Application of Integral-Equation Theory to Aqueous Two-Phase Partitioning Systems

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A molecular-thermodynamic model is developed for representing thermodynamic properties of aqueous two-phase systems containing polymers, electrolytes, and proteins. The model is based on McMillan-Mayer solution theory and the generalized mean-spherical approximation to account for electrostatic forces between unlike ions. The Boublik-Mansoori equation of state for hard-sphere mixtures is coupled with the osmotic virial expansion truncated after the second-virial terms to account for short-range forces between molecules.

Osmotic second virial coefficients are reported from low-angle laser-light scattering (LALLS) data for binary and ternary aqueous solutions containing polymers and proteins. Ion-polymer specific-interaction coefficients are determined from osmotic-pressure data for aqueous solutions containing a water-soluble polymer and an alkali chloride, phosphate or sulfate salt.

When coupled with LALLS and osmotic-pressure data reported here, the model is used to predict liquid-liquid equilibria, protein partition coefficients, and electrostatic potentials between phases for both polymer-polymer and polymer-salt aqueous two-phase systems. For bovine serum albumin, lysozyme, and α-chymotrypsin, predicted partition coefficients are in excellent agreement with experiment.

Introduction

In 1956, Per-Åke Albertsson (1986) introduced aqueous two-phase partition systems to the biological and engineering communities. Following Beijerinck (1896), Albertsson found that phase separation often occurs when two water-soluble polymers (for example, polyethylene glycol (PEG) and dextran) or when a polymer and a strong electrolyte (for example, PEG and an alkali phosphate) are dissolved in water. The aqueous two-phase systems formed contain mainly water, with each phase enriched with respect to one of the separation-inducing components. Due to their high-water content, both equilibrium phases provide a suitable environment for labile macromolecules, cells, membranes and organelles.

Aqueous two-phase partition systems provide a sensitive technique for fractionating and separating complex mixtures of bio-macromolecules (Walter et al., 1985; Kula et al., 1982). When a mixture of proteins is added to an aqueous two-phase system, each type of protein partitions uniquely between the phases. The partitioning behavior of a protein in a given two-phase system is governed predominantly by its size, surface chemistry and net charge, with other, usually smaller, contributions made by forces associated with the dipole moment and polarizability of the protein (Johansson, 1974b; Brooks et al., 1985; Haynes et al., 1989b). As shown by Hustedt et al. (1990), subtle changes in any of these properties can lead to noticeable changes in partitioning behavior.

Recently, attention has been given to the use of aqueous two-phase technology as an initial step in the (large-scale) isolation of genetically engineered proteins (Walter et al., 1991).

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To aid design and optimization of aqueous two-phase systems for recombinant-protein separations, several investigators have proposed models for predicting the thermodynamic properties of aqueous two-phase systems, giving special attention to prediction of protein partition coefficients. Most of these previous modeling efforts have focused on a description of nonelectrostatic forces, in particular excluded-volume forces, affecting phase diagrams and partition coefficients. For example, an early model was provided by Brooks et al. (1985), who used the Flory-Huggins theory to correlate phase-diagram and protein partition-coefficient data. Among others, Walter et al. (1991) have questioned the applicability of the Flory-Huggins theory, which assumes that all polymer segments are uniformly distributed and are free to assume a large number of configurations, to the description of protein partitioning since proteins generally have rigid structures and assume highly nonuniform segment densities in solution. However, Brooks et al. demonstrated that the Flory-Huggins theory could qualitatively describe phase diagrams and protein-partition coefficients in the absence of electrostatic effects (Albertsson et al., 1987).

Following Brooks, Tjerneld and coworkers used the Flory-Huggins theory to correlate a wide variety of ternary polymer/polymer/water phase diagrams (Gustafsson et al., 1986a; Gustafsson et al., 1986b; Sjöberg and Karlstrom, 1989). Also, Diamond and Hsu (1989) correlated an extensive set of dipeptide and low-molecular-weight protein partition-coefficient data using a simple, linearized form of Flory-Huggins theory.

A more sophisticated lattice-model approach was proposed by Baskir et al. (1987, 1989), who used the polymer adsorption theory of Scheutjens and Fleer to examine the nature of polymer-protein interactions in aqueous solution. In the same spirit, Abbott et al. (1991) applied the polymer-solution scaling theories of de Gennes (1979) to the description of interactions between nonionic phase-forming polymers and globular proteins. The novel physical pictures which emerged provide important criteria for testing existing and future models.

An alternate modeling approach was first proposed by King et al. (1988) who applied the theory of Edmond and Ogston (1968), based on the osmotic virial expansion truncated after the second-virial-coefficient terms, to the prediction of phase diagrams and protein partition coefficients; low-angle laser-light scattering measurements were used to determine osmotic second virial coefficients, the only nonelectrostatic model parameters. A short time later, we extended this theory to include three-body and higher-order interactions through application of differential-solvent-vapor-pressure data (Haynes et al., 1989a). Following the liquid-solution-theory approach of King et al., Cabezas et al. (1989) used the isothermal-isobaric virial expansion of Hill (1986) to predict ternary phase diagrams. Recently, Forciniti et al. (1991) used an osmotic-virial-expansion based theory, similar to that of King et al., to interpret an impressive set of protein-partition-coefficient data at the isoelectric point.

The excellent reviews of Walter et al. (1991) and Abbott et al. (1990) provide a critical analysis of these and other models describing the effects of the polymers on phase separation and isoelectric protein partitioning.

Unfortunately, little attention has been given to modeling aqueous two-phase systems containing ions (and electrostatic forces) despite their well-established importance (Johansson, 1974a; Reitherman et al., 1973; Bamberger et al., 1984; Brooks et al., 1984; Sharp et al., 1986; Zaslavsky et al., 1978–1983, 1986–1988, 1991). For realistic engineering applications, it is essential that electrostatic forces be included in any model for aqueous solutions containing proteins. To model protein partition coefficients in systems containing electrolytes, Brooks (Walter et al., 1985) and King et al. (1988) extended their theories to include the effect of the interfacial-electrostatic-potential difference. The resulting models follow classical electrochemical thermodynamics (Guggenheim, 1959) by assuming that the logarithm of the partition coefficient of the protein macroion, $\ln K_p$, is given by

$$\ln K_p = \ln K_p^* + \frac{z_p F}{RT}\Delta\Phi \tag{1}$$

where K_p^* is the protein partition coefficient in the absence of an interfacial-electrostatic-potential difference $\Delta\Phi$ (Albertsson, 1986); $\ln K_p^*$ is given, for example, by Flory-Huggins theory in Brooks' model and by the osmotic virial expansion in King's model. For most polymer/polymer/water two-phase systems, Eq. 1 captures the basic effects of added electrolytes (at low concentrations) on protein partitioning, probably because the formation of an interfacial-electrostatic-potential difference is the dominant effect of adding small amounts of strong electrolytes to such systems.

However, Eq. 1 does not consider the ions of added strong electrolytes or the counterions of protein macroions as molecular species; as a result, no account is given of the influence of ion hydration/solvation (including salting-out forces), hydrated-ion excluded-volume forces, and electrostatic screening (double-layer forces) on phase diagrams and protein partition coefficients. Neglect of these forces is satisfactory for many polymer/polymer/water two-phase systems containing strong electrolytes at low concentrations (King et al., 1988; Haynes et al., 1991; Haynes et al., 1992c). However, ion-specific forces and screening effects become increasingly important as the concentration and charges of ions increase (Robinson and Stokes, 1954). Moreover, models which neglect these effects cannot be used to describe polymer/salt/water two-phase systems, which are finding increasing industrial use in large-scale protein-purification trains.

This work is concerned with the influence of electrolytes and electrostatic forces on phase diagrams and protein partition coefficients. A few years ago, we presented results from a molecular-thermodynamic model for aqueous two-phase systems which specifically includes the molecular properties and effects of salts (Haynes et al., 1989b). That model, which draws from the concentrated-electrolyte-solution models of Guggenheim (1935) and Newman (1973), is based on the osmotic virial expansion coupled with extended Debye-Hückel theory to account for electrostatic forces between unlike ions. Predicted phase diagrams and interfacial-electrostatic-potential differences were in good agreement with experiment for polymer/polymer/water systems containing small amounts of strong electrolytes and globular proteins; however, predicted protein partition coefficients were in only fair agreement with the experiment, primarily because of the requirement that a single "effective" ion-size parameter must be used when applying

Debye-Hückel theory to multicomponent electrolytic solutions.

Cabezas et al. (1989) presented a similar model which combines Pailthorpe et al.'s (1982) extension to Debye-Hückel theory with the isothermal-isobaric virial expansion. With parameters fit from isopiestic vapor-pressure experiments and the low-angle laser-light scattering data of King et al. (1988), Cabezas' model accurately predicts polymer/polymer/water phase diagrams for systems containing strong electrolytes. However, Cabezas' model has not been used to predict protein partition coefficients.

