dx.

Table 2. Density versus Mass Fraction w for Polymer + Water at 298.15 K

polymer	$100w$	ρ/(g cm^{-3})	polymer	$100w$	ρ/(g cm^{-3})
Dx	6.62	1.0216	PEG	10.30	1.0113
	12.93	1.0442		20.25	1.0306
	18.96	1.0689		29.85	1.0526
	24.70	1.0976		39.14	1.0738
	30.20	1.1237		48.12	1.0898

Table 3. Effect of pH on Liquid−Liquid Equilibrium for PEG (1) + Dx (2) + Water (3) at 298.15 K

pH	$100w_1$ (initial)	$100w_2$ (initial)	$100w_1$ top	$100w_1$ bottom	$100w_2$ top	$100w_2$ bottom	ρ/(g cm^{-3}) top	ρ/(g cm^{-3}) bottom
9.6	4.22	7.01	7.6	−	0.22	15.56	1.0130	1.0614
7.1	4.63	7.06	8.1	−	0.17	16.79	1.0125	1.0668
5.3	4.51	6.71	7.9	−	0.18	16.41	1.0125	1.0653

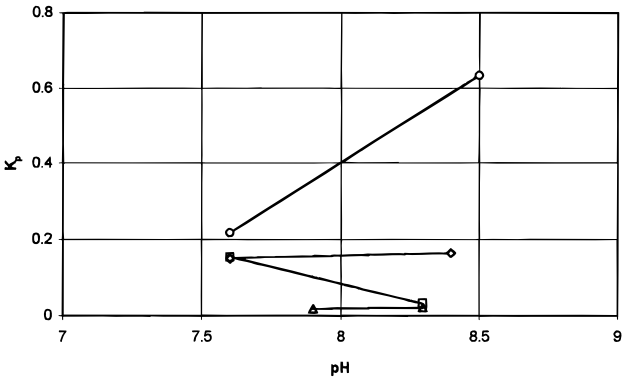

Figure 2. BSA partition coefficients as a function of pH at 298.15 K at different tie line lengths (TLL) (in mass fraction): (○) 17; (◇) 20; (□) 25; (△) 28. Lines connect points with approximately the same TLL. See the text for an explanation of TLL.

Table 4. Partition Coefficients of BSA and Lysozyme in PEG (1) + Dx (2) + Water (3) at 298.15 Comparison with Literature Data (Forciniti et al., 1992)

source	pH	$100w_1$ (initial)	$100w_2$ (initital)	partition coefficient	
				BSA	lysozyme
literature	4.6	4.37	6.75	0.21	2.1
this work	4.0	4.32	6.41		1.6
	5.2	4.32	6.41	0.24	

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.

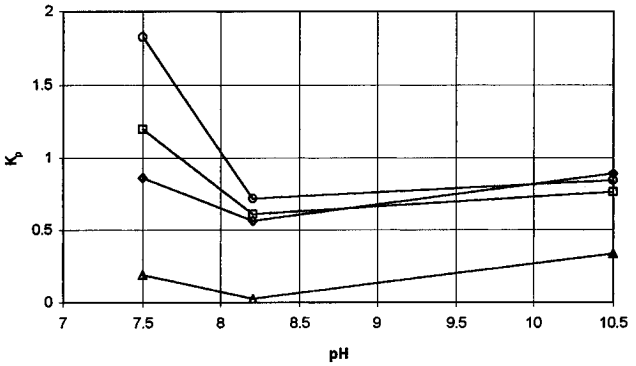

Figure 3. Lysozyme partition coefficients as a function of pH at 298.15 K at different tie line length (TLL) (in mass fraction): (○) 17; (◇) 20; (□) 24; (△) 28. Lines connects points with approximately the same TLL.

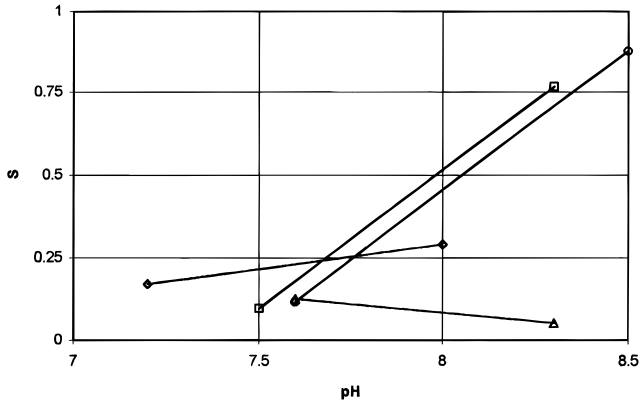

Figure 4. Selectivity ($S = K_{BSA}/K_{lysozyme}$) of the proteins as a function of pH at 298.15 K at different tie line lengths (TLL) (in mass fraction): (○) 16; (◇) 20; (□) 28; (△) 24. Lines connect points with approximately the same TLL.

