Protein Partitioning in PEG/Dextran Aqueous Two-Phase Systems

Utilizing a modified form of the Flory-Huggins theory of polymer solution thermodynamics, a generalized expression has been obtained for correlating protein partitioning in the polyethylene glycol (PEG)/dextran aqueous two-phase systems. The expression relates the natural logarithm of the partition coefficient of the protein to the polymer concentration difference between the phases. The parameters of the relationship are a function of protein and phase forming polymer molecular weight, protein-water, protein-polymer and polymer-water interaction parameters, and the electrostatic potential difference between the phases. The relationship was verified by partitioning 17 proteins covering a wide range of molecular weight in the tie lines of the above systems, along with data from the literature. This correlation will facilitate the selection and engineering scale-up of aqueous two-phase systems for biomolecule purification.

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Introduction

The downstream processing of biological materials requires purification techniques which are both economically feasible and delicate enough to preserve biological activity. One such bioseparation process which meets these criteria is liquid-liquid extraction using aqueous polymer two-phase systems.

Aqueous two-phase systems were first reported in the literature as early as 1896 (Beijerinck). He discovered that when appropriate combinations of water, agar, and gelatin were mixed, a turbid solution resulted which subsequently split into two aqueous phases. In 1955, P.-Å. Albertsson first applied an aqueous two-phase system consisting of polyethylene glycol (PEG), potassium phosphate, and water to the partitioning of plant cell particles (Albertsson, 1985). Albertsson's pioneering work eventually led to the application of aqueous two-phase systems to preparative biomolecule purification (Albertsson, 1986). The large-scale purification of proteins has also been successfully demonstrated by Kroner et al. (1982) and Tjerneld et al. (1987).

Aqueous two-phase systems are formed when two polymers, such as dextran (a carbohydrate) and PEG, are mixed at appropriate concentrations in the presence of water. The solvated polymers cause a phase separation: the upper phase is rich in PEG, whereas the lower phase is dextran-rich. The phases contain between 85 and 99% water, have interfacial tensions on the order of 0.0001 to 0.1 dyne/cm, and have been demonstrated to provide a protective environment for biological activity of biomolecules (Albertsson, 1986). In the other reported phase systems, the two polymers have been replaced by a polyelectrolyte and a nonionic polymer, two polyelectrolytes, or a polymer and a low molecular weight component (salt) such as the PEG/potassium phosphate/water system (Albertsson, 1986).

As discovered by Albertsson, the phases of aqueous two-phase systems offer different physical and chemical environments which allow for the selective partitioning of solutes such as proteins. The partition coefficient of a protein depends on many variables such as hydrophobicity, molecular size, electrochemistry, molecular conformation, biospecificity of the protein, as well as environmental conditions such as pH, buffer concentration, ionic strength, temperature, and protein concentration (Albertsson, 1986). Much work has been done to elucidate the effects of pH (Albertsson et al., 1970), the influence of salts (Johansson, 1970; Albertsson, 1971), hydrophobicity (Shanbhag and Axelsson, 1975; Zaslavsky et al., 1978), and biomolecule and polymer molecular weight (Hustedt et al., 1978; Albertsson et al., 1987) on protein partitioning.

Several theoretical models have been proposed for the thermodynamic behavior of aqueous two-phase systems and protein partitioning. Brooks et al. (1985) and Albertsson et al. (1987) have shown that the lattice model of Flory (1941) and Huggins

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(1941) could be used to qualitatively predict protein partition trends. Baskir et al. (1987) have modified the theory of Scheutjens and Fleer (1979, 1980) to predict protein partitioning, while King et al. (1988) extended the solution theory of Edmond and Ogston (1968) by taking into account the electrostatic potential difference between the phases. The advantages and disadvantages of the above models have been discussed in detail by Baskir et al. (1989). Gustafsson and Wennerström (1986) attempted to predict a binodial of the PEG/dextran two-phase system using Flory-Huggins theory, while Kang and Sandler (1987) attempted to analyze PEG/dextran binodials using both the Flory-Huggins theory and the UNIQUAC equation (Abrams and Prausnitz, 1975). However, a generalized correlation, which is needed to facilitate the use of aqueous two-phase systems, has not resulted.

Diamond and Hsu (1989a) presented a simple linear semilogarithmic relationship for correlating dipeptide and low-molecular-weight protein partitioning to the polymer concentration difference between the phases in the PEG 8000/Dextran T-500/water system based on Flory-Huggins polymer solution thermodynamics. However, the relationship was not applicable to high-molecular-weight proteins which exhibited nonlinear semilogarithmic partitioning trends. The purpose of this paper is to develop, based on a modified form of the Flory-Huggins theory, a generalized correlation which is applicable to proteins partitioned in PEG/dextran aqueous two-phase systems and which can be used to facilitate the engineering scale-up of aqueous two-phase systems for protein purification.