In this work, we relax the restriction of a single ion-size parameter by replacing the Debye-Hückel theory with the generalized mean-spherical approximation, a statistical-mechanical result from integral-equation theory providing analytical expressions for the thermodynamic properties of a multicomponent mixture of unequal-sized ions dilute in a liquid continuum (Blum, 1975). This result is coupled with a perturbed Boublik-Mansoori expansion for nonadditive hard-sphere mixtures (Mansoori et al., 1971) and the osmotic virial expansion with the hard-sphere contribution removed (McMillan and Mayer, 1945). This coupling yields a statistical-thermodynamic model useful for predicting phase diagrams, (globular) protein-partition coefficients and electrostatic potential differences in polymer/polymer and, more importantly, polymer/salt aqueous two-phase systems.

Modeling of Multicomponent Electrolytic Solutions

The correlation or prediction of thermodynamic properties of multicomponent electrolytic solutions requires an appropriate molecular-thermodynamic theory. Theories of ionic behavior in solution have been developed in a number of ways, including empirical correlation and statistical mechanics. Semiempirical theories, such as that of Meissner and Tester (1972) and Meissner (1980), are not our concern here. Instead, we focus on those theories which are derived from the primitive model of molecular fluids using statistical mechanics. In the primitive model, ions are considered as charges embedded in hard spheres of specified diameter. Solvent molecules are not explicitly modeled; instead, the effects of solvent granularity are removed by averaging over all possible configurations of the solvent molecules. Through this averaging process, known as the McMillan-Mayer solution theory, the solvent is replaced by a continuum having the same macroscopic (dielectric) properties as those of the solvent.

For liquid solutions at normal temperatures and pressures, phase equilibria can be described by an appropriate activity-coefficient model. Useful activity-coefficient models for multicomponent aqueous mixtures of strong electrolytes having ions of similar size include those of Guggenheim and Turgeon (1955), Newman (1973), Haynes et al. (1989b), Bromley (1974), Cruz and Renon (1978), Chen et al. (1982), Chen and Evans (1986), Haghtalab and Vera (1988) and, most notably, Pitzer (1973, 1974). All of these models are based on the Debye-Hückel theory; therefore, they use a single ion-size (distance-of-closest-approach) parameter to describe all ion-ion interactions in solution. This approximation is satisfactory for systems where ion diameters differ by less than a factor of two, but agreement with the experiment quickly deteriorates as the ion-size asymmetry increases above two (Zemaitis et al., 1986). Therefore, activity-coefficient models based on the Debye-Hückel theory are of limited use in describing charge-charge interactions in aqueous two-phase systems, where the ratio of protein-macroion diameter to strong-electrolyte ion diameter is typically between 5 and 25.

Fortunately, integral-equation theory provides a powerful method for developing activity-coefficient models for multicomponent electrolytic solutions which explicitly include the (hydrated) diameter of each ion in the mixture (Hansen and McDonald, 1977; Friedman, 1985; Rasaiah et al., 1972; McQuarrie, 1976). Integral-equation theory provides a means for determining the pair correlation function $g_{ij}(r)$ describing the distribution of ions of type i around a central ion of type j from knowledge of the pair potential function $u_{ij}(r)$; the pair correlation functions so derived can then be used to determine useful thermodynamic properties of the solution.

The primitive model for electrolyte solutions belongs to a more general class of liquid theories based on solution of the Ornstein-Zernike integral equation:

$$h_{ij}(r) = C_{ij}(r) + \sum_k \rho_k \int_0^r C_{kj}(|r-r'|)\, h_{ik}(r')\, dr \tag{2}$$

where ρ_k is the number density of species k, and $C_{ij}(r)$ is the direct correlation function between particle i and particle j when the distance between these particles is r; $h_{ij}(r)$, the total correlation function, is a measure of the total influence of particle i on particle j; it is related to the pair correlation function by $h_{ij}(r) = g_{ij}(r) - 1$. Since $C_{ij}(r)$ and $h_{ij}(r)$ are both unknown, a second, approximate relation, known as a closure relation, is needed to transform Eq. 2 into a closed equation for $h_{ij}(r)$. A number of closure relations consistent with the primitive model can be found in the literature; the most successful of these are the mean-spherical approximation (MSA and its modifications) and the hypernetted-chain (HNC) approximation, which can be thought of as a second-order correction to the MSA. For a description of the aqueous solutions of 1-1 and 1-2 electrolytes, the HNC theory is slightly superior to the MSA theory for predicting thermodynamic properties. However, the HNC theory, which must be solved numerically for each solution condition, is not well suited for use in multicomponent phase-equilibrium calculations. The MSA theory is more appropriate for such calculations, in part, because its solution, even for unequal-sized ions, is analytical.

The mean-spherical approximation has been applied to the primitive model of electrolytes (Waisman and Lebowitz, 1970, 1972; Blum, 1975, 1980; Blum and Høye, 1977), primarily to correlate activity coefficients in aqueous electrolyte solutions (Triolo et al., 1976, 1978; Watanasiri et al., 1982; Ball et al., 1985; Corti, 1987). From this earlier work, the MSA with contributions from hard-sphere interactions explicitly included is known to yield good results for activity coefficients of 1-1 and 1-2 electrolytes up to moderate ion concentrations. However, at very dilute salt concentrations ($<1\times10^{-6}$ M), inaccuracies in the MSA can occur as a result of improper account of coupling forces.

In this work, we examine the applicability of the generalized MSA theory to the description of charge-charge interactions in aqueous solutions containing polymers, strong electrolytes, and globular-protein macroions (Anderson and Chandler, 1971, 1972; Chandler and Anderson, 1972).

The MSA closure relation is given by (McQuarrie, 1976)

$$\begin{aligned} C_{ij}(r) &= -u_{ij}(r)/kT \quad & r > d_{ij} \\ h_{ij}(r) &= -1, & r < d_{ij} \end{aligned} \tag{3}$$

where d_{ij} is the distance of closest approach between ions i and j. Results from MSA theory reveal an intricate interplay between ion size, charge and the thermodynamic properties of electrolytes in solution which is not captured by Debye-Hückel theory (Lee, 1988; Olivares and McQuarrie, 1975). As shown later, this interplay is essential to a quantitative description of protein and salt partitioning in aqueous two-phase systems.

For a multicomponent aqueous solution containing ions of arbitrary diameters and charges, MSA theory (obtained from simultaneous solution of Eqs. 2 and 3 through Fourier transforms) yields the following expression for the charge-charge contribution to the excess modified Helmholtz energy, $A'_{Ex,cc}$ (Blum, 1980; Harvey et al., 1988),

$$\frac{A'_{Ex,cc}}{V} = -\frac{e^2}{\epsilon}\left(\Gamma \sum_i \frac{\rho_i z_i^2}{1+d_i\Gamma} + \frac{\pi}{2\Delta}\Omega P_n^2\right) + \frac{\Gamma^3 kT}{3\pi} \tag{4}$$

$$4\Gamma^2 = \frac{\kappa^2 \sum_i \rho_i (1+d_i\Gamma)^{-2} (z_i - \pi d_i^2 P_n/2\Delta)^2}{\sum_i \rho_i z_i^2}$$

$$P_n = \frac{1}{\Omega}\sum_j \frac{d_j \rho_j z_j}{1+d_j\Gamma} \quad \text{and} \quad \Omega = 1 + \frac{\pi}{2\Delta}\sum_j \frac{\rho_j d_j^3}{1+d_j\Gamma}$$

$$\Delta = 1 - \frac{\pi}{6}\sum_j \rho_j d_j^3 \quad \text{and} \quad \kappa^2 = \frac{4\pi e^2}{\epsilon kT}\sum_j \rho_j z_j^2$$

where κ is the reciprocal Debye screening length, e is the electronic charge, k is Boltzmann's constant, ϵ is the permittivity of the solvent continuum, T is the absolute temperature, z_i is the valence of species i, d_j is the hard-sphere diameter of ion j and the sums extend over all ionic species. Γ is the MSA screening parameter, which reduces to $\kappa/2$ (that is, the Debye-Hückel limit) at infinite dilution. $A'_{Ex,cc}$ is relative to a mixture of uncharged hard spheres in a dielectric continuum.

Modified Helmholtz Energy of an Aqueous Mixture of Polymers, Salts, and Globular Proteins

The statistical-mechanical basis for ionic-solution models derived from the primitive model and for dilute-polymer-solution models such as the osmotic virial expansion lies ultimately in the dilute-liquid-solution theory developed by McMillan and Mayer. The independent variables for McMillan-Mayer theory are temperature, volume, chemical potential of the solvent (μ_o) and numbers of moles and solutes (n_i's). As recently established (Haynes et al., 1992a), application of statistical mechanics to electrolytic or dilute-polymeric solutions yields a modified Helmholtz energy $A' = A - n_o\mu_o$ (where A is the Helmholtz energy of the mixture). Thus, all other thermodynamic properties are derived from A', not A. The use of models derived in the McMillan-Mayer framework for correlating and predicting phase-equilibrium data therefore requires an understanding of the thermodynamic connection between the semigrand canonical ensemble (Hill, 1986), where the independent variables are T, V, μ_o, and all solute mole numbers, and experimental data, where temperature, pressure, and composition are the independent variables. Establishment of this connection is not merely of academic interest. Correct conversion of model equations to the constant-temperature constant-pressure framework often leads to quantitative improvements in the ability of models derived in the McMillan-Mayer framework to describe real-solution properties.

