Molecular Thermodynamics of Aqueous Two-Phase Systems for Bioseparations

Aqueous polymer-polymer two-phase systems provide a powerful method for separating biomolecules by extraction. When a complex mixture of biomolecules (e.g., a fermentation broth or a solution of lysed cells) is added to such a system, biomolecules partition uniquely between the two phases, achieving separation.

A thermodynamic framework is presented for optimizing extraction performance in biological separations. First, a molecular-thermodynamic model, based on the osmotic virial equation, is proposed to describe phase equilibria for dilute aqueous mixtures containing polymers and protein. Second, experimental phase-equilibrium data (protein partition coefficients) are reported for a number of model proteins including albumin, lysozyme, and α-chymotrypsin. To interpret and correlate the experimental data, Low-Angle Laser-Light Scattering (LALLS) measurements were made to determine osmotic second virial coefficients for aqueous mixtures containing polymers, proteins, salts (KCl, KH_2PO_4 and K_2SO_4 at concentrations of 50 and 100 mM) and several combinations of polymer-polymer and polymer-protein pairs. Combined with electrochemical measurements (differences in potential between the two phases and protein net charge), these data provide parameters for the model to calculate the desired phase equilibria. A comparison of calculated and experimental results indicates that the virial-equation model provides good prediction of binodals and a reliable basis for estimating infinite-dilution protein partition coefficients for biotechnical process design.

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Introduction

Aqueous two-phase, polymer-polymer systems provide a powerful method for separating mixtures of biomolecules by extraction. A biocompatible liquid-liquid system can arise when two polymers [e.g., poly(ethylene glycol) and dextran] are dissolved in water, with one polymer predominating in each phase (Albertsson, 1986; Walter et al., 1985). When a mixture of biomolecules [e.g., a fermentation broth or a solution of lysed cells] is added to an aqueous two-phase system, each type of biomolecule partitions uniquely between the two phases.

A wide range of biomolecules (proteins, lipids, nucleic acids, viruses and whole cells) have seen separated using this technique (Albertsson, 1986). In addition, aqueous two-phase systems have been used: for extractive bioconversions [fermentation or enzyme reaction with simultaneous product removal] (Mattiasson, 1983); for characterization of hydrophobicity (Shanbhag and Axelsson, 1975; Zaslavsky et al., 1983) and binding interactions (Shanbhag et al., 1973; Ling and Mattiasson, 1982); and for assaying a wide range of biomolecules using Partition Affinity Ligand Assay (PALA) (Ling, 1983).

Advantages of aqueous two-phase systems include: 1) high biocompatibility and low interfacial tension, since each phase contains predominantly water (75–90 wt %), thereby minimizing product degradation often observed in nonaqueous systems; 2) good resolution and yields which can be enhanced dramatically, if needed, by covalently binding affinity ligands to the phase polymers (Birkenmeier et al., 1984); 3) high capacity; 4) linear scale-up of up to 2×10^4 times that of lab scale (Kula, 1979); and 5) direct use of available chemical engineering technology (liquid-liquid extraction equipment) for industrial-scale separations (Kula, 1979).

The partition coefficient of the desired biomolecule specifies

the type of extraction equipment required (Kula, 1979; Fauquex et al., 1985). At present, industrial equipment designs are based on trial-and-error experiments. As aqueous two-phase systems become increasingly popular in a number of large-scale and analytical applications, the need for a design-oriented correlation becomes exceedingly more important. When affinity ligands are not incorporated, the major factors which influence biomolecule partitioning include the concentration, molecular weight and type of polymers used and a proposed electric-potential difference between the two phases caused by the addition of certain buffer ions (Kula, 1979). Other factors include pH, ionic strength, temperature, relative surface and interfacial tension and relative hydrophobicity (Johansson et al., 1973; Johansson, 1974; Kula, 1979; Zaslavsky et al., 1982, 1983; Brooks, 1984; and Albertsson, 1986). Much empirical research has been devoted to the effects of selected independent factors and to practical applications. However, previous workers have only studied individual effects which are important to protein partitioning, e.g., the electric-potential difference (Brooks, 1984). Moreover, each previous study has used a different set of conditions, such as salt concentration, temperature and molecular weight of polymer; little attention has been given to systematic investigation. To provide a rational method for process optimization, this work provides a molecular-thermodynamic framework for interpreting, correlating and ultimately predicting phase separation and protein partition coefficients.

To establish a design-oriented correlation, it is necessary to characterize and quantify polymer-polymer and polymer-protein interactions in aqueous systems with and without salt. The most efficient method for obtaining such data is provided by Low-Angle Laser Light-Scattering (LALLS) measurements. The data obtained are used in conjunction with an osmotic virial expansion to predict polymer-polymer-water phase diagrams (binodals) and protein partition coefficients, K_p. While a preliminary report (King et al., 1986) verified that this method can be used to predict binodals, to predict K_p, it is necessary to incorporate the effects caused by certain common buffer salts. In this work, we focus on the effects of phase polymers and buffer salts on binodals and K_p.

Thermodynamic Framework

Several theories have been proposed for thermodynamic properties of polymer solutions; the best known is the lattice model of Flory (1942) and Huggins (1942). However, most of these theories apply only to simple nonpolar systems with no specific interactions such as hydrogen bonding (e.g., Scott, 1948; Blanks and Prausnitz, 1964; Patterson, 1967; Konigsveld et al., 1968; and Kumar et al., 1987). Kim (1987), Grossman (1987), and Grossman and Gainer (1988) have proposed exclusion mechanisms to correlate protein partitioning in aqueous two-phase systems derived from one salt, one polymer and water. Kabiri-Badr et al. (1987) have attempted using fluctuation theory to describe salt-polymer aqueous two-phase systems. Although progress made in understanding the physical picture of protein partitioning in these systems is encouraging, the forces driving partitioning may be different between salt/polymer/water and polymer/polymer/water aqueous two-phase systems.

Recently, attempts have been made to modify classical theories to apply to polymer-polymer-water two-phase systems. Kang and Sandler (1987) have applied the UNIQUAC equation (Abrams and Prausnitz, 1975) to predict binodals; however, many adjustable parameters are required. McGee and Benge (1986) reviewed the use of a number of models including Flory-Huggins lattice model, Heil and Prausnitz (1966) segment-interaction model, a modified UNIFAC model proposed by Hershkowitz and Gottlieb (1985) and an osmotic virial expansion approach (Edmond and Ogston, 1968). Baskir et al. (1987) have proposed a modified lattice theory to predict protein partitioning; although progress has been made toward prediction of protein partition coefficients (Baskir et al., 1988), salt effects are not taken into account. Moreover, experimental evaluation of the parameters required for their model is quite difficult, if not impossible. That work is useful, however, because it confirms previous studies (Edmond and Ogston, 1968; Knoll and Hermans, 1983) by showing that exclusion effects are the dominant forces between nonionic polymers and proteins in aqueous solutions. Other, more sophisticated, theories (Prigogine, 1957; Yamakawa et al., 1963) are either not applicable to aqueous systems or require parameters which are difficult to evaluate; they are of little use for engineering purposes. Further progress in understanding protein partitioning in aqueous two-phase systems will require fundamental thermodynamic data.