With this objective, 17 proteins covering a broad range of molecular weight up to 669,000 were partitioned in PEG 8000/Dextran T-500/water systems at 4°C. In addition, the effect of phase forming polymer molecular weight on protein partitioning was investigated by using the above systems along with PEG 8000/Dextran T-40/water, PEG 8000/Dextran T-70/water, and PEG 3400/Dextran T-500/water systems at 4°C. The effect of temperature was investigated by using PEG 8000/Dextran T-500 systems at 0, 4, 10 and 22°C. A correlation was obtained which enabled the linearization of protein partitioning data regardless of protein molecular weight or the system utilized. The correlating parameters were also shown to be a function of reciprocal absolute temperature.

Theoretical Development of Aqueous Two-Phase Systems

Brooks et al. (1985) were the first to suggest the use of the Flory-Huggins theory to describe protein partitioning in aqueous two-phase systems. The relationship they presented gave a successful qualitative description of protein partitioning, but was not applied quantitatively. To apply the Flory-Huggins theory of polymer solution thermodynamics to protein partitioning in aqueous two-phase systems, several fundamental assumptions must be made including:

1. The liquid lattice model provides an adequate representation of an aqueous solution.
2. An ideal entropy of mixing is suitable.
3. Deviations from ideal solution behavior can be simply accounted for in terms of (enthalpic) solute-solvent and solute-solute interaction parameters.
4. The polymer chains are fully flexible.

Inherent in the first three assumptions is that molecular interactions are almost exclusively of the van der Waals type. However, aqueous two-phase systems containing polymers, proteins, and salts will undoubtedly possess, among others, hydrogen bonding and ionic interactions. To help alleviate some of the problems associated with the first three assumptions, we utilize the nonionic-phase forming polymers, PEG and dextran, along with relatively low salt (buffer) concentrations. The fourth assumption presents a limitation to the description of protein structure. Many proteins tend to be globular in nature with their structures being held together by intrachain covalent bonding, hydrogen bonding, ionic interactions, and hydrophobic interactions (Lehninger, 1982). Proteins, however, are not rigid spheres and their chains still possess some degree of flexibility. Despite the above assumptions, it will be shown that the Flory-Huggins theory can be modified and used as a simple approach to describe the partition phenomenon in aqueous two-phase systems.

The chemical potential for species i, μ_i, in an aqueous polymer two-phase system may be expressed as (Albertsson, 1986):

$$\mu_i - \mu_i^o = N_A \left(\frac{\partial \Delta G_m}{\partial n_i} \right)_{n_j,T,P} + z_b F \psi \tag{1}$$

where

μ_i^o = chemical potential in the standard state, taken as the pure component species at the temperature and pressure of the mixture
ΔG_m = Gibbs free energy of mixing
N_A = Avogadro's number
n_i = number of molecules of species i
T, P = absolute temperature and pressure, respectively
z_b = net charge of the species
F = Faraday constant
ψ = electrostatic potential

Many researchers (Johansson, 1974; Zaslavsky et al., 1982; Brooks et al., 1984; King et al., 1988; Albertsson, 1986) have found that the addition of salts to aqueous two-phase systems induces an electrostatic potential difference between the phases. For this reason, the electrostatic potential term is present in the chemical potential expression. Flory's (1953) expression for the Gibbs free energy of mixing in a multicomponent polymer solution can be inserted into Eq. 1, which can then be substituted into the equilibrium relations:

$$\mu_i' = \mu_i'' \tag{2}$$

where single and double prime superscripts refer to the bottom and top phase, respectively. If the following simplifying assumptions are made (Diamond and Hsu, 1989a): 1) the biomolecule concentration is small relative to that of the polymers and water, 2) a proportionality factor, α_i, exists between the volume and weight fraction difference between the phases for species i, and 3) the parameter, ϕ, defined as the ratio of the weight fraction difference between the phases for dextran to that of PEG, is constant, we can obtain an expression for the partition coefficient of a biomolecule (component 3) in a system composed of water-salt (component 0), PEG (component 1) and dextran (component 2). It should be noted that water and salt are being treated as a single component (King et al., 1988). The partition expression is:

$$\ln(K) = m_3\left\{\left[\frac{\alpha_1}{m_1} + \frac{\alpha_2\phi}{m_2} + (\alpha_1 + \alpha_2\phi)(\chi_{03} - 1) - \alpha_1\chi_{13} - \alpha_2\phi\chi_{23} + \alpha_1\chi_{01} + \alpha_2\phi\chi_{02}\right](w_1'' - w_1') + (\chi_{02}\alpha_2^2\phi^2 - \chi_{01}\alpha_1^2)(w_1'' - w_1')^2 - 2\chi_{01}\alpha_1^2 w_1'(w_1'' - w_1') - 2\chi_{02}\alpha_2^2\phi w_2''(w_1'' - w_1') + (\chi_{12} - \chi_{01} - \chi_{02})\alpha_1\alpha_2(w_1''w_2'' - w_1'w_2')\right\} + \frac{z_bF\Delta\psi}{RT} \quad (3)$$

where $K = w_3''/w_3'$, w_i is the weight fraction of species i, m_i is the molar volume ratio of species i to that of water, R is the gas law constant, χ_{ij} represents the Flory-Huggins interaction parameter for species i and j, and $\Delta\psi$ is the electrostatic potential difference between the phases.