Here, we use the key results from Haynes et al. (1992a) to develop a free-energy model for aqueous solutions containing polymers, salts, and globular proteins.

Following conventional practice (for example, Prausnitz et al., 1986), we divide the modified Helmholtz energy A' into its ideal and excess contributions:

$$A' = A'_{id} + A'_{Ex} \tag{5}$$

where A'_{id}, the ideal modified Helmholtz energy for an incompressible liquid mixture, is given by (Haynes et al., 1992a)

$$\begin{aligned} A'_{id} = {} & \sum_{j\neq 0} n_j \left[a_j + \mathrm{RT}\ln\left(\frac{\overline{V}_j^\theta n_j}{V}\right)\right] \\ & + \frac{V - \sum_{j\neq 0} \overline{V}_j^\theta n_j}{\overline{V}_o^\theta}\left[a_o - \mu_o + \mathrm{RT}\ln\left(1 - \frac{\sum_{j\neq 0}\overline{V}_j^\theta n_j}{V}\right)\right] \\ = {} & \sum_{j\neq 0} n_j [a_j + \mathrm{RT}\ln\phi_j] + \frac{1 - \sum_{j\neq 0}\phi_j}{\overline{V}_o^\theta} \\ & \times \left[a_o - \mu_o + \mathrm{RT}\ln\left(1 - \sum_{j\neq 0}\phi_j\right)\right] \end{aligned} \tag{6}$$

In Eq. 6, $\phi_j\,(= c_j \overline{V}_j^\theta)$ and $\overline{V}_j^\theta$ are the volume fraction and partial molar volume of component j, respectively, and, by definition, $a_j = \mu_j^\theta - P\overline{V}_j^\theta$; a_j (which can be interpreted as a molar Helmholtz energy) is a function of temperature but, since the system was taken to be incompressible, not of pressure; μ_j^θ is the standard-state chemical potential of component j defined at the limit of vanishing solutes.

The excess modified Helmholtz energy, A'_{Ex}, is divided into five parts:

$$A'_{Ex} = A'_{Ex,hs} + A'_{Ex,na} + A'_{Ex,ic} + A'_{Ex,cc} + A'_{Ex,ve} \tag{7}$$

$A'_{Ex,hs}$ is the contribution to the excess modified Helmholtz energy due to mixing unequal-sized hard spheres in a continuum solvent. $A'_{Ex,na}$ provides a first-order hard-sphere nonadditivity correction to $A'_{Ex,hs}$. The inclusion of this term follows Abbott et al. (1991), who suggested that significant penetration of protein macroions into the volume occupied by polymer coils may occur in systems where the sizes of the two molecules are similar, or where the polymer occupies a larger volume than the protein macroion. $A'_{Ex,ic}$ is the contribution due to the ion-charging process; $A'_{Ex,cc}$ accounts for charge-charge interactions in solution and is given by Eq. 4. Finally, $A'_{Ex,ve}$ accounts for short-range interactions between solute molecules in solution; as shown later, $A'_{Ex,ve}$ is derived from the osmotic virial expansion truncated after the second-virial coefficient terms.

Additive hard-sphere contribution to A'_{Ex}

An accurate equation of state (EOS) for mixtures of additive hard spheres in vacuum has been derived by Boublik (1970) and by Mansoori et al. (1971). We can extend Boublik's EOS to account for the presence of continuum by applying the principles developed in Haynes et al. (1992a). The resulting EOS for a mixture of additive hard spheres in a continuum is given by

$$\frac{P}{kT}=\frac{P_o}{kT}+\frac{6}{\pi}\left[\frac{\xi_o'}{V-\xi_3'}+\frac{3\xi_1'\xi_2'}{(V-\xi_3')^2}+\frac{3(\xi_2')^3}{(V-\xi_3')^3}-\frac{\xi_3'(\xi_2')^3}{V(V-\xi_3')^3}\right] \tag{8}$$

where

$$\xi_m'=V\xi_m=\frac{\pi N_{Av}\sum_i n_i d_i^m}{6} \quad \text{with} \quad m=0,\ 1,\ 2,\ \text{or}\ 3$$

$P_o=(\mu_o-a_o)/\overline{V}_o^\theta$, and N_{Av} is Avogadro's number. In Eq. 8, the presence of the continuum is reflected in the P_o/kT term which, at infinite dilution, forces the total pressure to converge to the vapor pressure of the solvent. Integration of Eq. 8 with respect to volume gives A'_{hs}, the modified Helmholtz energy of a mixture of additive hard-spheres in a continuum. The constant of integration is determined by expanding the expression for A'_{hs} for large V and comparing the result with the corresponding form of A'_{id} for an ideal dilute fluid. $A'_{Ex,hs}$ is then given by

$$\begin{aligned}A'_{Ex,hs}&=A'_{hs}-A'_{id}\\&=-\mathrm{RT}\sum_i n_i\ln(1-\xi_3)-\frac{\mathrm{RT}}{\overline{V}_o^\theta}\sum_{i\neq 0}n_i\overline{V}_i^\theta\\&\quad-\frac{\mathrm{RTV}}{\overline{V}_o^\theta}\left(1-\sum_{i\neq 0}\phi_i\right)\ln\left(1-\sum_{i\neq 0}\phi_i\right)\\&\quad+\frac{6k\mathrm{TV}}{\pi}\left[\frac{3\xi_1\xi_2-(\xi_2)^3/\xi_3}{1-\xi_3}+\frac{(\xi_2)^3}{(\xi_3)^2}\ln(1-\xi_3)+\frac{(\xi_2)^3}{(1-\xi_3)^2}\right]\end{aligned} \tag{9}$$

Haynes et al. (1992a) provide a derivation of Eq. 9.

Nonadditive hard-sphere mixture correction contribution to A'_{Ex}

The Boublik-Mansoori EOS assumes that the distance of closest-approach between two molecules (like or unlike) is given by the sum of their hard-sphere radii:

$$d_{ij}=\frac{1}{2}(d_i+d_j) \tag{10}$$

where d_{ij} is the hard-sphere distance of closest approach between solutes i and j. This approximation seems reasonable for electrostatic interactions, which, in dilute solutions, occur over distances that are large compared to the size of the solutes. However, in aqueous two-phase systems, where the volume occupied by a random-coil polymer (for example, polyethylene glycol) chain can be comparable to that of a protein, the "true" distance of closest approach σ_{ij}, characterizing the nonelectrostatic interaction between polymer i and protein (or polymer) j, may be significantly less than d_{ij} since the large number of polymer-chain configurations allow the protein to penetrate partially the average volume occupied by the polymer (Abbott et al., 1991). The demonstrated importance of excluded-volume forces in aqueous two-phase systems (Haynes et al., 1989b; Cabezas et al., 1989; King et al., 1988; Forciniti and Hall, 1990) suggests that polymer-penetration effects can significantly influence phase diagrams and protein-partition coefficients.

Polymer-penetration effects are accounted for by incorporating a nonadditivity correction into the model. Perturbation theory can be used to relate the thermodynamic properties of a nonadditive mixture of hard spheres in a continuum to those of an additive hard-sphere system. Here, perturbation theory is used to expand the modified Helmholtz energy of a hard-sphere mixture in a continuum in a Taylor series with a set of expansion parameters $(\sigma_{ij}-d_{ij})$. Truncation of the expansion after the first-order perturbation terms gives (Haynes, 1992b; Harvey, 1988)

$$A'_{Ex,na}=A'_{na}-A'_{hs}=\frac{2\pi\mathrm{RT}}{V}\sum_{i\neq 0}\sum_{j\neq 0}n_i\frac{n_j}{\sum_{k\neq 0}n_k}d_{ij}^2g_{ij}(d_{ij})(\sigma_{ij}-d_{ij}) \tag{11}$$

where A'_{na} is the modified Helmholtz energy of a nonadditive hard-sphere mixture in a continuum solvent; $g_{ij}(d_{ij})$ is the contact value of the pair correlation function for species i and j in the *additive* hard-sphere system. A good estimate of $g_{ij}(d_{ij})$ for additive hard-sphere mixtures is given by Grundke and Henderson (1972):

$$g_{ij}(d_{ij})=\frac{1}{1-\xi_3}+\frac{3\xi_2}{2(1-\xi_3)^2}\left(\frac{d_id_j}{d_{ij}}\right)+\frac{\xi_2^2}{2(1-\xi_3)^3}\left(\frac{d_id_j}{d_{ij}}\right)^2 \tag{12}$$

Originally derived by Grundke and Henderson for hard-spheres in vacuo, this expression for $g_{ij}(d_{ij})$ is also applicable to a mixture of uncharged solute molecules in an uncharged continuum (Haynes, 1992b).