A more suitable description (King et al., 1986; McGee and Benge, 1986) of dilute aqueous two-phase systems is provided by Edmond and Ogston (1968) who developed a theory for dilute ternary aqueous solutions of polymers (or proteins) based on the osmotic virial equation truncated at the second-virial-coefficient term. Vink (1976), Shiskov and Frenkel (1979), and Shiskov et al. (1981) have suggested that truncation of the osmotic virial equation after the third virial coefficient may be more accurate. This suggestion has been verified by Haynes et al. (1988). Recently, Mansoori and Ely (1987) outlined a method to account for the effects of polydispersity. However, in this work, to reduce the complexity of the equations and the number of parameters that must be evaluated we restrict ourselves to use of number-average molecular weights with the virial equation truncated at the second virial coefficient. For our purposes, the latter is a reasonable approximation, since we are interested in systems that are relatively dilute in polymer and highly dilute in protein.

Following Edmond and Ogston, we write the chemical potentials of polymer(2) and polymer(3) as a function of molality:

$$\Delta\mu_2 = RT(\ln m_2 + a_{22}m_2 + a_{23}m_3) \tag{1}$$

$$\Delta\mu_3 = RT(\ln m_3 + a_{33}m_3 + a_{23}m_2) \tag{2}$$

From the Gibbs-Duhem relation, the chemical potential for water (1) is then given by:

$$\Delta\mu_1 = -RTV_1\rho_1\left(m_2 + m_3 + \frac{a_{22}}{2}(m_2)^2 + \frac{a_{33}}{2}(m_3)^2 + a_{23}m_2m_3\right) \tag{3}$$

The chemical potentials are given relative to those in a standard state which is the same in both aqueous phases.

In Edmond and Ogston's notation, interaction coefficients, a_{22}, a_{33} and a_{23}, are directly related to the traditional second virial coefficients, A_{ij}, in units of mL · mol/g^2 (Tompa, 1956),

respectively, by:

$$2A_{22} = 1{,}000\,\frac{a_{22}}{(M_2)^2} \tag{4a}$$

$$2A_{33} = 1{,}000\,\frac{a_{33}}{(M_3)^2} \tag{4b}$$

$$2A_{23} = 1{,}000\,\frac{a_{23}}{M_2 M_3} \tag{4c}$$

To determine the phase diagram, Eq. 1–3 and mass-balance relations are substituted into the phase-equilibrium equations:

$$\mu_1' = \mu_1'' \tag{5a}$$

$$\mu_2' = \mu_2'' \tag{5b}$$

$$\mu_3' = \mu_3'' \tag{5c}$$

When a protein (p) is placed in an aqueous two-phase system, we have a four-component system. To predict the protein partition coefficient, K_p, we extend Edmond and Ogston's equation to:

$$\Delta\mu_2 = RT(\ln m_2 + a_{22}m_2 + a_{23}m_3 + a_{2p}m_p) \tag{6}$$

$$\Delta\mu_3 = RT(\ln m_3 + a_{33}m_3 + a_{23}m_2 + a_{3p}m_p) \tag{7}$$

$$\Delta\mu_p = RT(\ln m_p + a_{pp}m_p + a_{2p}m_2 + a_{3p}m_3) \tag{8a}$$

The Gibbs-Duhem equation then gives,

$$\Delta\mu_1 = -RTV_1\rho_1\left(m_2 + m_3 + m_p + \frac{a_{22}}{2}(m_2)^2 + \frac{a_{33}}{2}(m_3)^2 + \frac{a_{pp}}{2}(m_p)^2 + a_{23}m_2m_3 + a_{2p}m_2m_p + a_{3p}m_3m_p\right) \tag{9}$$

Equations 6–9, however, do not provide a satisfactory account of the effect of electrostatic charges. Johansson (1974) and Albertsson (1986) have shown that certain common buffer salts may affect the partition of a highly charged biomolecule; it was observed that, when added to a two-phase system, phosphates, sulfates and citrates also partition, possibly creating an electric potential difference between the phases. If the effect of an electric potential Φ is included, Eq. 8a becomes:

$$\Delta\mu_p = RT(\ln m_p + a_{pp}m_p + a_{2p}m_2 + a_{3p}m_3) + z_pF\Phi \tag{8b}$$

Incorporation of this effect introduces additional terms in Eqs. 6, 7 and 9; however, for phase-equilibrium calculations of interest here, we assume that these additional terms are approximately the same in both phases and therefore cancel in Eq. 5.

In our development, we consider salt-water as a single component. When salts are present, all polymer and biomolecule-interaction parameters and concentrations are based on the salt-water pseudosolvent. However, as previously stated, some salts also partition slightly between the phases and therefore, the standard state chemical potential, μ_i^o, is not exactly the same in both phases. For our purposes here, we assume that this small difference has a negligible effect on our phase equilibrium calculations. Moreover, although our equations do not explicitly take into account interactions between ions and the phase polymers, in principle, the interaction parameters are now also a function of the salt concentration. Fortunately, for the uncharged polymers studied here, dependence of the virial coefficient on salt concentration is negligible at the low salt concentrations considered here.

For a solution very dilute in protein, as $m_p \to 0$, where $m_p \ll m_2$ and $m_p \ll m_3$, the protein partition coefficient is given by:

$$\ln K_p = \ln\frac{m_p'}{m_p''} = a_{2p}(m_2'' - m_2') + a_{3p}(m_3'' - m_3') + \frac{z_pF(\Phi'' - \Phi')}{RT} \tag{10}$$

Since Kula (1979) has shown that the protein partition coefficient is independent of its own concentration up to approximately 30 wt. %, it is likely that this equation could be easily extended to apply to concentrated biomolecule solutions.

Experimental

Materials

Polymers. Poly(ethylene glycols) (PEG) were purchased from Union Carbide Corporation, Chemical and Plastics Division, New York, NY as Carbowax 3350 and Carbowax 8000. Dextrans were purchased from Pharmacia Inc., Piscataway, NJ as Dextran T fractions: T-70 (lot #00356); and T-500 (lot #38624).

PEG standards for estimating molecular weight were purchased from Polysciences, Warrington, PA.

Proteins. Bovine Serum Albumin (Σ # A-7030, lot #75f-0006); α-chymotrypsin (Σ # c-4129, lot #85f-8130); and Lysozyme (Σ # L-6876, lot #65f-8170) were purchased from Sigma Chemical Company, St. Louis, MO.

Salts. Potassium Chloride (lot #855933) and Potassium Phosphate (lot #870603A) were obtained from Fisher Scientific, Fair Lawn, NJ. Potassium Sulfate (lot #T182J) was obtained from Allied Chemical, New York, NY.

Radionucleotide S^{35} (as H_2SO_4, 100 μc in 100 ml) and Atomlight liquid scintillation solution were purchased from New England Nuclear, Boston, MA.

Silver. Silver Chloride microelectrodes were purchased from Microelectrodes Inc., Londonderry, NH.