To further simplify Eq. 3, it is appropriate to compare the magnitude of the five terms: $(w_1'' - w_1')$, $(w_1'' - w_1')^2$, $(w_1''w_2'' - w_1'w_2')$, $w_1'(w_1'' - w_1')$, and $w_2''(w_1'' - w_1')$. Each of these terms has been plotted in Figure 1 vs. w_2'' (dextran concentration in the bottom phase) for the phase systems of the PEG 8000/Dextran T-500/water phase diagram at 4°C. It is apparent from Figure 1 that $(w_1'' - w_1')$ and $(w_1'' - w_1')^2$ are of greatest importance, while the three other terms are negligible. This trend has also been found to be true for other PEG/dextran systems. The order of significance of the absolute value of the terms was found to be $(w_1'' - w_1') > (w_1'' - w_1')^2 > (w_1''w_2'' - w_1'w_2') > w_1'(w_1'' - w_1') > w_2''(w_1'' - w_1')$. Therefore, if $(w_1'' - w_1')$ and $(w_1'' - w_1')^2$ are maintained in the partition expression, while the remaining three terms are cancelled, Eq. 3 becomes:

$$\ln(K) = m_3\left\{\left[\frac{\alpha_1}{m_1} + \frac{\alpha_2\phi}{m_2} + (\alpha_1 + \alpha_2\phi)(\chi_{03} - 1) - \alpha_1\chi_{13} - \alpha_2\phi\chi_{23} + \alpha_1\chi_{01} + \alpha_2\phi\chi_{02}\right](w_1'' - w_1') + (\chi_{02}\alpha_2^2\phi^2 - \chi_{01}\alpha_1^2)(w_1'' - w_1')^2\right\} + \frac{z_bF\Delta\psi}{RT} \quad (4)$$

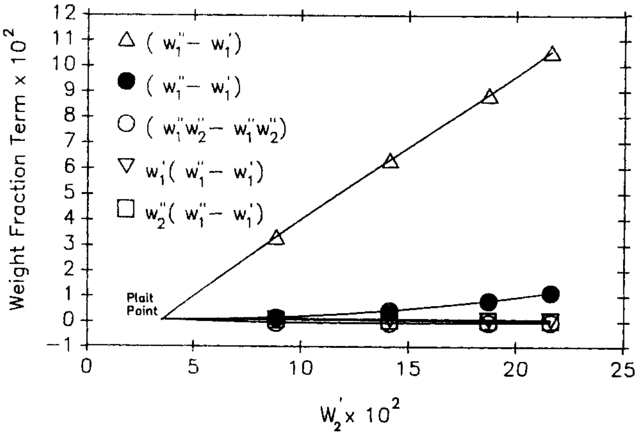

Figure 1. Comparison of weight fraction terms present in the Flory-Huggins partition expression.

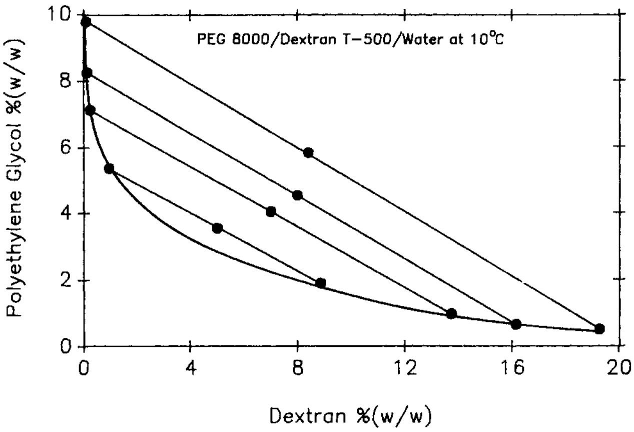

Figure 2. Phase diagram for the systems composed of PEG 8000, Dextran T-500 and water at 10°C.

Equation 4 may be simplified by making the following substitutions:

$$A^* = m_3\left[\frac{\alpha_1}{m_1} + \frac{\alpha_2\phi}{m_2} + (\alpha_1 + \alpha_2\phi)(\chi_{03} - 1) - \alpha_1\chi_{13} - \alpha_2\phi\chi_{23} + \alpha_1\chi_{01} + \alpha_2\phi\chi_{02}\right] \quad (5)$$

and

$$b = m_3(\chi_{02}\alpha_2^2\phi^2 - \chi_{01}\alpha_1^2) \quad (6)$$

Therefore, Eq. 4 becomes:

$$\ln(K) = A^*(w_1'' - w_1') + b(w_1'' - w_1')^2 + \frac{z_bF\Delta\psi}{RT} \quad (7)$$