Ion-charging contribution to A'_{Ex}

By modeling ions as charged hard spheres in a continuum of uniform dielectric constant, Born (1920) calculated the reversible work required to charge an ion in solution. Following Born, we write the ion-charging contribution to the excess modified Helmholtz energy as

$$A'_{Ex,ic}=\frac{N_{Av}e^2}{\epsilon}\sum_i\frac{n_iz_i^2}{d_i} \tag{13}$$

where the sum extends over all ionic species.

Osmotic-virial-expansion contribution to A'_{Ex}

Several authors (Edmond and Ogsten, 1968; King et al., 1988; Haynes et al., 1989b; Forciniti et al., 1991; Cabezas et al., 1989) have demonstrated the applicability of models based on the osmotic virial expansion (or on the isobaric-isothermal virial expansion of Hill) to the prediction of polymer/polymer/water phase equilibria. For a multicomponent mixture of solutes dilute in a solvent, the osmotic virial expansion is given by (McMillan and Mayer, 1945; Krigbaum and Flory, 1953)

$$\Pi = \mathrm{RT}\left[\sum_{i\neq 0}\frac{n_i}{V} + N_{Av}\sum_{i\neq 0}\sum_{j\neq 0}\frac{n_i n_j}{V^2}B^*_{ij}(\mu_o, T)\right] \tag{14}$$

where Π is the osmotic pressure of the solution; $B^*_{ij}(\mu_o, T)$, which has units of liters, is the molecular osmotic second virial coefficient characterizing short-range two-body interactions between solutes i and j, and the expansion has been truncated after the second virial coefficient terms (Hill, 1986). Statistical-mechanical arguments provide a relation between $B^*_{ij}(\mu_o, T)$ and the potential of mean force $W_{ij}(\mu_o, T, r)$:

$$B^*_{ij}(\mu_o, T) = -\frac{1}{2}\int_0^\infty [e^{-W_{ij}(\mu_o, T, r)/kT} - 1]4\pi r^2 dr \tag{15}$$

where $W_{ij}(\mu_o, T, r)$ describes the interaction between molecules i and j held a fixed distance r apart when the remaining molecules, all solvent molecules, are canonically averaged over all configurations.

In our model, the hard-sphere contribution to $B^*_{ij}(\mu_o, T)$ is already accounted for in the nonadditive hard-sphere mixture terms (Eqs. 9 and 11). The hard-sphere contribution to $B^*_{ij}(\mu_o, T)$ can be determined by solving Eq. 15 for a mixture of hard spheres; the result is given by $[B^*_{ij}(\mu_o, T)]_{hs} = (1{,}000)2\pi\sigma^3_{ij}/3$. For example, when both i and j are nonionic polymers, we can split $B^*_{ij}(\mu_o, T)$ into two terms:

$$B^*_{ij}(\mu_o, T) = 1{,}000\frac{2\pi\sigma^3_{ij}}{3} + \beta^*_{ij}(\mu_o, T) \tag{16a}$$

where $\beta^*_{ij}(\mu_o, T)$ is a residual osmotic second virial coefficient describing specific nonelectrostatic and nonhard-sphere interactions between solutes i and j; $\beta^*_{ij}(\mu_o, T)$ is directly related to $\beta_{ij}(\mu_o, T)$, the specific-interaction coefficient (kg/mol) of Guggenheim. Similarly, for two-body interactions between protein macroions and/or ions of added electrolyte, we can define $\beta^*_{ij}(\mu_o, T)$ by splitting $B^*_{ij}(\mu_o, T)$ into three terms:

$$B^*_{ij}(\mu_o, T) = 1{,}000\frac{2\pi\sigma^3_{ij}}{3} + [B^*_{ij}(\mu_o, T)]_{el} + \beta^*_{ij}(\mu_o, T) \tag{16b}$$

where $[B^*_{ij}(\mu_o, T)]_{el}$ represents all contributions made to $B^*_{ij}(\mu_o, T)$ by electrostatic forces which are taken into account in the model by $A'_{Ex,cc}$ and $A'_{Ex,ic}$ (Eqs. 4 and 13).

Equation 14, with $B^*_{ij}(\mu_o, T)$ replaced by $\beta^*_{ij}(\mu_o, T)$, can be used to determine the virial-expansion contribution to the excess modified Helmholtz energy; the resulting expression for $A'_{Ex,ve}$ is given by

$$A'_{Ex} = \mathrm{RTN}_{Av}\sum_{i\neq 0}\sum_{j\neq 0} n_i\frac{n_j}{V}\beta^*_{ij}(\mu_o, T) \tag{17}$$

The procedure for deriving Eq. 17 from Eq. 14 is given in Haynes et al. (1992a).

Equations 4 through 17 provide a modified Helmholtz energy model applicable to the description of equilibrium properties of moderately concentrated aqueous solutions containing random-coil nonionic polymers, strong electrolytes, and globular proteins.

Table 1. Solute Hard-Sphere Diameters in Dilute Aqueous Solution at 25°C

Ion	d_{ii} (Å)	Polymer	d_{ii} (Å)	Protein	d_{ii} (Å)
Na^+	2.32	PEG 3350	24.6	Bovine serum albumin	59.6
K^+	3.04	PEG 8000	30.8	α-Chymotrypsin	36.0
Li^+	1.86	Dextran T-70	46.8	Lysozyme	30.4
Ca^{2+}	2.28	Dextran T-500	88.4		
Mn^{2+}	1.98				
Cl^-	3.62				
$H_2PO_4^-$	4.44				
HPO_4^{2-}	3.82				
HSO_4^-	3.66				
SO_4^{2-}	3.28				

Determination of Model Parameters

Application of Eq. 7 to a specific aqueous two-phase system requires knowledge of several parameters, including the hard-sphere diameter of each solute, the molar volume of the solvent, the partial molar volume at infinite dilution of each solute, the electrokinetic charge and valence of each ionic (or macroionic) species, the solvent dielectric constant, the osmotic second virial coefficient characterizing each solute i-solute i interaction, and the cross osmotic second virial coefficient characterizing each solute i-solute j interaction.

Charges and valences for ions of strong electrolytes are determined from the stoichiometry of the dissociation reaction; all strong electrolytes are assumed to be fully dissociated in aqueous solution. Protein macroion electrokinetic charges as a function of solution pH were taken from the literature (Tanford and Wagner, 1954; Tanford et al., 1955; Tanford, 1961; Sakakibara and Hamaguchi, 1968; Shiao et al., 1972; Horn and Heuck, 1983). The charge and hard-sphere diameter of the counterions to each protein were assumed to be the same as those of the corresponding ion of the added strong electrolyte.

Ion-ion (salt) specific-interaction coefficients, $\beta_{ij}(\mu_o, T)$'s, at 25°C were taken from the data of Guggenheim and Turgeon (1955), Guggenheim and Stokes (1958), and Newman (1973).

Hard-sphere diameters

Table 1 shows hard-sphere diameters for a number of ions of added strong electrolytes, nonionic random-coil polymers, and (globular) protein macroions. Cation hard-sphere diameters were taken from Robinson and Stokes (1954), Kraus (1949), Walden (1936), Harned and Owen (1958), and Burgess (1988). Anion hard-sphere diameters were regressed from mean-ionic activity-coefficient data for binary aqueous solutions containing the electrolyte at 25°C (Robinson and Stokes, 1954; Harned and Owen, 1958; Guggenheim and Stokes, 1958; Zemaitis et al., 1986).

The "hard-sphere" diameter of each random-coil polymer was determined by fitting to differential solvent vapor-pressure data (Haynes et al., 1989a) for the binary aqueous polymer solution; Eq. 5 yields the following expression for the solvent vapor-pressure difference ΔP between pure solvent and aqueous polymer solution

$$\frac{P_o-\Delta P}{P_o}=\exp\left\{-\frac{V_o}{N_{Av}}\frac{N_1}{V}\frac{(1+\phi_1+\phi_1^2)}{(1-\phi_1)^3}-\frac{V_o}{N_{Av}}\frac{N_1^2}{V^2}[B_{11}^*(\mu_o,\ T)-(1{,}000)2\pi\sigma_{11}^3]\right\} \tag{18}$$

where P_o is the vapor pressure of pure water at 25°C, N_1 is the number of polymer 1 chains, and V_o is the molar volume of the solvent; the first term inside the exponential is the Carnahan-Starling equation of state for a monodisperse hard-sphere system (which the Boublik-Mansoori hard-sphere EOS reduces to when applied to a single-solute-in-continuum system); ϕ_1 is the volume fraction of solute 1 based on the partial molar volume of the solute at infinite dilution; B_{11}^* is the molecular osmotic second virial coefficient (in units of liters) for the polymer obtained from low-angle laser-light scattering data (Rathbone et al., 1990; King et al., 1988).

Protein macroion hard-sphere diameters were taken from Tyn and Gusek (1990), who present effective hydrodynamic radii and diffusion coefficients for 86 globular proteins in water at 25°C.

Hard-sphere distance-of-closest-approach parameters, d_{ij}, are determined from the Boublik-Mansoori (Boublik, 1970; Mansoori, 1971) combining rule

$$d_{ij}=\frac{1}{2}(d_i+d_j) \tag{19}$$

which assumes that the hard-sphere diameters are additive.