Methods

The following experiments were performed: Size-Exclusion High-Performance Liquid Chromatography (SEC) to measure phase compositions in ternary polymer(2)-polymer(3)-water and polymer(2)-polymer(3)-buffer systems; LALLS measurements to determine interaction parameters (osmotic virial coefficients); ultraviolet spectrophotometry to determine protein partition coefficients; liquid scintillation to determine salt partition coefficients; and voltammetry to determine electric-potential differences between the phases. All measurements were performed at 25°C ± 0.01°C. Since these experiments were

performed using the same polymers, proteins and salts, they provide a consistent basis for drawing conclusions.

Phase-diagram measurements

Two-phase systems are made by dissolving PEG and dextran in water in volumetrically marked centrifuge tubes at several concentrations, or by mixing different ratios of known stock solutions of PEG in water (or salt-water) and dextran in water (or salt-water). Water (or a salt-water stock solution) is then added to a total system weight of 10 grams. The phases are then allowed to separate and equilibrate in a constant-temperature bath until both phases become transparent. In addition, the samples are centrifuged for 5 minutes just before the phases are separated. To determine the total original solution concentration of the polymers, the phase volumes are measured and then separated by first pipetting away most of the top phase and then removing the rest of the top phase along with the interface and part of the bottom phase. The total solution concentration of the dextran must be determined using polarimetry, since the dextran supplied retains water in amounts varying from 2–10 wt. %. If known stock solutions are used, the total original solution concentration of the polymers can be calculated directly. Phase densities are determined by weighing 1-ml samples. Samples of each phase are then diluted and the PEG and dextran concentrations in each phase are measured by SEC (a variation of chromatography which separates macromolecules by size) using a three-column system comprised of one Bio-Gel TSK-40 30-cm column and two Bio-Gel TSK-30 30-cm columns in series. The samples are diluted to a concentration range in which the calibration curve (peak area versus concentration) is linear for the refractive-index detector. Results are independent of the method of preparation of the two-phase systems.

Low-angle laser-light scattering

Static light-scattering measurements are made with a LDC/Milton Roy KMX-6 LALLS Photometer, which employs a 2-mW He-Ne laser at a wavelength of 633 nm. Refractive index increments were measured on a LDC/Milton-Roy KMX-16 Laser Differential Refractometer. Water used in preparing protein and polymer solutions is filtered through a Barnsted-Nanopore water-purification system. Protein and polymer solutions are made individually or diluted from known stock solutions. After equilibration, the solutions are passed through a 0.22-μm Millipore filter and into the cell at a flow rate of approximately 0.2 mL/min. Results are independent of the method of sample preparation or flow rate through the LALLS photometer.

From experimental values of the reduced Rayleigh ratio at several solute concentrations, the single-solute polymer and protein second virial coefficients, A_{ii}, are determined from (LDC/Milton Roy, 1978):

$$\frac{KC_i}{\overline{R_\theta}} = \frac{1}{M_i} + 2A_{ii}c_i \tag{11}$$

The weight-average molecular weight is obtained from the inverse of the intercept.

Once the single-solute polymer and protein second virial coefficients are determined, the polymer(2)-polymer(3) and polymer-protein second virial cross coefficients, A_{ij}, either in water or salt-water, are calculated from (Comper and Laurent, 1978):

$$\frac{K^*}{\overline{R_\theta}} = \left(\sum_i^n M_i v_i^2 c_i\right)^{-1} + \frac{2\sum_i^n \sum_j^n A_{ij} M_i M_j c_i c_j v_i v_j}{\left(\sum_i^n M_i v_i^2 c_i\right)^2} \tag{12}$$

The reduced Rayleigh ratio is now measured for a two-solute aqueous (or salt-water) solution containing either two different polymers or a polymer and a protein. Since all necessary quantities have been fixed or previously measured, we now solve for A_{ij} where $i \neq j$. Light scattering of protein solutions generally requires the presence of a salt to reduce effects of protein denaturation and long-range electrostatic interactions between proteins (Huglin, 1972).

Partition-coefficient measurements

Identical two-phase polymer-polymer systems are prepared, each containing a different protein, and one without protein to serve as a blank. Protein partition coefficients are measured in aqueous and in salt solutions containing either KCl, KH_2PO_4 or K_2SO_4 in concentrations of 50 or 100 mM. As in the phase diagram measurements, the phases are prepared by mixing quantities of known polymer and salt stock solutions and then adding a small known amount of protein. Since the protein partition coefficient is independent of its own concentration, concentrations were chosen to assure a measurable UV absorbance and economical use of protein. The amounts of protein added were approximately 0.5, 1.5 and 2.5 mg/mL for lysozyme, chymotrypsin and albumin, respectively. The phases are allowed to equilibrate, are centrifuged and then separated and diluted for analysis. The protein concentrations in each phase are determined spectrophotometrically at 280 nm. The polymer concentrations are unchanged by the dilute concentrations of added solutes.

Two-phase systems containing salts (without proteins) are prepared in the same manner; however, sulfate-concentration measurements for each phase are made using liquid scintillation counting (Brooks et al., 1984). One-mL samples of each phase are mixed with 1 mL of water and 10 mL of Atomlight scintillation counting cocktail. The counting efficiency of each phase is measured by adding known amounts of radionucleotide to blank samples of the separated phases.

Voltammetry

Figure 1 shows a schematic of the microelectrode apparatus used to measure an electric potential difference between the two aqueous phases. The protocol followed is the same as that of Brooks et al. (1984) and Sharp (1987).

Analytical methods

Protein concentrations are measured spectrophotometrically with either a Bausch & Lomb Spectronic 710 System 400-4, a Beckman DU-7 or a Bausch & Lomb 1201. Values used for the extinction coefficients (cm^2/mg) at 280 nm are 0.666 for Bovine Serum Albumin, 2.0 for α-chymotrypsin, 2.55 for Hen Lysozyme (Fasman, 1976).

Dextran concentrations are measured with a Perkin-Elmer 241 Polarimeter using a specific optical rotation of +199.5 for

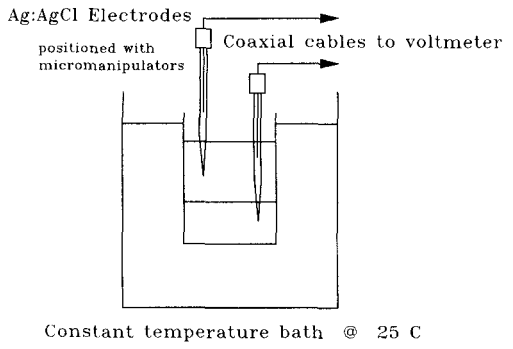

Figure 1. Schematic of microelectrode apparatus.

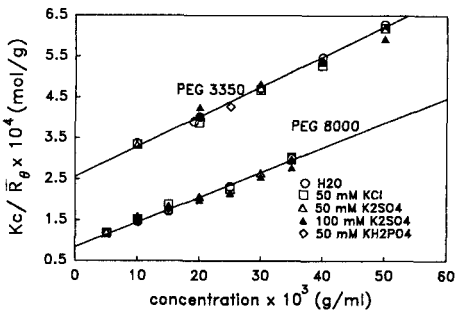

Figure 3. LALLS data for poly(ethylene glycols) in different salt-water systems.
Concentrations are in grams of solute per mL of solution.

all molecular weights of dextran above 10,000 Daltons (Albertsson, 1986).