To further simplify Eq. 7, a means is needed by which $\Delta\psi$ can be related to the PEG concentration difference between the phases, $(w_1'' - w_1')$. King et al. (1988) measured $\Delta\psi$ for various phase compositions of the PEG 3350/Dextran T-70/salt-water and PEG 8000/Dextran T-500/salt-water systems at 25°C, and plotted $\Delta\psi$ vs. the tie line length [defined as $\sqrt{(w_1'' - w_1')^2 + (w_2'' - w_2')^2}$]. For both phase diagrams, plots of $\Delta\psi$ vs. tie line length showed a linear trend, with extrapolation of the data approximately originating at the point $\Delta\psi = 0$ at tie line length equal to zero, i.e., the plait point (King et al., 1988). A different linear relationship was observed for the different salt types and concentrations. When we plotted King's data vs. the PEG concentration difference between the phases, a nonlinear trend was exhibited. Further analysis led us to correlate the electrostatic potential difference and PEG concentration difference with second-order polynomials according to the following empirical relationship:

$$\Delta\psi = g(w_1'' - w_1') + h(w_1'' - w_1')^2 \quad (8)$$

where g and h are regression constants, which are a function of salt type and concentration. These two parameters, as obtained from King's data, along with the regression coefficient for each polynomial are presented in Table 1. Substituting Eq. 8 to Eq. 7 gives:

$$\ln(K) = \left(A^* + \frac{z_b F g}{RT}\right)(w_1'' - w_1') + \left(b + \frac{z_b F h}{RT}\right)(w_1'' - w_1')^2 \quad (9)$$

At a constant pH, temperature, and pressure, the charge, z_b, of the biomolecule will be constant, and the terms, z_bFg/RT and z_bFh/RT, may be incorporated with A^* and b, respectively. The following relation is then obtained when Eq. 9 is divided by $(w_1'' - w_1')$:

$$\frac{\ln(K)}{(w_1'' - w_1')} = A^{**} + b^*(w_1'' - w_1') \quad (10)$$

where

$$A^{**} = A^* + \frac{z_b F g}{RT} \quad (11)$$

and

$$b^* = b + \frac{z_b F h}{RT} \quad (12)$$

Equation 10 represents a simple second-order semilogarithmic relationship for correlating protein partitioning in aqueous two-phase systems, where the intercept A^{**} is a function of protein and phase forming polymer molecular weight, and the protein-water, protein-polymer, polymer-water interaction parameters, pH and salt type, and concentration. Similarly, the slope b^* is a function of protein molecular weight, the polymer-water interaction parameters, pH and the salt type, and concentration. It is quite interesting that A^{**} and b^* contain χ_{01} and χ_{02}, which represent the interactions between water and PEG, and water and dextran, respectively. Zaslavsky et al. (1989) have shown that phase separation in aqueous two-phase systems results from the structural effect on water provided by the PEG and dextran. The interaction parameters χ_{01} and χ_{02} range from 0.43 to 0.51. Therefore, it is not too surprising that a general partition expression would contain the phase forming polymer-solvent interaction parameters.

Table 1. Parameters *g* and *h* of Eq. 8 Obtained from the Electrostatic Potential Data of King et al. (1988)

System	$g \times 10^{-2}$	$h \times 10^{-4}$	r^2
PEG 3350/Dextran T-70/Water with:			
50 mM K_2SO_4	0.22	0.018	0.997
100 mM K_2SO_4	0.29	0.015	0.999
50 mM KH_2PO_4	0.15	0.014	0.996
PEG 8000/Dextant T-500/Water with:			
50 mM K_2SO_4 and 100 mM K_2SO_4*	0.25	0.014	0.949
50 mM KH_2PO_4	0.11	0.020	0.991
50 mM KCl	0.038	0.0012	0.762
50 mM NaAc	0.0035	0.015	0.987

*The electrostatic potential data for these two salt concentrations were combined for the regression analysis. This was done because the two sets of data were very close to each other and overlapped at some points.

If the second-order and electrostatic potential terms of Eq. 9 had been neglected, the first-order semilogarithmic relationship presented by Diamond and Hsu (1989a) would have been obtained:

$$\ln(K) = A(w_1'' - w_1') \quad (13)$$

where

$$A = m_3\left[\frac{\alpha_1}{m_1} + \frac{\alpha_2\phi}{m_2} + (\alpha_1 + \alpha_2\phi)(\chi_{03} - 1) - \alpha_1\chi_{13} - \alpha_2\phi\chi_{23}\right] \quad (14)$$

This simplified relationship could adequately describe the partition data for dipeptides and some low-molecular-weight proteins, but was not applicable for many other high-molecular-weight proteins.