Partial molar volumes

At infinite dilution, partial molar volumes for salts and proteins are widely available; for example, experimental values for salts can be found in Harned and Owen (1958), Guggenheim and Stokes (1958), and Millero (1971, 1972). For proteins, partial molar volumes at infinite dilution can be found in a number of sources including Creighton (1984), Jones (1979), Tanford (1961), and Goldberg (1984).

Infinite-dilution partial molar volumes for nonionic random-coil polymers were regressed from data for solution density as a function of polymer concentration using the relation (Newman, 1973)

$$\overline{V}_1^\theta=\frac{M_{n_1}-\left(\frac{\partial\rho}{\partial\hat{c}_1}\right)_{\hat{c}_1\to 0}}{\rho-\hat{c}_1\left(\frac{\partial\rho}{\partial\hat{c}_1}\right)_{\hat{c}_1\to 0}} \tag{20}$$

where ρ is the solution density (g/L) and M_{n_1} is the number-average molecular weight of the polymer fraction (g/mol). Measured infinite-dilution partial molar volumes are 3.12±0.20 L/mol for PEG 3350 ($M_{n_1}=3{,}790$), 8.56±0.25 L/mol for PEG 8000 ($M_{n_1}=9{,}037$), 25.7±0.50 L/mol for dextran T-70 ($M_{n_1}=29{,}630$), and 151.0±2 L/mol for dextran T-500 ($M_{n_1}=167{,}000$). Each measurement was done at 25°C and the data represent the arithmetic average of four independent experiments.

Polymer and protein osmotic second virial coefficients

Static low-angle laser-light scattering (LALLS) measurements were made with an LDC/Milton-Roy KMX-6 LALLS photometer according to the procedure of Rathbone et al. (1990). Refractive-index increments were measured with an LDC/Milton-Roy KMX-16 laser differential refractometer following the procedure given by Rathbone et al. (1990). All experiments were for dilute, binary, aqueous polymer solutions and for aqueous electrolyte (buffer) solutions containing a globular protein. All measurements were at 25°C.

Rayleigh theory provides the relation for determining the polymer (molar) osmotic second virial coefficient, B_{ii} (mL mol/g^2) from experimental values of the reduced Rayleigh ratio at several polymer concentrations:

$$\frac{Kc_i}{\overline{R}_\theta}=-\frac{1}{1{,}000\,\mathrm{RTV}_o}\left(\frac{\partial\mu_o}{\partial c_i}\right)_{T,P}=\frac{1}{M_{w_i}}+2B_{ii}c_i \tag{21}$$

where $\overline{R}_\theta$ is the reduced Rayleigh ratio, c_i is the mass concentration of solute i, M_{w_i} is the weight-average molecular weight of the polymer, and K (cm^2 mol/g^2), an optical constant characteristic of the polymer and the solvent, is given by

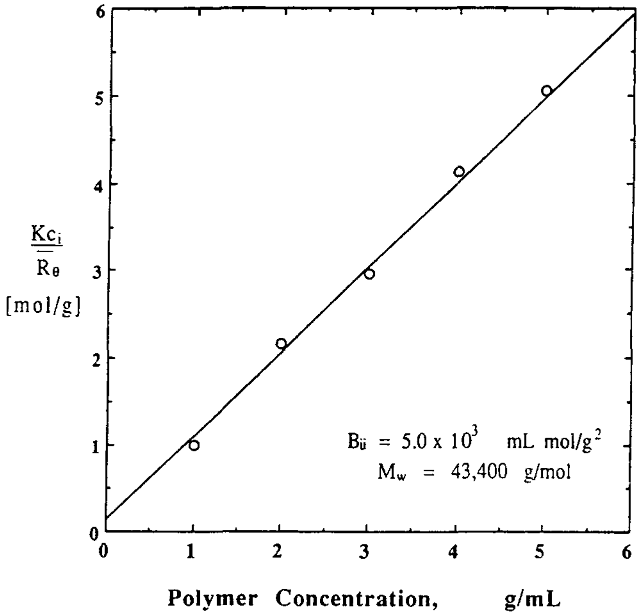

Figure 1. Low angle laser-light scattering data for dextran T-40 in water at 25°C.

Table 2. Osmotic Second Virial Coefficients, Specific Refractive Index Increments, and Weight-Average Molecular Weights for Polyethylene Glycol and Dextran Fractions in Water at 25°C*

Polymer	M_w (g/mol)	$B_{ii} \times 10^4$ (mL·mol/g^2)	ν_i (cm^3/g)
PEG 3350	3,860	36.3	0.131
PEG 8000	11,700	30.3	0.132
Dextran T-10 (lot# 30170)	10,100	8.2	0.152
Dextran T-40 (lot# 38205)	43,400	5.0	0.151
Dextran T-70 (lot# 07340)	68,100	4.0	0.147
Dextran T-500 (lot# 38624)	508,900	1.3	0.147

*From LALLS measurements.

$$K=\frac{2n_o^2\pi^2\nu_i^2(1+\cos^2\theta)}{\lambda^4 N_{Av}} \tag{22}$$

where n_o is the refractive index of the solvent, λ is the wavelength of the incident light (cm), and ν_i (mL/g) is the refractive-index increment at infinite dilution.

As shown in Figure 1 for dextran T-40 in water at 25°C, the osmotic second virial coefficient and weight-average molecular weight for each polymer fraction i were determined from a plot of $\overline{Kc_i/R_\theta}$ as a function of polymer concentration.

Table 2 reports experimental osmotic second virial coefficients, weight-average molecular weights and specific refractive-index increments for two polyethylene glycol fractions and four dextran fractions. Additional nonionic polymer virial coefficients and weight-average molecular weights from LALLS measurements have been reported previously (Rathbone et al., 1990; King et al., 1988).

Osmotic second virial coefficients and weight-average molecular weights were also measured for 15 PEG fractions chemically modified to carry charge on the ends of each chain. The chemically modified PEG fractions were kindly provided by Dr. Maria Kula (1990), who used them to study polyelectrolyte charge effects on protein partitioning. Table 3 shows osmotic second virial coefficients and weight-average molecular weights for all of the modified polymers in water and for several of the modified polymers in 10-mM and 100-mM potassium phosphate buffer.

Table 3. Osmotic Second Virial Coefficients and Weight-Average Molecular Weights for Several Polyethylene-Glycol Based Polyelectrolytes in Water and in Potassium Phosphate Buffer at 25°C

		$B_{ii} \times 10^4$ (mL·mol/g^2)		
Polymer Fraction	M_w (g/mol)	Pure Water	10-mM Buffer	0.1-M Buffer
NH_2-PEG 1550	2,380	2.49	1.94	1.85
″ 4000	3,840	2.14	1.69	1.20
″ 6000	5,780	2.07	1.95	1.85
″ 10000	18,700	2.69		1.35
″ 20000	29,000	2.77		1.22
″ 35000	36,400	2.48		1.44
Cl-PEG 10000	12,300	1.18		
″ 20000	22,700	1.42		
Br-PEG 20000	23,700	1.45		
COOH-PEG 20000	22,200	1.32		
NH_2-MPEG 5000	5,610	3.37		
Cl-MPEG 5000	5,760	2.21		
Br-MPEG 5000	6,620	1.73		

Table 4. Osmotic Second Virial Coefficients and Weight-Average Molecular Weights for *Bovine Serum Albumin* in Aqueous Buffer (or Salt) Solutions at 25°C*

Buffer Salt (or Salt)	Salt Conc. (mol/kg)	Molal Ionic Strength (mol/kg)	pH	$B_{ii} \times 10^4$ (mL·mol/g^2)	M_w (g/mol)
Potassium Phosphate	0.050	—	6.0	1.8	86,300
″	0.100	—	6.0	1.6	″
Potassium Phosphate	—	0.075	7.0	8.3	86,600
″	—	0.140	7.0	6.1	″
″	—	0.280	7.0	3.7	″
″	—	0.400	7.0	2.5	″
″	—	0.500	7.0	2.4	″
Potassium Phosphate	0.050	—	8.0	6.9	86,300
″	0.100	—	8.0	6.1	″
Sodium Phosphate	—	0.075	7.0	8.6	88,200
″	—	0.150	7.0	6.4	″
″	—	0.300	7.0	3.9	″
″	—	0.400	7.0	2.8	″
″	—	0.500	7.0	2.6	″
″	—	1.000	7.0	2.6	″
KCl	0.050	—	~7	8.7	89,000
NaCl	0.050	—	~7	8.9	89,000

*From LALLS measurements.