A Beckman LS-230 Liquid Scintillation system is used for radionucleotide counting.

pH's were measured using an Ingold micro-pH electrode with a Fisher Accumet pH meter.

Results and Discussion

Figure 2 shows a phase diagram for a poly(ethylene glycol)/dextran/water system. The predicted binodal is calculated from the osmotic-virial-coefficient equations with virial coefficients determined by LALLS (King et al., 1986). The tie-line length provides a useful parameter for correlating properties of aqueous two-phase systems; it is a representative measure of the composition difference between the two liquid phases at equilibrium and is defined in this figure.

Figures 3–5 show the effects of different salts on the second virial coefficients (slopes of the figures) of the polymers and proteins studied here. For all polymers and for albumin, at the salt concentrations used in this study, the interaction between like solutes are unaffected by the type of salt added. However, for both lysozyme and α-chymotrypsin, the type of salt added has a marked effect on the protein-protein interaction in solution. This effect could be due to salt "bridging" between the proteins (attractive interactions between proteins caused by electrostatic interactions with the salts) or to conformational changes in the structure of the protein caused by different salts. Changes in pH, which may cause changes in protein surface charge, can also drastically alter protein-protein interactions in solution. pH's measured for these unbuffered aqueous salt-polymer solutions were approximately 5.5 for 50-mM KH_2PO_4 and 7.5 for both 50-mM KCl and 50- and 100-mM K_2SO_4. If surface charge were the only factor, we would expect virial coefficients in sulfate and chloride to be similar. This is clearly not true for α-chymotrypsin. Most likely, salt effects on protein-protein interactions in solution are a combination of all above mentioned factors.

Figures 6a and 6b show electric-potential differences measured between the phases as a function of the type of salt and the tie-line length. The potentials measured are in the range 0–7 mV. Previous studies (Reitherman, 1973; Johansson, 1973; Brooks et al., 1984; Sharp, 1987) have also reported values in this range, although for phase systems with different polymer molecular weights and salt concentrations. As expected, extrapolation of these plots result in zero potential difference (within experimental error = 0.1 mV) at a tie-line length of zero (a homogeneous solution). For a given tie line, the electric-potential difference increases depending on the anion in the order chloride, acetate, phosphate, sulfate. To compare systematically salts which gave measurable potentials with one that did not, we chose to focus on chlorides, sulfates and phosphates for all LALLS and partition-coefficient studies.

Figure 6c shows sulfate partition coefficients in PEG/dextran/salt-water systems as a function of tie-line length. When Figures 6a, 6b and 6c are compared, a direct proportionality is seen between trends in the salt partition coefficients and electric potential difference; as salt partition coefficient decreases, electric potential difference increases. This observation has led previous workers to the conclusion that salt partitioning causes an electrical potential difference between the two phases; however, it is still uncertain whether the measured potentials can be

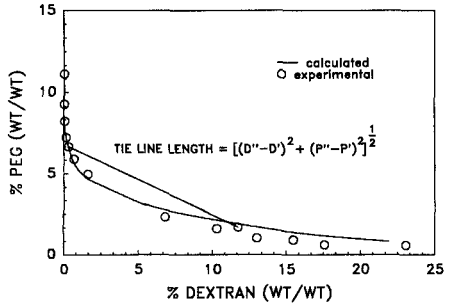

Figure 2. Experimental and predicted binodal for PEG 8000/dextran T-500/water system.
D = wt. % dextran; P = wt. % PEG.

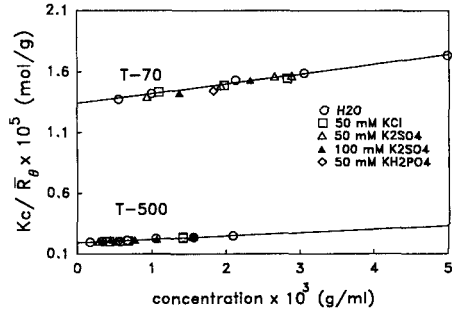

Figure 4. LALLS data for dextrans in different salt-water systems.
Concentrations are in grams of solute per mL of solution.

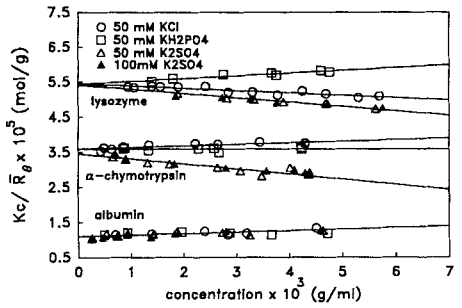

Figure 5. LALLS data for proteins in different salt-water systems.

Concentrations are in grams of solute per mL of solution.

equated with true thermodynamic potentials or even whether salt partitioning alone can cause any type of electric potential difference between the phases. It is clear, however, that salt partitioning is an important factor in protein partitioning (Albertsson, 1986; Johansson, 1973; and King et al., 1986, 1988).

Table 1 presents data obtained from LALLS measurements. The molecular-weight data for PEG and for dextrans are in good agreement with manufacturer's estimates. The virial coefficients fall within the range of values found in previous studies (Senti, 1955; Chernyak and Polushina 1961; Lakhanpal 1968; Edmond and Ogston 1968; Vink, 1971; Comper and Laurent, 1978; LDC Milton-Roy, 1978, 1985; Gerstein; Tweeten; Hefford, 1984; King et al., 1986). Although fundamental thermodynamic data for proteins are scarce, albumin has been studied in detail (Dandliker, 1954; Comper and Laurent, 1978; Edmond and Ogston, 1968; Knoll and Hermans, 1983; LDC Milton-Roy, 1978, 1985). The virial coefficient reported here for albumin is within the range presented in these previous studies. The molecular weight reported for lysozyme, although higher than the generally accepted value of 14,500, is in good agreement with previous work by Jones and Midgley (1984). This higher value can be attributed to protein self-association or dimerization. The sign of the virial coefficient is also important. A positive virial coefficient indicates repulsive interactions such as excluded volume effects or repulsive electrostatic effects. A negative virial coefficient indicates attractive two-body interactions, such as hydrophobic or attractive electrostatic effects. Negative second virial coefficients are common for highly charged macromolecules such as proteins, which associate easily. On the other hand, second virial coefficients for nonionic polymers should always be positive due to excluded-volume considerations.