The effect of temperature on A^{**} can be obtained by considering the general form of the Flory-Huggins interaction parameters which comprise them (Flory, 1953):

$$\chi_{ij} = \frac{z\Delta w_{ij} m_i}{kT} \quad (15)$$

where Δw_{ij} is the change in energy for the formation of a contact between species i and j, z is the lattice coordinate number, and k, T and m_i have previously been defined. By introducing this definition into Eq. 10 and rearranging, A^{**} can be expressed as:

$$A^{**} = \gamma^{**} + \frac{\delta^{**}}{T} \quad (16)$$

where

$$\gamma^{**} = m_3\left[\frac{\alpha_1}{m_1} + \frac{\alpha_2\phi}{m_2} - (\alpha_1 + \alpha_2\phi)\right] \quad (17)$$

and

$$\delta^{**} = m_3\frac{z}{k}\left[(\alpha_1 + \alpha_2\phi)\Delta w_{03} - \alpha_1\Delta w_{13} m_1 - \alpha_2\phi\Delta w_{23} m_2 + \alpha_1\Delta w_{01} + \alpha_2\phi\Delta w_{02} + \frac{z_b F g}{m_3 z N_A}\right] \quad (18)$$

The parameters γ^{**} and δ^{**}, as will later be shown by experimental proof, are essentially constant. This will be demonstrated by a plot of A^{**} vs. reciprocal absolute temperature for a variety of proteins partitioned in PEG/dextran aqueous two-phase systems.

Experimental Study

Materials

Dextran T-500 (Lot 06905) was purchased from Pharmacia, Piscataway, NJ. Polyethylene glycol of molecular weight 3,400 (Lot 00304 EV) and 8,000 (Lot 02316 EV) was obtained from Aldrich Chemical Company, Milwaukee, WI.

Ribonuclease (bovine pancreas), trypsin, lysozyme (chicken egg), rhodanese (bovine liver), hexokinase (bakers yeast), invertase (bakers yeast), transferrin (human), bovine serum albumin (BSA), thyroglobulin (bovine), cytochrome c (horse heart), alcohol dehydrogenase (bakers yeast), ovalbumin (turkey egg), α-amylase (Bacillus species), lipase (wheat germ), conalbumin (chicken egg white), myoglobin (horse skeletal muscle), and protease were purchased from Sigma Chemical Company, St. Louis, MO.

Protein partitioning

The phase compositions of the PEG 8000/Dextran T-40/ water, PEG 8000/Dextran T-70/ water, PEG 8000/Dextran T-500/water, and PEG 3400/Dextran T-500/water systems at 4°C were obtained from Diamond and Hsu (1989a). The phase compositions for the PEG 8000/Dextran T-500 systems at 0°C and 22°C were obtained from Albertsson (1986) and Diamond and Hsu (1989b), respectively. The PEG 8000/Dextran T-500/ water phase equilibrium data at 10°C are given in Figure 2. The phase diagram was obtained according to the procedure presented in detail by Diamond and Hsu (1989a).

Partition experiments were performed as previously described (Diamond and Hsu, 1989a). All experiments were performed at pH 7.0 using 0.01 molal (m) potassium phosphate buffer; 10 mL of phase system was poured into 15 mL polypropylene centrifuge tubes; 10 mg of protein was added; and the tubes tightly sealed. The phases of the PEG/dextran system were allowed to settle for 24–48 hours at either 0, 4, or 10°C in a temperature-controlled refrigerator. Experiments at 22°C were permitted to settle for 24 hours in the laboratory environment. A pasteur pipet was used to collect the top phase, while the lower phase was drained from the tube by piercing a hole at its bottom.

Protein concentration was determined by diluting the phase aliquot with water and measuring absorbance by Shimadzu UV-VIS spectrophotometer, at 280 nm vs. an appropriately diluted-phase blank.

Results and Discussion

To verify the applicability of Eq. 10 for correlation of partition data, proteins covering a wide range of molecular weight were partitioned in four systems of the PEG 8000/ Dextran T-500/water phase diagram at 4°C. In Figures 3–5, ln $(K)/(w_1'' - w_1')$ for 17 proteins is plotted vs. $(w_1'' - w_1')$ according to Eq. 10. Figure 3 contains low-molecular-weight protein partitioning (lipase, cytochrome c, ribonuclease, lysozyme, myoglobin, and trypsin), while Figures 4 and 5 contain high-molecular-weight proteins. We have established the distinction between low- and high-molecular-weight proteins to be a molecular weight of 25,000. The lines drawn through the data points in each of the plots represent a first-order regression, and the A^{**} and b^* parameters, along with protein molecular weight, are recorded in Table 2.

Diamond and Hsu (1989a) had previously shown that a linear semilogarithmic relationship could be used to correlate the partitioning of some dipeptides and low-molecular-weight proteins, but high-molecular-weight proteins could not be correlated by this relation. Figures 3–5 show that Eq. 10 provides a generalized means for correlating protein partitioning regardless of molecular weight. Since several of the low-molecular-weight proteins, e.g., cytochrome c, ribonuclease, lysozyme and trypsin, exhibited a semilogarithmic linear trend when correlated according to Eq. 13 (Diamond and Hsu; 1989a), it is expected that relatively small magnitude b values (slopes) would be obtained when the data are regressed according to Eq. 10. Figure 3 and Table 2 indicate that, although the regression curves are not perfectly horizontal, the b values for these proteins are of relatively small magnitude.