Table 5. Osmotic Second Virial Coefficients and Weight-Average Molecular Weights for *Lysozyme* in Aqueous Buffer (or Salt) Solutions at 25°C*

Buffer Salt (or Salt)	Salt Conc. (mol/kg)	Molal Ionic Strength (mol/kg)	pH	$B_{ii} \times 10^4$ (mL·mol/g^2)	M_w (g/mol)
Potassium Phosphate	0.050	—	6.0	0.4	17,900
″	0.100	—	6.0	−2.7	″
Potassium Phosphate	—	0.075	7.0	4.1	17,900
″	—	0.140	7.0	−2.5	″
″	—	0.280	7.0	−7.2	″
″	—	0.400	7.0	−10.0	″
″	—	0.500	7.0	−10.0	″
Potassium Phosphate	0.050	—	8.0	−3.6	18,200
″	0.100	—	8.0	−7.4	″
Sodium Phosphate	—	0.075	7.0	4.8	18,000
″	—	0.150	7.0	−1.9	″
″	—	0.300	7.0	−6.7	″
″	—	0.400	7.0	−9.8	″
″	—	0.500	7.0	−11.0	″
″	—	1.000	7.0	−11.0	″
KCl	0.050	—	~7	5.8	18,200
NaCl	0.050	—	~7	6.1	18,200

*From LALLS measurements.

Table 6. Osmotic Second Virial Coefficients and Weight-Average Molecular Weights for *α-chymotrypsin* in Aqueous Buffer (or Salt) Solutions at 25°C*

Buffer Salt (or Salt)	Salt Conc. (mol/kg)	Molal Ionic Strength (mol/kg)	pH	$B_{ii}\times 10^4$ (mL·mol/g²)	M_w (g/mol)
Potassium Phosphate	0.050	—	6.0	−6.3	26,600
"	0.100	—	6.0	−3.3	"
Potassium Phosphate	—	0.075	7.0	−26.0	26,600
"	—	0.140	7.0	−13.0	"
"	—	0.280	7.0	−4.9	"
"	—	0.400	7.0	−3.5	"
"	—	0.500	7.0	−2.8	"
"	—	1.000	7.0	−2.3	"
"	—	1.500	7.0	−2.2	"
Potassium Phosphate	0.050	—	8.0	−16.0	26,600
"	0.100	—	8.0	−5.8	"
Sodium Phosphate	—	0.075	7.0	−21.0	26,800
"	—	0.150	7.0	−9.8	"
"	—	0.300	7.0	−3.9	"
"	—	0.400	7.0	−2.9	"
"	—	0.500	7.0	−2.2	"
"	—	1.000	7.0	−1.9	"
KCl	0.050	—	~7	−13.0	26,200
NaCl	0.050	—	~7	−13.0	26,200

* From LALLS measurements.

For LALLS measurements on proteins, the aqueous buffer solution serves as the solvent. The specific refractive-index increment was determined for each aqueous buffer (or salt-enriched) solution. Then, $Kc_i/\overline{R}_\theta$ values were measured by LALLS over a range of protein concentrations. Here, $\overline{R}_\theta$ is the reduced Rayleigh ratio describing the intensity of the protein-protein interaction at the given solution pH.

Tables 4, 5 and 6 report osmotic second virial coefficients and weight-average molecular weights for bovine serum albumin, lysozyme, and α-chymotrypsin, respectively, in a number of aqueous buffer (salt) solutions at 25°C.

Table 7. Cross Osmotic Second Virial Coefficients for Several Polymer Pairs in Water at 25°C

Polymer Pair	$B_{ii}\times 10^4$ (mL·mol/g²)
PEG 3350: Dextran T-70	17.3
PEG 3350: Dextran T-500	13.6
PEG 8000: Dextran T-500	12.7
Dextran T-10: MOPEG 5	12.1
Dextran T-70: MOPEG 5	9.5
Dextran T-70: PVP 10	9.1
Dextran T-70: PVP 40	5.6
Dextran T-70: PVP 360	3.0
Dextran T-70: PVA 14	6.1
Dextran T-70: MC 41	7.5
Dextran T-70: Aquaphase PPT	2.8
Dextran T-500: MOPEG 5	9.6
MOPEG 5: PVP 10	15.3
MOPEG 5: PVP 40	10.9
MOPEG 5: PVP 360	8.0

Table 8. Cross Osmotic Second Virial Coefficients for Several Polymer-Protein Pairs in Aqueous Potassium-Phosphate Buffer Solutions at 25°C*

Polymer *i* Protein *j*	PEG 3350	PEG 8000	Dextran T-70	Dextran T-500
Albumin	(8.0±0.1)	(5.5±0.2)	(2.6±0.1)	(1.5±0.1)
pH 7: 50 mM buffer	8.0	5.6	2.6	1.5
pH 7: 100 mM buffer	8.0	5.6	2.6	1.5
pH 6: 50 mM buffer	8.0	5.5	2.6	1.5
pH 6: 100 mM buffer	7.9	5.5	2.6	1.6
Lysozyme	(3.3±0.1)	(3.0±0.2)	(2.3±0.1)	(1.9±0.1)
pH 7: 50 mM buffer	3.3	3.0	2.3	1.9
pH 7: 100 mM buffer	3.4	2.9	2.2	1.9
pH 6: 50 mM buffer	3.3	3.1	2.3	1.8
pH 6: 100 mM buffer	3.4	3.0	2.3	1.9
α-Chymotrypsin	(5.6±0.2)	(4.2±0.1)	(2.4±0.1)	(1.6±0.1)
pH 7: 50 mM buffer	5.5	4.2	2.3	1.6
pH 7: 100 mM buffer	5.6	4.2	2.4	1.6
pH 6: 50 mM buffer	5.7	4.1	2.4	1.6
pH 6: 100 mM buffer	5.6	4.2	2.4	1.6

* For each polymer-protein pair, an average $B_{ij}\times 10^4$ (mL·mol/g²), determined from the set of LALLS data, is reported in parentheses.

The specific-interaction coefficient, β_{ii}, for each polymer *i* or protein *i* can be regressed from its osmotic second virial coefficient and (number-average) molecular weight by relating the model (as written for a binary solution) and the osmotic virial expansion truncated after the second virial coefficient term:

$$\Pi = 1{,}000\,\mathrm{RT}\,c_i\left(\frac{1}{M_{n_i}} + B_{ii}c_i\right) = \mathrm{RT}\,\hat{c}_i - \left(\frac{\partial A'_{Ex}}{\partial V}\right)_{\mu_o, n_j (j\neq 0), \Gamma} \tag{23}$$

where Π is the osmotic pressure of the mixture, and A'_{Ex} is a function of β_{ii} as shown in Eqs. 7 and 17. All other model parameters for the binary system must be known to regress a β_{ii} parameter using Eq. 23; for binary dilute polymer solutions, the hard-sphere diameter of the polymer is the only other model parameter. For dilute aqueous protein solutions at a given pH, the hard-sphere diameter and electrokinetic charge of the protein must be known, as well as the total ionic strength of the solution.

Cross osmotic second virial coefficients for macromolecules

Tables 7, 8, and 9 report experimental cross osmotic second virial coefficients for several polymer pairs, polymer-protein pairs, and (globular) protein pairs, respectively. With the individual osmotic second virial coefficients, weight-average molecular weights and specific refractive-index increments known for the two macromolecules, the cross osmotic second virial coefficient, B_{ij}, was determined from the limiting slope of a plot of $K'(c_i+c_j)/\overline{R}_\theta$ as a function of total polymer concentration (c_i+c_j) according to the linear relationship (Kratochvil et al., 1975),

$$\frac{K'(c_i+c_j)}{\overline{R}_q} = m(c_i+c_j) + b \tag{24}$$

where

$$m \equiv \frac{2\nu_1^2 M_{w_i}^2 w_1^2 B_{ii}^2 + 4\nu_i\nu_j M_{w_i} M_{w_j} w_i w_j B_{ij} + 2\nu_j^2 M_{w_j}^2 w_j^2 B_{jj}^2}{(\nu_1^2 M_{w_i} w_i + \nu_j^2 M_{w_j} w_j)}$$

Table 9. Cross Osmotic Second Virial Coefficients for Several Protein-Protein Pairs in Aqueous Potassium-Phosphate Buffer Solutions at 25°C*

Protein *j* \ Protein *i*	*α-Chymotrypsin*	*Lysozyme*	*Albumin*
Albumin			
pH 7: 50 mM buffer	−2.3	−4.3	6.5
pH 7: 100 mM buffer	−1.1	−2.1	4.7
pH 6: 50 mM buffer	−1.6	−3.8	1.8
pH 6: 100 mM buffer	−0.5	−1.7	1.6
Lysozyme			
pH 7: 50 mM buffer	0.5	2.5	
pH 7: 100 mM buffer	−0.2	−1.9	
pH 6: 50 mM buffer	0.8	0.4	
pH 6: 100 mM buffer	0.3	−2.7	
α-Chymotrypsin			
pH 7: 50 mM buffer	−18.0		
pH 7: 100 mM buffer	−11.0		
pH 6: 50 mM buffer	−6.3		
pH 6: 100 mM buffer	−3.3		

* All data are determined from LALLS measurements and are reported as $B_{ij} \times 10^4$ (mL·mol/g^2).

and

$$b \equiv \frac{1}{v_i^2 M_{w_i} w_i + v_j^2 M_{w_j} w_j}$$

In Eq. 24, K' (mol/cm^4) is K/v_i^2 and w_i is the weight fraction of polymer fraction i, defined as $c_i/(c_i+c_j)$. Equation 24 is valid only when the ratio of polymer concentrations, c_i/c_j, is held constant for all measured values of $\overline{R}_\theta$.