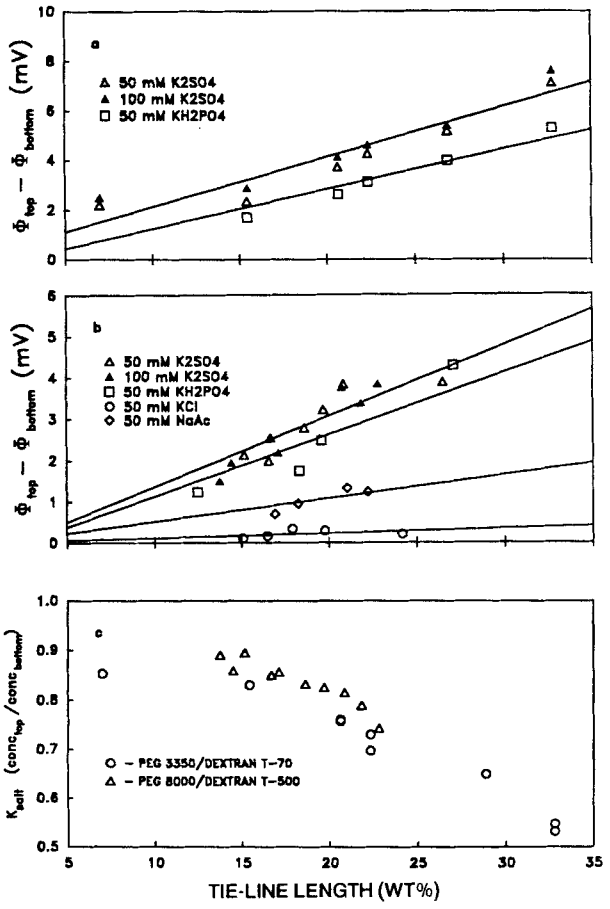

Figure 6. a) Potential difference vs. tie-line length for PEG 3350/dextran T-70/salt-water systems; b) potential difference vs. tie-line length for PEG 8000/dextran T-500/salt-water systems; c) salt partition coefficient vs. tie-line length for both systems.

Table 2 presents polymer-protein interaction coefficients in several dilute salt solutions. Fundamental thermodynamic data for polymer-protein interactions are also scarce; however, the data presented here are in the range of values reported previously (Edmond and Ogston, 1968; Comper and Laurent, 1978; Knoll and Hermans, 1983). Reliable interaction coefficients for polymer-protein pairs in potassium sulfate are difficult to obtain due to a large amount of scatter; for lysozyme and for α-chymotrypsin, values are uncertain because of the small values of their interaction coefficients with polymers. At the salt concentrations studied, albumin-polymer interaction coefficients appear to be independent of the type of salt. Moreover, for lysozyme and α-chymotrypsin, the magnitude of the protein-polymer interaction in aqueous potassium chloride is similar to that in aqueous potassium-phosphate solutions.

Figure 7 shows equilibrium phase diagrams (binodals) for the systems PEG 3350/dextran T-70 and PEG 8000/Dextran T-500. This figure also shows the effects of added salts on the PEG 8000/dextran T-500 binodal. At the concentrations studied here, the effect is negligible; however, at higher salt concentrations (>0.1 M), phosphates and sulfates have been shown to reduce polymer concentrations at the critical point (Bamberger

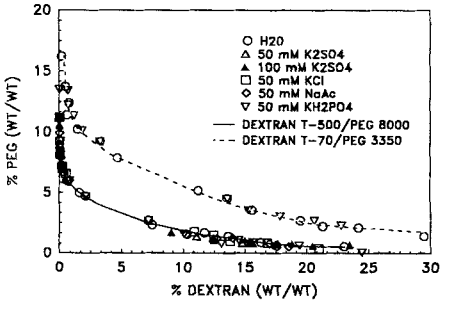

Figure 7. Binodals for PEG 8000/dextran T-500/salt-water and PEG 3350/dextran T-70/salt-water systems.

Table 1. LALLS Data for Single-Solute (Polymer or Protein) Solutions Using Water or Salt-Water

Solute	Solvent	M_n*	M_w	$A_{ii} \times 10^4$ (mL · mol/g^2)
PEG 3350	all	3,690	3,860	36.36
PEG 8000	all	8,920	11,800	30.50
Dextran T-70	all	37,000	74,540	4.04
Dextran T-500	all	167,000	509,000	1.25
Lysozyme	50 mM KCl		18,350	−3.30
Lysozyme	50 mM K_2SO_4		18,350	−6.00
Lysozyme	100 mM K_2SO_4		18,350	−6.00
Lysozyme	50 mM KH_2PO_4		18,350	3.90
α-chymotrypsin	50 mM KCl		28,500	2.25
α-chymotrypsin	50 mM K_2SO_4		28,500	−6.73
α-chymotrypsin	100 mM K_2SO_4		28,500	−6.73
α-chymotrypsin	50 mM KH_2PO_4		28,500	0.09
Albumin	all		90,000	1.60

*From differential vapor-pressure measurements (Haynes et al., 1988) and manufacturer's data (Pharmacia).

Table 2. Polymer-Protein and Polymer-Polymer Interaction Coefficients, A_{ij}, in Several Salt-Water Systems, Units as in Table 1

	PEG 3350	PEG 8000	Dextran T-70	Dextran T-500
Lysozyme				
in 50 mM KCl	3.07	1.60	1.77	1.40
in 50 mM KH_2PO_4	2.87	3.40	2.00	2.50
α-Chymotrypsin				
in 50 mM KCl	5.10	3.40	2.40	1.55
in 50 mM KH_2PO_4	6.00	4.34	2.50	1.61
Albumin				
in KCl or KH_2PO_4 or K_2SO_4 at 50–100 mM	8.00	5.60	2.58	1.50
Dextian T-40	19.0	—	—	—
Dextian T-70	17.0	—	—	—
Dextian T-500 lot #26066	14.0	13.4	—	—
Dextian T-500 lot #38624	12.5	10.0	—	—

Table 3. Phase-Diagram Data for PEG 8000/Dextran T-500 Lot #38624/H_2O at 25°C

Total (wt. %)			Top (wt. %)			Bottom (wt. %)		
Dextran	PEG	H_2O	Dextran	PEG	H_2O	Dextran	PEG	H_2O
4.90	3.44	91.66	1.63	4.98	93.39	6.85	2.33	90.82
4.94	4.10	90.96	0.68	5.91	93.41	10.31	1.60	88.09
4.99	4.30	90.71	0.34	6.68	92.98	11.75	1.70	86.55
5.93	4.49	89.58	0.18	7.23	92.59	13.67	1.42	85.91
5.00	5.70	89.30	0.10	7.90	92.00	15.00	1.10	83.90
7.02	5.07	87.91	0.07	8.24	91.69	16.30	1.00	83.70
7.39	5.86	86.75	0.03	9.29	90.68	17.57	0.61	82.88
8.32	7.16	84.52	0.01	11.14	88.85	23.05	0.58	76.37

Table 4. Phase-Diagram Data for PEG 3350/Dextran T-70 Lot #00356/H_2O at 25°C

Total (wt. %)			Top (wt. %)			Bottom (wt. %)		
Dextran	PEG	H_2O	Dextran	PEG	H_2O	Dextran	PEG	H_2O
6.47	7.22	86.31	4.70	7.87	87.43	11.25	5.14	83.61
6.47	7.22	86.31	3.63	8.69	87.68	13.73	3.39	82.88
6.57	7.64	85.79	1.47	10.23	88.30	15.56	2.85	81.59
5.58	9.15	85.27	0.59	11.42	87.99	19.50	2.01	78.49
8.19	8.70	83.11	0.79	12.40	86.81	21.32	2.25	76.43
8.21	9.76	82.03	0.48	13.73	85.79	24.19	2.14	73.67
9.78	11.39	78.83	0.19	16.22	83.59	29.44	1.37	69.19

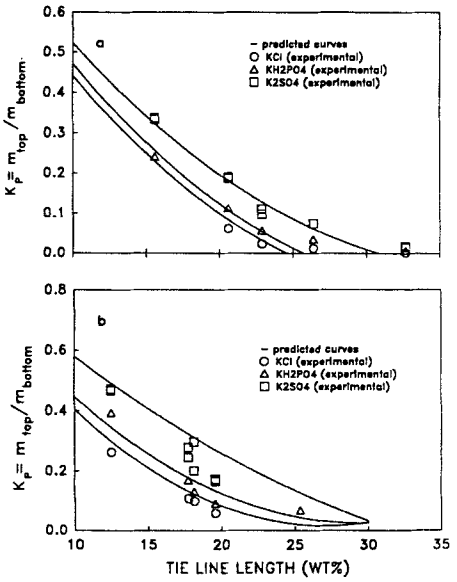

Figure 8. Experimental and predicted protein partition coefficient vs. tie line length for albumin in a) PEG 350/dextran T-70/salt-water system and b) PEG 8000/dextran T-500/salt-water system.

et al., 1984). Tables 3 and 4 present the phase-diagram data for the two two-phase systems studied.