To provide further proof of the applicability of our correlation, we attempted to apply it to the data obtained from the literature. In Figure 6 we have plotted the partition data of Johansson and Andersson (1984) for the proteins glucose-6-phosphate dehydrogenase and alcohol dehydrogenase. The system conditions are 25 mM sodium phosphate buffer, pH 7.0 and 0°C. It is apparent that these two proteins follow the linear trend when correlated according to Eq. 10. It is also interesting to note that the two-phase system used in their study contained more than double the salt concentration of our systems. This indicates that our correlation can be applied to systems containing appreciable quantities of salt. In a similar fashion, we have plotted, according to Eq. 10, the protein partition data from King et al. (1988) in Figure 7. The data from King et al., like

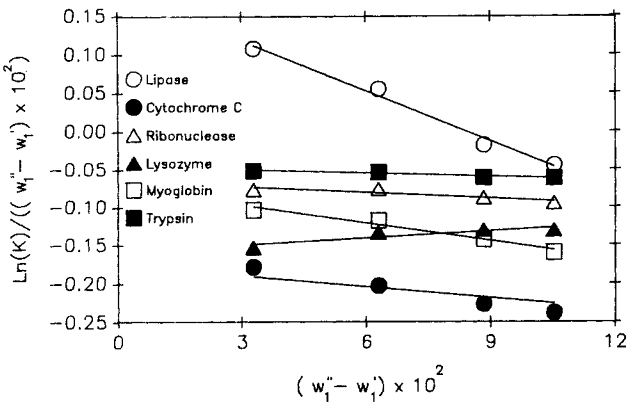

Figure 3. Correlation of low-molecular-weight protein partitioning according to Eq. 10 in four phase systems of the PEG 8000/Dextran T-500/ water phase diagram, pH 7.0, 10 m*m* potassium phosphate buffer at 4°C.

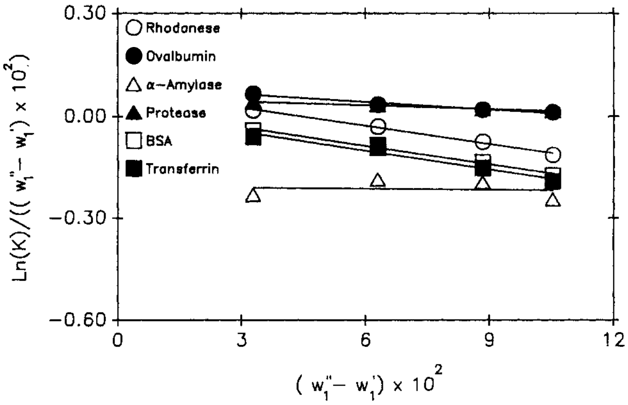

Figure 4. Correlation of high-molecular-weight protein partitioning according to Eq. 10 in four systems of the PEG 8000/Dextran T-500/water phase diagram, pH 7.0, 10 m*m* potassium phosphate buffer at 4°C.

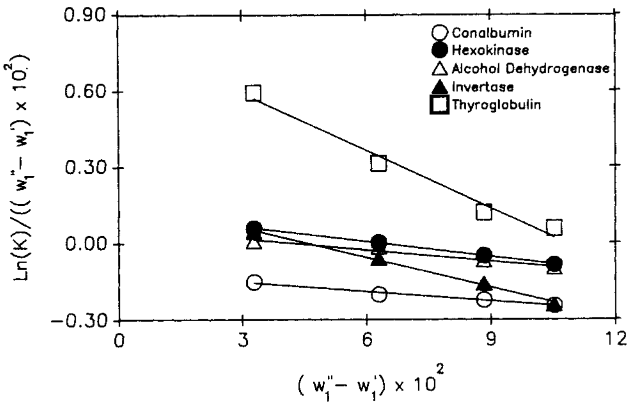

Figure 5. Correlation of high-molecular-weight protein partitioning according to Eq. 10 in four systems of the PEG 8000/Dextran T-500/water phase diagram, pH 7.0, 10 m*m* potassium phosphate buffer at 4°C.

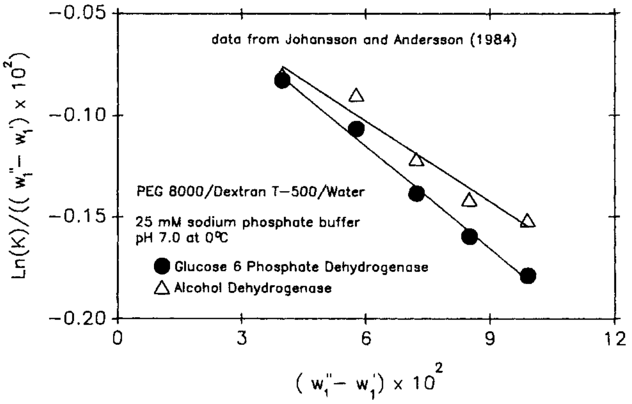

Figure 6. Correlation of protein partitioning data from Johansson and Andersson (1984) according to Eq. 10.

those of Johansson and Andersson, show a linear trend. It should also be noted that King's data contained salt concentrations five times as great as our work, indicating, once again, the applicability of our relationship in salt-containing two-phase systems.