The cross specific-interaction coefficient, β_{ij}, is regressed from the cross osmotic second virial coefficient for the macromolecule pair by relating the model (as written for a ternary solution) with the osmotic virial expansion truncated after the second virial coefficient term:

$$\Pi = 1{,}000\,\mathrm{RT}\sum_i c_i\left(\frac{1}{M_{n_i}} + \sum_j B_{ij} c_j\right)$$

$$= \mathrm{RT}\sum_i \hat{c}_i - \left(\frac{\partial A'_{\mathrm{Ex}}}{\partial V}\right)_{\mu_o, n_j\,(j\neq 0),\,\Gamma} \qquad (25)$$

Table 10. Salt-Polymer Specific-Interaction Coefficients, β_{ij} (kg/mol), Characterizing Nonelectrostatic Interactions Between Electrolyte *i* and Polymer *j* in Aqueous Solution at 25°C

Salt \ Polymer	PEG 3350	PEG 8000	Dextran T-70	Dextran T-500
NaH_2PO_4	2.69	11.45	0.69	1.96
Na_2HPO_4	1.94	8.15	0.48	1.71
KH_2PO_4	2.67	11.36	0.68	1.90
K_2HPO_4	1.93	8.10	0.48	1.61
$NaHSO_4$	3.44	14.43	0.72	2.48
Na_2SO_4	2.47	10.30	0.68	1.88
$KHSO_4$	3.42	14.33	0.72	2.40
K_2SO_4	2.45	10.21	0.68	1.75
NaCl	0.24	1.30	0.01	0.04
KCl	0.00	0.00	0.00	0.00

where the regression also requires values for the individual solute virial coefficients and specific-interaction coefficients.

Macromolecule-salt cross specific-interaction coefficients

Studies by Huglin (1972) showed that low-angle laser-light scattering can be used to obtain a qualitative understanding of the strength of salt-macromolecule interactions in aqueous solution. However, a quantitative interpretation of the light-scattering data is hindered by the relatively weak scattering intensity of the ions of the strong electrolyte, whose molecular size is commensurate with that of the solvent.

Fortunately, Scatchard and coworkers (1946, 1954), Cassassa and Eisenberg (1960, 1964), and Tombs and Peacocke (1974) have established a thermodynamic framework for regressing macromolecule-salt cross coefficients from vapor-pressure osmometry or membrane-osmometry data. Table 10 reports cross specific-interaction coefficients for nonionic polymer-salt pairs. Polymer-salt β_{ij} parameters were regressed directly from vapor-pressure osmometry data for ternary aqueous solutions containing nonionic polymer i and strong electrolyte j. A Knauer model A-0280 vapor-pressure osmometer was used for all solvent vapor-pressure measurements.

Table 11 reports cross specific-interaction coefficients for (globular) protein-salt pairs. Protein-salt β_{ij} parameters were regressed directly from membrane-osmometry data for ternary aqueous solutions containing protein i and strong electrolyte j. A Knauer membrane osmometer was used for all osmotic-pressure measurements. Experimental details of all new osmotic-pressure and vapor-pressure data can be found elsewhere (Haynes, 1992b; Haynes et al. 1992c; Haynes et al., 1989b; Knauer A-0280 vapor-pressure osmometer operations manual, 1988; Knauer membrane-osmometry operations manual, 1990).

Following Scatchard et al. (1946, 1954), the solvent vapor pressure and the osmotic pressure of a dilute ternary aqueous solution containing one diffusible solute (3) and one nondiffusible macroion (2) are related to the chemical potentials of the solute species by

$$-\frac{RT}{V_o}\left[\frac{d\ln\left(\dfrac{P_o-\Delta P}{P_o}\right)}{dc_2}\right] = \frac{d\Pi}{dc_2}$$

$$= \frac{m_2}{M_{n_2}}\left[\left(\frac{\partial \mu_2}{\partial m_2}\right)_{T,P,m_3} - \frac{\left(\dfrac{\partial \mu_2}{\partial m_3}\right)^2_{T,P,m_2}}{\left(\dfrac{\partial \mu_3}{\partial m_3}\right)_{T,P,m_2}}\right] \times 10^6 \qquad (26)$$

where m_i is the molality (mol/kg solvent) of solute i. Equation 26 was used to regress macromolecule-salt cross specific-interaction coefficients from solvent vapor-pressure data or osmotic-pressure data; each solute chemical potential was determined from appropriate differentiation of A'. In deriving Eq. 26, Scatchard assumed that the solution was dilute and incompressible, and that contributions from the partial molar volume of the small diffusible solute are negligible.

Figure 2 shows experimental and calculated osmotic pressures as a function of protein concentration for a ternary

Table 11. Cross Specific-Interaction Coefficients, β_{ij} (kg/mol), for(Globular) Protein-Salt Pairs in Aqueous Solution at 25°C

Salt	*Bovine Serum Albumin*	*α-Chymotrypsin*	*Lysozyme*
NaH_2PO_4	3.48	1.96	1.69
Na_2HPO_4	3.36	1.75	1.53
KH_2PO_4	3.46	1.89	1.63
K_2HPO_4	3.30	1.70	1.47
$NaHSO_4$	4.83	2.31	1.88
Na_2SO_4	4.36	2.18	1.65
$KHSO_4$	4.76	2.25	1.83
K_2SO_4	4.31	2.10	1.60
NaCl	0.83	0.46	0.29
KCl	0.76	0.40	0.25

aqueous system at pH 8 and 25°C containing bovine serum albumin and 50-mM potassium-phosphate buffer. Osmotic pressures were calculated using Eqs. 4 through 17 and the parameters in Tables 1, 4 and 11. The excellent agreement of the model calculations with experiment suggests that the model may also be useful for correlating osmotic pressures of other aqueous (globular) protein solutions.

Calculation of Liquid-Liquid Equilibria

Consider a liquid (α)-liquid (β) system at temperature T and pressure P containing nonelectrolytes and electrolytes. At equilibrium, the chemical potential of each component present in both phases must be the same in the two phases. The chemical potential for any nonelectrolytic solute j is determined from A' by

$$\mu_j = \left(\frac{\partial A'}{\partial n_j}\right)_{\mu_o, n_i (i \neq j,0), T, V} \tag{27}$$

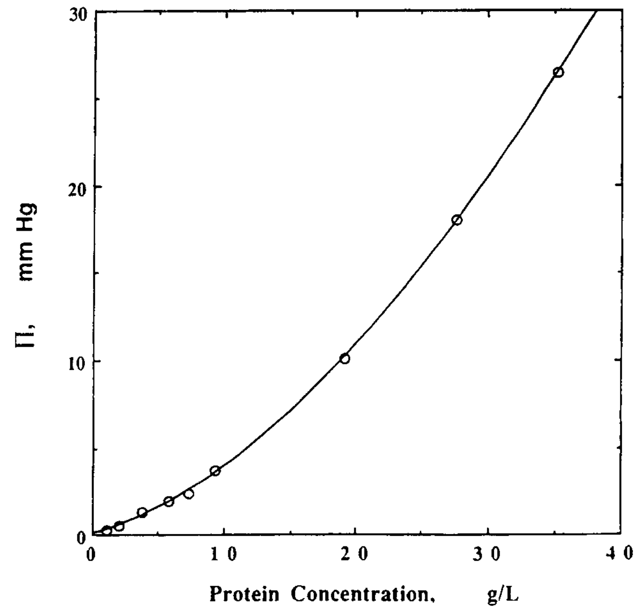

Figure 2. Experimental and calculated osmotic pressures for *bovine serum albumin* in a ternary aqueous solution at pH 8.0 and 25°C containing 50-mM potassium phosphate buffer.

Equation 27 also applies to any ion j present in both phases; in that case, however, μ_j is the electrochemical potential of ion j (as defined by Guggenheim (1959)) which depends on the electrical state of phase.

Since each bulk phase is, on average, electrically neutral, ions must move from phase α to phase β in neutral combinations. Although the work of transferring an ion i from one phase to another depends on the electrical states of the two phases, the work of reversibly transferring at constant temperature and volume a *neutral combination of ions* from phase α to phase β does not. For a solution containing a strong electrolyte (or protein) j which has dissociated into a cationic species ($i = +$) and an anionic species ($i = -$), we define the molar *chemical* potential of the neutral salt μ_j as

$$\begin{aligned} \mu_j &= \nu_+\mu_+ + \nu_-\mu_- \\ &= \nu_+\mu_+^\theta + \nu_-\mu_-^\theta + \mathrm{RT}\ln(\nu_+^{\nu_+}\nu_-^{\nu_-}) + \nu\mathrm{RT}\ln(\hat{c}_j\gamma_{\pm j}) \end{aligned} \tag{28}$$

where $\gamma_\pm$ is the molar mean-ionic activity coefficient. Equation 28 is used to determine the chemical potential of each neutral salt formed from the set of ions in solution. Equilibrium tie-lines and compositions are calculated by forcing the chemical potential of each neutral component to be the same in the two phases.