The proteins studied here are well characterized with respect to surface charge as a function of pH (Tanford and Wagner, 1954; Tanford et al., 1955; Sakakibara and Hamaguchi, 1968; Shiao et al., 1972; Horn and Heuck, 1983). As stated above, chlorides and sulfates added to the phase systems do not alter the pH of the phases significantly from neutral pH; however, the

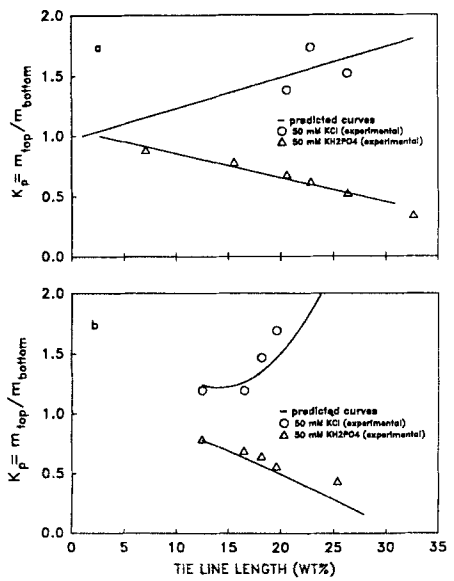

Figure 9. Experimental and predicted protein partition coefficients vs. tie line length for α-chymotrypsin in a) PEG 3350/dextran T-70/salt-water system and b) PEG 8000/dextran T-500/salt-water system.

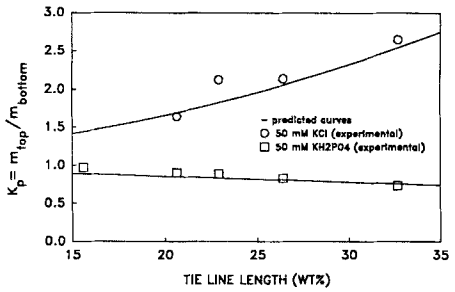

Figure 10. Experimental and predicted protein partition coefficient vs. tie line length for lysozyme in PEG 3350/dextran T-70/salt-water system.

pH of 50 mM potassium phosphate is 5.5. Studies using a micro pH electrode show negligible (~0.1 pH unit) difference in pH between the phases. Thus, we can assume that the protein has the same charge in both phases.

Equation 10 can now be used to predict protein partition coefficients. Figures 8–10 present comparisons between experimental and predicted protein partition coefficients. Salts have a marked effect on partitioning. For example, albumin, which is negatively charged close to neutral pH, partitions almost totally to the bottom phase when in KCl, a salt which does not partition and does not generate a measurable electric potential difference between the phases. When phosphate or sulfate (salts which partition to the bottom phase and therefore generate a measurable potential difference) replace chloride, a higher partition coefficient is measured. Sulfate partitions into the bottom phase slightly more than phosphate (Brooks et al., 1984), producing slightly higher potential difference; moreover, in dilute aqueous phosphate solutions, albumin is less negatively charged, due to a difference of 2 pH units between sulfate and phosphate solutions. Equation 10 predicts that phosphate and sulfate increase the protein partition coefficient from that in KCl. Lysozyme and α-chymotrypsin are both positively charged; therefore, we expect the opposite trend: as the potential difference rises, the partition coefficient should decrease. Experimental results verify these trends. Without the electric-potential difference term in Eq. 10, calculated protein partition-coefficient data are highly inaccurate; however, this term is not always dominant. The polymer molecular weight, concentration and polymer-protein interactions, which are accounted for in the first two terms on the right of Eq. 10, are in most cases equally important. Recent work has also shown Eq. 10 to be valid for systems with controlled pH (King et al., 1988). Thus, if a desired protein is characterized with respect to its interaction with the phase polymers and if its surface charge is known, it is possible to design phase systems with respect to pH and salt, to optimize extraction performance.

Conclusions

Osmotic virial coefficients for several combinations of polymers and proteins in water and in dilute salt-water are reported. Electric-potential difference measurements for the corresponding aqueous two-phase systems were also determined. These fundamental data, combined with an appropriate molecular-thermodynamic model for polymers and/or proteins in dilute aqueous solutions, facilitates correlation and prediction of infinite-dilution partition coefficients for proteins in aqueous two-phase sys-

tems. Although some questions remain concerning the exact relationship between salt partitioning and the measured electric-potential difference between the phases, it is clear that for reliable results, the model must incorporate terms which can account for the effects of added buffer salts on protein partition coefficients. Finally, in order to develop further the theory and mechanisms for biomolecule partitioning in aqueous two-phase systems, it is essential to establish a database, consisting of protein-protein and polymer-protein interaction data in aqueous solutions differing in salt, pH and ionic strength along with protein-charge data in aqueous polymer-salt solutions.

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Notation

a_{ij} = second virial coefficient for interaction between i and j in a solvent, L/mol
A_{ij} = second virial coefficient for interaction between i and j in a solvent, mL · mol/g^2
c_i = solute concentration, g/mL
F = Faraday constant
K = single-component optical constant = $2\pi^2 n^2 v^2/\lambda^4 N(1 + \cos^2\theta)$
K^* = multicomponent optical constant = K/v^2
m_i = concentration, molality, mol/1,000g solvent
M_i = molecular weight of solute i
M_n = number-average molecular weight
M_w = weight-average molecular weight
N = Avogadro's number
n_o = refractive index of solvent
n = total number of components
R = gas constant
$\overline{R}_\theta$ = reduced Rayleigh ratio = (R_θ) solution − (R_θ) solvent
T = temperature, K
V_1 = molar volume of water, L/mol
z_p = net surface charge of biomolecule

Greek letters

ϕ = electrical potential relative to some reference, mV
λ = wavelength of laser light, 633 nm
$\Delta\mu$ = difference between chemical potential and that measured at a specified standard state
μ = chemical potential
ν = refractive-index increment
ρ = density of solvent, g/mL
θ = scattering angle of collected light

Superscripts

$'$ = lighter phase
$''$ = heavier phase

Subscripts

1 = solvent
2 = polymer
3 = polymer
p = protein

Literature Cited

Abrams, D. S. and J. M. Prausnitz, "Statistical Thermodynamics of Liquid Mixtures: A New Expression for the Excess Gibbs Energy of Partly or Completely Miscible Systems," *AIChE J.*, **21**(1), 116 (1975).