Diamond and Hsu (1989a) demonstrated that when the low-molecular-weight proteins lysozyme and trypsin were partitioned in the PEG 8000/Dextran T-40, PEG 8000/Dextran T-70, PEG 8000/Dextran T-500, and PEG 3400/Dextran T-500 aqueous systems at 4°C, a semilogarithmic linear trend was observed, according to Eq. 13, depending on the molecular weight of the phase forming polymers. The A values for each of the proteins increased as dextran molecular weight increased or PEG molecular weight decreased. In addition, BSA was partitioned in the four systems indicated above, and a series of curves obtained when ln (K) was plotted vs. the PEG concentration difference. When the same BSA data are replotted according to the relationship of Eq. 10, each phase system yields a different trend, with the values of A^{**} being -5.8, -4.2, 2.4, and 1.9, and the b values being -199, -207, -183, and -121 for the above four systems, respectively. It can be seen that A^{**} like A increased as dextran molecular weight was increased, while it also increased as PEG molecular weight was decreased from 8,000 to 3,400.

The effect of polymer molecular weight on A^{**}, like A, is mainly due to the term $[(\alpha_1/m_1) + (\alpha_2\phi/m_2)]$. Since α_1, α_2, m_1, and m_2 are positive while ϕ is negative, then (α_1/m_1) is positive and $(\alpha_2\phi/m_2)$ is negative. Since the molar volume ratios of PEG and dextran, m_1 and m_2, are directly proportional to polymer molecular weight, then as dextran molecular weight is increased, $(\alpha_2\phi/m_2)$ becomes less negative, and A^{**} increases. Similarly, as PEG molecular weight is increased, (α_1/m_1) becomes less positive, causing a decrease in A^{**}. Both these trends were observed witt the BSA partitioning. The effect of polymer molecular weight on b^* is not as obvious as that of A^{**}. The interaction parameters χ_{01} and χ_{02} should be functions of PEG and dextran molecular weight, but these functions are not yet known. However, from the data it can be seen that b^* is

Table 2. Parameters A^{**} and b^* of Eq. 10 for Proteins Partitioned in the PEG 8000/Dextran T-500/Water Phase Systems

Protein	Molecular Weight	A^{**}	b^*
Lipase	6,669	18.1	−217.0
Cytochrome *c*	12,400	−17.7	−47.9
Ribonuclease	12,600	−6.7	−25.3
Lysozyme	13,900	−16.1	31.3
Myoglobin	16,900	−7.6	−78.0
Trypsin	23,200	−4.8	−14.8
Rhodanese	37,570	8.0	−178.0
Ovalbumin	44,000	8.7	−74.5
α-Amylase	45,000	−20.8	−10.0
Protease	48,410	5.5	−37.3
BSA	67,500	2.4	−183.0
Transferrin	77,000	1.1	−186.0
Conalbumin	86,810	−12.0	−122.0
Hexokinase	102,000	11.9	−194.0
Alc. Dehydrogenase	145,000	5.8	−147.0
Invertase	270,000	17.3	−387.0
Thyroglobulin	669,000	81.2	−753.0

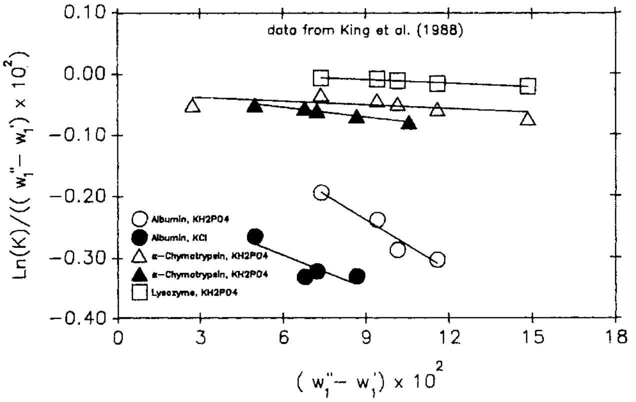

Figure 7. Correlation of protein partitioning data from King et al. (1988) according to Eq. 10.

All partition data are at 25°C. Open symbols represent partitioning in the PEG 3350/Dextran T-70/water systems. Closed symbols represent partitioning in the PEG 8000/Dextran T-500/water systems. Salt concentration was 50 or 100 mM for albumin partitioning, and 50 mM for α-chymotrypsin and lysozyme partitioning.