Conclusions

The experimental methods and the procedure described in this work have been proved to be valid for the determi-

Table 5. Partition Coefficients K_p of BSA and Lysozyme in PEG (1) + Dx (2) + Water (3) at 298.15 K

protein	K_p	TLL	pH	$100w_1$ (initial)	$100w_2$ (initial)	$100w_1$ top	$100w_1$ bottom	$100w_2$ top	$100w_2$ bottom
		16.78	10.6	4.23	6.69	6.9	–	0.26	15.53
		17.10	10.6	4.23	6.72	7.3	–	0.14	15.58
BSA	0.63	15.38	8.5	3.56	6.98	6.9	–	0.37	14.13
	0.22	17.04	7.6	4.23	6.85	7.7	–	0.25	15.43
lysozyme	0.85	16.77	10.5	4.38	8.08	7.3	–	0.27	15.36
	0.72	16.26	8.2	4.37	8.17	7.0	–	0.29	14.94
	1.83	17.27	7.5	4.39	8.12	7.7	–	0.22	15.66
		19.77	10.6	4.87	8.44	9.2	–	0.11	18.73
		20.54	10.6	4.83	8.43	8.9	–	0.10	18.62
BSA	0.16	20.69	8.4	4.86	8.60	9.1	–	0.16	18.75
	0.15	20.47	7.6	4.84	8.59	9.2	–	0.11	18.42
lysozyme	0.89	20.60	10.5	5.02	10.22	8.9	–	0.11	18.70
	0.56	19.95	8.2	5.06	10.36	8.8	–	0.11	17.99
	0.86	20.83	7.5	5.11	10.38	9.2	–	0.09	18.80
		23.60	10.6	5.44	9.93	10.6	–	0.04	21.13
		23.68	10.6	5.42	9.74	10.5	–	0.05	21.25
BSA	0.03	25.33	8.3	6.17	10.09	11.7	–	0.05	22.52
	0.15	23.46	7.6	5.48	10.09	10.6	–	0.08	20.99
lysozyme	0.76	23.55	10.5	5.63	12.01	10.8	–	0.06	21.01
	0.61	22.09	8.2	5.64	12.03	10.4	–	0.05	19.57
	1.20	24.25	7.5	5.64	12.02	10.6	–	0.05	21.88
		27.80	10.6	7.78	10.67	11.7	–	0.08	25.31
		28.10	10.6	7.37	10.18	12.6	–	0.01	25.13
BSA	0.02	27.95	8.3	7.40	10.19	13.1	–	0.02	24.73
	0.02	28.56	7.9	7.38	10.41	13.2	–	0.03	25.34
lysozyme	0.34	26.93	10.5	7.67	12.38	12.5	–	0.03	23.87
	0.03	28.63	8.2	7.65	12.39	13.2	–	0.02	25.41
	0.19	28.34	7.5	7.66	12.37	13.1	–	0.00	25.12

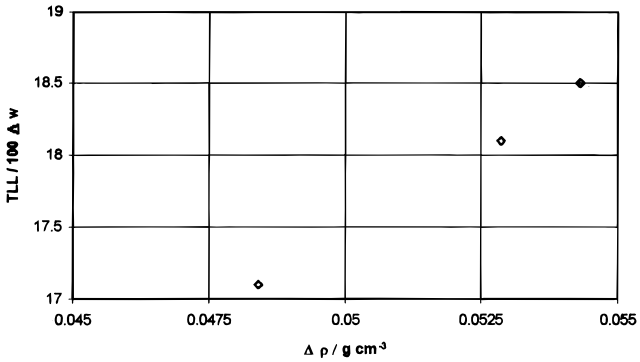

Figure 5. Tie lines length (TLL) as a function of the density phase difference between the top and the bottom phases.

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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