Albertsson, P-A., *Partition of Cell Particles and Macromolecules*, 3rd ed., Wiley-Interscience, New York (1986).

Bamberger, S., G. V. F. Seaman, J. A. Brown, and D. E. Brooks, "The Partition of Sodium Phosphate and Sodium Chloride in Aqueous Dextran Poly(ethylene glycol) Two-Phase Systems," *J. Colloid Interf. Sci.*, **99**, 1 (1984).

Baskir, J. N., T. A. Hatton, and U. W. Suter, "Thermodynamics of the Separation of Biomaterials in Two-Phase Aqueous Polymer Systems: Effect of the Phase Forming Polymers," *Macromol.*, **20**, 1300 (1987).

———, "Thermodynamics of the Partitioning of Biomaterials in Two-Phase Aqueous Polymer Systems: Comparison of Lattice Model to Experimental Data," personal communication (1988).

Birkenmeier, G., E. Usbeck, and G. Kopperschlager, "Affinity Partitioning of Albumin and α-Fetoprotein in an Aqueous Two-Phase System Using Poly(ethylene glycol)-Bound Triazine Dyes," *Anal. Biochem.*, **136**, 264 (1984).

Blanks, R. F., and J. M. Prausnitz, "Thermodynamics of Polymer Solubility in Polar and Non-Polar Systems," *Ind. Eng. Chem. Fund.*, **3**(1), 1 (1964).

Brooks, D. E., K. A. Sharp, S. Bamberger, C. H. Tamblyn, G. V. F. Seaman, and H. Walter, "Electrostatic and Electrokinetic Potentials in Two Polymer Aqueous Phase Systems," *J. Colloid Interf. Sci.*, **102**, 1 (1984).

Chernyak, V. Y., and T. V. Polushina, "Determination of the Molecular Weight of Poly(ethylene glycols)," *Med. Prom. S.S.S.R.*, **8**, 39 (1961).

Comper, W. D., and T. C. Laurent, "An Estimate of the Enthalpic Contribution to the Interaction between Dextran and Albumin," *Biochem. J.*, **175**, 703 (1978).

Dandliker, W. B., "Light Scattering of Isoionic Bovine Albumin," *J. Amer. Chem. Soc.*, **76**, 6036 (1954).

Edmond, E., and A. G. Ogston, "An Approach to the Study of Phase Separation in Ternary Aqueous Systems," *Biochem. J.*, **109**, 569 (1968).

Fauquex, P-F., H. Hustedt, and M.-R. Kula, "Phase Equilibration in Agitated Vessels During Extractive Enzyme Recovery," *J. Chem. Technol. Biotechnol.*, **35B**, 51 (1985).

Fasman, G. D., ed., *Handbook of Biochemistry and Molecular Biology: Proteins*, CRC Press, Cleveland, OH (1976).

Flory, P. J., "Thermodynamics of High Polymer Solutions," *J. Chem. Phys.*, **10**, 51 (1942).

Gerstein, A., Molecular Biology Division, Pharmacia Inc., personal communication.

Grossman, P., "A Model for Phase Separation and Partitioning in Aqueous Two-Phase Systems," M. S. Thesis, Univ. of Virginia (1987).

Grossman, P. D., and J. L. Gainer, "Correlation of Aqueous Two-Phase Partitioning of Proteins with Changes in Free Volume," *Biotechnol. Prog.*, **4**, 1, 6 (1988).

Haynes, C. A., R. V. Beynon, R. S. King, H. W. Blanch, and J. M. Prausnitz, "Thermodynamic Properties of Aqueous Polymer Solutions: Polyethylene glycol or Dextran," accepted for publication in *J. Phys. Chem.* (1988).

Hefford, R. J., "Polymer Mixing in Aqueous Solution," *Polym.*, **25**, 979 (1984).

Heil, J. F., and J. M. Prausnitz, "Phase Equilibria in Polymer Solutions," *AIChE J.* **12**, 678 (1966).

Hershkowitz, M., and M. Gottlieb, "Vapor-Liquid Equilibria in Aqueous Solutions of Various Glycols and Poly)ethylene glycols): 3. Poly(ethyleneglycols)," *J. Chem. Eng. Data*, **30**(2), 233 (1985).

Horn, D., and C-C. Heuck, "Charge Determination of Proteins with Polyelectrolyte Titration," *J. Biol. Chem.*, **258**, 3, 1665 (1983).

Huggins, M. L., "Some Properties of Solutions of Long-Chain Compounds," *J. Phys. Chem.*, **46**, 151 (1942).

Huglin, M. B., ed., *Light Scattering from Polymer Solutions*, Academic Press, New York (1972).

Johansson, G., A. Hartman, and P-A. Albertsson, "Partition of Proteins in Two-Phase Systems Containing Charged Poly(ethylene glycol)," *Eur. J. Biochem.*, **33**, 379 (1973).

Johansson, G., "Effects of Salts on the Partition of Proteins in Aqueous Polymeric Biphasic Systems," *Acta Chem. Scand. Ser. B.*, **28**, 873 (1974).