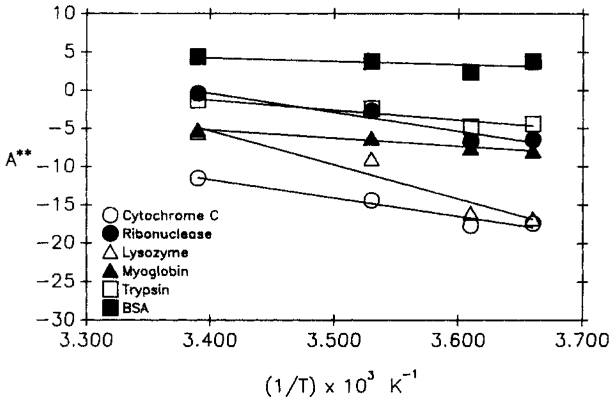

Figure 8. Parameter A^{**} as a function of temperature based on Eq. 16.

relatively constant at dextran molecular weights of 40,000 and 70,000, and then increases as the dextran molecular weight is increased to 500,000. As PEG molecular weight is increased from 3,400 to 8,000, the value of b becomes more negative.

To verify the effect of temperature on partitioning, as indicated by the relationship of Eq. 16, the proteins cytochrome *c*, ribonuclease, lysozyme, myoglobin, trypsin, and BSA were partitioned in the tie line compositions of the PEG 8000/Dextran T-500 system at 0, 4, 10 and 22°C. For each protein, a plot of $\ln (K)/(w_1'' - w_1')$ vs. $(w_1'' - w_1')$ at the four different temperatures yielded a different linear relationship and distinct value of A^{**}. In Figure 8, which summarizes the temperature, partition data for each of the proteins, A^{**} is plotted vs. $(1/T)$ according to Eq. 18. The data indicate a linear trend suggesting that Eq. 16 is useful for summarizing the variation of A^{**} over the given temperature range. It is interesting to note that, for each of the proteins in Figure 8, the data point at 4°C consistently provides some deviation from the linear trend. Although there is no definite explanation for this, it may be due to the fact that water is the densest at this temperature.

Equation 16, which was derived from a modified form of the Flory-Huggins theory, provides a simple means of correlating protein partitioning in aqueous polymer two-phase systems. The relationship contained two parameters, A^{**} and b^*, and was verified for PEG/dextran systems utilizing 17 proteins covering a broad range of molecular weight, along with protein partition data from the literature. The effect of phase forming polymer molecular weight on the parameters was investigated. The A^{**} parameter of the correlating equation was also shown to be linearly related to reciprocal absolute temperature. Equation 16 will facilitate the selection of an appropriate aqueous two-phase system for protein purification, since only two partition coefficients need to be measured to obtain the parameters of the equation and thus have a clear picture of partition trends in an aqueous two-phase system. Since the partition coefficient of a protein is independent of its concentration (Albertsson, 1986) or the volume of the two-phase system (Hustedt et al., 1985), Eq. 16 will be useful for the scale-up of aqueous two-phase systems for protein purification.

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Notation

$A = m_3[(\alpha_1/m_1) + (\alpha_2\phi/m_2) + (\alpha_1 + \alpha_2\phi)(\chi_{03} - 1) - \alpha_1\chi_{13} - \alpha_2\phi\chi_{23}]$
$A^* = m_3[(\alpha_1/m_1) + (\alpha_2\phi/m_2) + (\alpha_1 + \alpha_2\phi)(\chi_{03} - 1) - \alpha_1\chi_{13} - \alpha_2\phi\chi_{23} + \alpha_1\chi_{01} + \alpha_2\phi\chi_{02}]$
$A^{**} = A^* + z_bFg/RT$
$b = m_3(\chi_{02}\alpha_2^2\phi^2 - \chi_{01}\alpha_1^2)$
$b^* = b + z_bFh/RT$
F = Faraday constant
g = regression parameter in the relation between ψ and $(w_1'' - w_1')$
h = regression parameter in the relation between ψ and $(w_1'' - w_1')$
$K = w_3''/w_3'$
m_i = molar volume ratio of species *i* to that of water
M = molarity
m = molality
N_A = Avogadro's number
n_i = number of molecules of species *i*
P = pressure
R = gas law constant
T = absolute temperature
w_i = weight fraction of species *i*
z = lattice coordinate number
z_b = charge of a biomolecule

Greek letters

α_i = proportionality factor between volume and weight fraction for species *i*
$\gamma^{**} = m_3[(\alpha_1/m_1) + (\alpha_2\phi/m_2) - (\alpha_1 + \alpha_2\phi)]$
$\delta^{**} = m_3z/k[(\alpha_1 + \alpha_2\phi)\Delta w_{03} - \alpha_1\Delta w_{13}m_1 - \alpha_2\phi\Delta w_{23}m_2 + \alpha_1\Delta w_{01} + \alpha_2\phi\Delta w_{02} + (z_bFg/m_3zN_A)]$
ΔG_m = Gibbs free energy of mixing
Δw_{ij} = energy change for the formation of a contact between species *i* and *j*
$\Delta\psi$ = electrostatic potential difference between the phases
μ_i = chemical potential for species *i*
μ_i^o = standard state chemical potential for species *i*
$\phi = w_2'' - w_2'/ w_1'' - w_1'$
χ_{ij} = Flory-Huggins interaction parameter between species *i* and *j*
ψ = electrostatic potential

Superscripts

$''$ = top phase
$'$ = bottom phase

Subscripts

0 = water-salt
1 = PEG
2 = dextran
3 = biomolecule

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