Jones, M. N., and P. J. W. Midgley, "Light-scattering from detergent-complexed biological macromolecules," *Biochem. J.*, **219,** 875 (1984).
Kabiri-Badr, M., H. Cabezas, Jr., and R. A. Perkins, "A Fluctuation Theory Model of Salt-Polymer Aqueous Two-Phase Systems," AIChE Mtg., New York (Nov 17, 1987).
Kang, C. H., and S. I. Sandler, "Phase Behavior of Aqueous Two-Polymer Systems," *Fluid Phase Equil.*, **38** (1987).
Kim, C. W., "Interfacial Condensation of Biologicals in Aqueous Two-Phase Systems," Ph.D. Thesis MIT (1987).
King, R. S., H. W. Blanch, and J. M. Prausnitz, "Thermodynamics of Aqueous Polymer-Polymer Two-Phase Systems," ACS Mtg. Anaheim, CA (Sept. 10, 1986).
King, R. S., C. A. Haynes, S. J. Rathbone, H. W. Blanch, and J. M. Prausnitz, "Molecular Thermodynamics of Aqueous Two-Phase Systems for Bioseparations," Engineering Foundation Conf. on Recovery of Bioproducts, Kailua-Kona, HI (Apr. 21, 1988).
Knoll, D., and J. Hermans, "Polymer-Protein Interactions," *J. Biol. Chem.*, **258,** (9), 5710 (1983).
Konigsveld, R., and A. J. Staverman, "Liquid-Liquid Phase Separation in Multicomponent Polymer Solutions: I. Statement of the Problem and Description of Methods of Calculation," *J. Polym. Sci., part A-2,* **6,** 305 (1968).
Kula, M.-R., "Extraction and Purification of Enzymes," *Appl. Biochem. Bioeng.*, Wingard, L. B. Jr., E. Katchalski-Katzir, and L. Goldstein, eds., **2,** Academic Press, New York p.71 (1979).
Kula, M.-R., K. H. Kroner, and H. Hustedt, "Purification of Enzymes of Liquid-Liquid Extraction," *Adv. Biochem. Eng.*, **24,** 73 (1982).
Kumar, S. K., R. C. Reid, and U. W. Suter, "A Statistical Mechanics Based Lattice Model Equation of State: Applications to Mixtures with Supercritical Fluids," *ACS Symp. Ser.*, **329,** 88 (1987).
Lakhanpal, M. L., K. S. Chinna, and S. C. Sharma, "Thermodynamic Properties of Aqueous Solutions of Polyoxyethylenes," *Indian J. Chem.*, **6,** 505 (1968).
LDC/Milton Roy Application note LS1 (1978).
LDC/Milton Roy Application note LS4 (1985).
Ling, T. G. I., and B. Mattiasson, "Comparison between Binding Analyses Performed by Equilibrium Dialysis and Partitioning in Aqueous Two-Phase Systems Exemplified by the Binding of Cibacron Blue to Serum Albumin," *J. Chromatogr.*, **252,** 159 (1982).
Ling, T. G. I., "Partition Affinity Ligand Assay," Ph.D. Thesis, Dept. Biochem., Univ. of Lund, Sweden (May, 1983).
Mansoori, G. A., and J. F. Ely, "Partitioning of Monodispersed/Polydispersed Polymers and Biological Macromolecules in Aqueous Two-Phase Systems (Statistical Mechanical Modeling and Data Requirements for Bioprocess Engineering Applications," AIChE Mtg., New York (Nov. 17, 1987).
Mariani, E., A. Ciferri, and M. Maraghini, "Osmotic Measurements on Dilute Dextran Solutions," *J. Polym. Sci.*, **XVIII**(88), 303 (1955).
McGee, H., and G. G. Benge, "A Thermodynamic Model for Predicting the Phase Behavior of Aqueous-Polymer Two-Phase Systems," Virginia Polytechnic and State Univ., personal communication.
Ogston, A. G., "Some Thermodynamic Relationships in Ternary Systems, with Special Reference to the Properties of Systems Containing Hyaluronic Acid and Protein," *Arch. Biochem. Biophys.*, **Suppl. 1,** 39 (1962).
Patterson, D., "Thermodynamics of Non-Dilute Polymer Solutions," *Rubber Chem. Technol.*, **40,** 1 (1967).
Prigogine, I., A. Bellmans, and V. Mathot, *The Molecular Theory of Solutions,* Amsterdam, North-Holland Publishing Co. (1957).
Reitherman, R., S. D. Flanagan, and S. H. Barondes, "Electromotive Phenomena in Partition of Erythrocytes in Aqueous Polymer Two Phase Systems," *Biochim. Biophys. Acta,* **297,** 193 (1973).
Sakakibara, R., and K. Hamaguchi, "Structure of Lysozyme XIV: Acid-Base Titration of Lysozyme," *J. Biochem.*, **64,**(5), 613 (1968).
Scott, R. L., "The Thermodynamics of High Polymer Solutions: V. Phase Equilibria in the Ternary System: Polymer 1 - Polymer 2 - Solvent," *J. Chem. Phys.*, **17,** 3 (1949).
Senti, F. R., N. N. Hellman, N. H. Ludwig, G. E. Babcock, R. Tobin, C. A. Glass, and B. L. Lamberts, "Viscosity, Sedimentation and Light-Scattering Properties of Fractions of an Acid-Hydrolyzed Dextran," *J. Polym. Sci.*, **XVII,** 527 (1955).
Shanbhag, V. P., R. Sodergard, H. Carstensen, and P-A. Albertsson, "A New Method for the Determination of the Binding Capacity of Testosterone-Estradiol-Binding Globulin in Human Plasma," *J. Steroid Biochem,* **4,** 537 (1973).
Shanbhag, V. P., and C-G. Axelsson, "Hydrophobic Interaction Determined by Partition in Aqueous Two=Phase Systems," *Eur. J. Biochem.*, **60,** 17 (1975).
Sharp, K. A., "Protocol for Measurement of Electrostatic Potentials," personal communication (1987).
Shiao, D. D. F., R. Lumry, and S. Rajender, "Modification of Protein Properties by Change in Charge Succinylated Chymotrypsinogen," *Eur. J. Biochem.*, **29,** 377 (1972).
Shiskov, A. K., and S. Ya. Frenkel, "The Theory of Phase Separation in an Non-concentrated Solution of Several Polydispersed Polymers," *Polym. Sci. U.S.S.R. (Engl. Trans.),* **20,** 2941 (1979).
Shiskov, A. K., V. V. Krivobokov, Ye. V. Chubarova, and S. Ya. Frenkel, "Separation of Phases in Aqueous Solutions of Polyethylene glycol and Dextrane," *Polym. Sci. U.S.S.R. (Engl. Trans.),* **23,**(6), 1330 (1981).
Tanford, C., and M. L. Wagner, "Hydrogen Ion Equilibria of Lysozyme," *J. Amer. Chem. Soc.*, **76,** 3331 (1954).
Tanford, C., S. A. Swanson, and W. S. Shore, "Hydrogen Ion Equilibria of Bovine Serum Albumin," *J. Amer. Chem. Soc.*, **77,** 6414 (1955).
Tompa, H., *Polymer Solutions,* Butterworths Scientific Publications, London (1956).
Tweeten, T. N., Polymer Laboratories Inc., personal communication.
Vink, H., "Precision Measurements of Osmotic Pressure in Concentrated Polymer Solutions," *Eur. Polym. J.*, **7,** 1411 (1971).
Vink, H., "Phase Equilibria in Polymer Solutions," *Eur. Polym. J.*, **12,** 77 (1976).
Walter, H., D. E. Brooks, and D. Fisher, Eds., *Partitioning in Aqueous Two-Phase Systems,* Academic Press, London (1985).
Yamakawa, H., S. A. Rice, R. Corneliussen and L. Kotin, "On the Thermodynamic Properties of Solutions of Polar Polymers. Theory," *J. Chem. Phys.*, **38,** 7 (1963).
Zaslavsky, B. Yu., L. M. Miheeva, N. M. Mestechkina, and S. V. Rogozhin, "Physico-chemical Factors Governing Partition Behavior of Solutes and Particles in Aqueous Polymeric Biphasic Systems: I. Effect of Ionic Composition on the Relative Hydrophobicity of the Phases," *J. Chromatogr.*, **253,** 139 (1982).
Zaslavsky, B. Yu, L. M. Miheeva, N. M. Mestechkina, and S. V. Rogozhin, "Physico-chemical Factors Governing the Partition Behavior of Solutes and Particles in Aqueous Polymeric Biphasic Systems: II. Effect of Ionic Composition on the Hydration Properties of the Phases," *J. Chromatogr.*, **253,** 149 (1983).
Zaslavsky, B. Yu., L. M. Miheeva, N. M. Mestechkina, and S. V. Rogozhin, "Physico-chemical Factors Governing the Partition Behavior of Solutes and Particles in Aqueous Polymeric Biphasic Systems: III. Features of Solutes and Biological Particles Detected by the Partition Technique," *J. Chromatogr.*, **256,** 49 (1983).

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