Experimental Phase Diagrams and Partition Coefficients

Experimental phase diagrams

Equilibrium phase compositions in polymer/polymer and polymer/salt aqueous two-phase systems were determined using size-exclusion high-performance liquid chromatography (HPLC). The HPLC used a three-column configuration comprised of one Bio-Gel TSK-40 30-cm column and two Bio-Gel TSK-30 30-cm columns connected in series. Dextran concentrations were measured with a Perkin-Elmer 241 Polarimeter using a specific optical rotation of +199.5°. The protocol followed was the same as that of King et al. (1988).

Experimental solute partition coefficients

Salt partition coefficients were determined by measuring cation concentrations in each phase using either a Perkin-Elmer model 2280 or model 2380 atomic-absorption spectrophotometer. Cation emission intensity was detected at a wavelength of 589.5 nm and a slit width of 0.2 or 0.4 nm. Potassium chloride was added to each sample containing Na^+ to prevent ion pairing, and an ionization impact bead was used to improve sensitivity.

Protein concentrations in aqueous buffer solutions were measured spectrophotometrically with a Milton-Roy Spectronic 1201 spectrophotometer at a static wavelength of 280 nm. The extinction coefficients, $E_{280}^{1\%}$ (cm^2/mg), used for lysozyme, α-chymotrypsin, and bovine serum albumin are 2.55, 2.00 and 0.66, respectively.

Model Predictions

Table 12 reports σ_{ij} values, which represent the true distance of closest approach between solutes i and j, regressed from equilibrium-composition data for ternary and quaternary

Table 12. True Distance of Closest Approach Parameters, σ_{ij} (Å), for Polymer-Solute Interactions in Aqueous Solution at 25°C

Polymer *i* Solute *j*	PEG 3350	PEG 8000	Dextran T-70	Dextran T-500
Na^+	13.1	16.1	16.7	16.9
K^+	13.2	16.4	17.6	17.6
Cl^-	13.3	16.5	17.9	18.4
$H_2PO_4^-$	13.8	17.1	18.4	18.8
HPO_4^{2-}	13.8	17.0	18.6	18.6
HSO_4^-	13.6	16.7	18.1	18.5
SO_4^{2-}	13.7	16.7	18.5	18.8
PEG 3350	22.9	25.3	27.3	41.2
PEG 8000	25.3	28.1	29.4	44.2
Dextran T-70	27.3	29.4	33.6	45.5
Dextran T-500	41.2	44.2	45.5	53.6
Bovine serum albumin	41.6	43.9	45.3	59.2
Chymotrypsin	28.1	31.3	27.5	44.6
Lysozyme	24.6	28.3	24.4	43.8

aqueous two-phase systems. PEG-PEG and dextran-dextran σ_{ij} values were regressed from dilute ternary osmotic-pressure data, and Dextran-PEG σ_{ij}s from the minimum experimental tie-line length for the salt-free system. All salt-polymer and salt-protein σ_{ij} values were regressed from dilute ternary vapor-pressure-osmometry data. Finally, protein-polymer σ_{ij}s were regressed from the experimental partition coefficient for the protein at the minimum tie-line length studied.

The A' model given in Eqs. 4 through 17, the model parameters shown in Tables 1 through 12, and the liquid-liquid equilibria framework outline above were used to calculate phase diagrams, salt and protein partition coefficients, and (as discussed below) interfacial-electrostatic potential differences for a number of industrially important aqueous two-phase systems. All of the data in Tables 1 through 12 have been used in model calculations on aqueous two-phase systems. However, for brevity, we present here only a sample of calculated results which serve to illustrate the flexibility and accuracy of the model.

All model calculations are based on simultaneous solution of the equilibrium criteria

$$\mu_j^\alpha = \mu_j^\beta \tag{29}$$

for each neutral component j present in the two phases. For charges species (such as proteins), the neutral component j is formed by the appropriate combination of protein macroions and counterions. Equation 28 gives μ_j for each neutral combination so defined. The phase rule gives the total number of independent neutral combinations which can be formed from the charged species in solution.

Since the concentration of each protein appears explicitly in the A' model, the calculations presented below consider the influence of proteins and protein-protein interactions on phase behavior and salt partition coefficients. This is an important departure from previous models, such as the models of King et al. (1988), Forciniti and Hall (1990), and Abbott et al. (1991), which rely on the assumptions that the protein is infinitely dilute and that salts have no effect on the polymer distribution coefficients. As shown below, proteins, at concentrations 0.1 wt. % and above, can noticeably influence phase diagrams and salt partition coefficients. These effects may prove important in industrial aqueous two-phase extraction systems, where protein concentrations are often much larger than 1 wt. % (Kula et al., 1982).

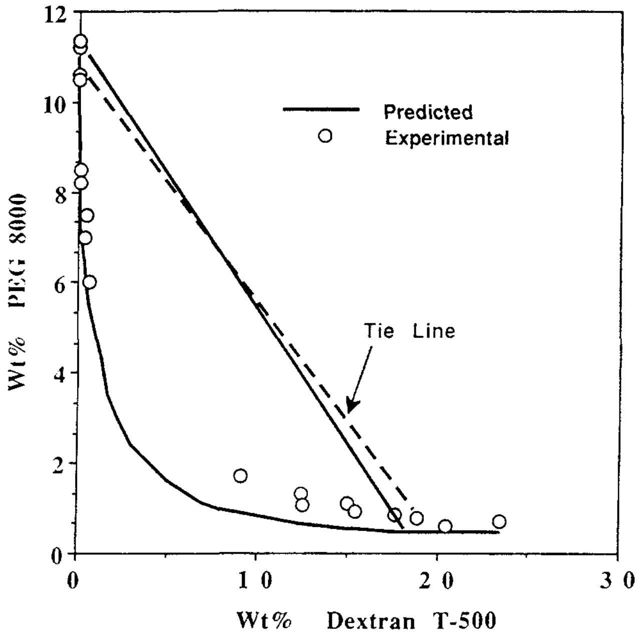

Figure 3. Experimental and calculated phase diagrams for the poly(ethylene glycol) 8000/dextran T-500 aqueous two-phase system at 25°C.

Phase diagrams

Figure 3 shows experimental and calculated phase diagrams for the polyethylene glycol 3350/dextran T-70 aqueous two-phase system at 25°C. Aqueous mixtures of PEG 3350 and dextran T-70 having a total composition which lies above the coexistence curve separate into two liquid phases, while mixtures with total compositions below the coexistence curve give one liquid phase. The length of the tie-line which connects the total composition with the compositions of the resulting equilibrium phases provides a convenient parameter for correlating data. The tie-line length TLL is a measure of the composition difference between the two equilibrium phases; it is defined as

$$TLL = \sqrt{(\text{wt. }\%_{PEG}^{\alpha} - \text{wt. }\%_{PEG}^{\beta})^2 + (\text{wt. }\%_{dextran}^{\alpha} - \text{wt. }\%_{dextran}^{\beta})^2} \tag{30}$$

Model predictions are in good agreement with the experiment over the entire range of tie-line lengths applicable to protein-extraction systems. Moreover, model predictions are significantly better than those based on our earlier model calculations using the osmotic virial expansion truncated after the second virial coefficient terms (King et al., 1988; Haynes et al., 1989b). The superiority of this model for calculating phase diagrams for polymer/polymer aqueous two-phase systems is partly due to the incorporation of polymer penetration effects. As shown in Table 12, for all random-coil polymer pairs, the "true" distance of closest approach σ_{ij} between polymer coils is smaller than the corresponding hard-sphere distance of the closest approach indicating partial penetration of one polymer chain into the average volume occupied by the other chain.

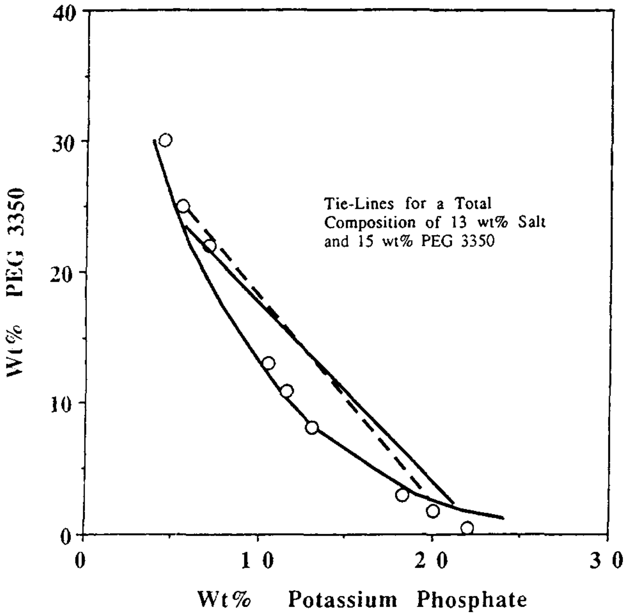

Figure 4. Experimental and calculated phase diagrams for the poly(ethylene glycol) 3350/potassium phosphate aqueous two-phase system at 25°C.

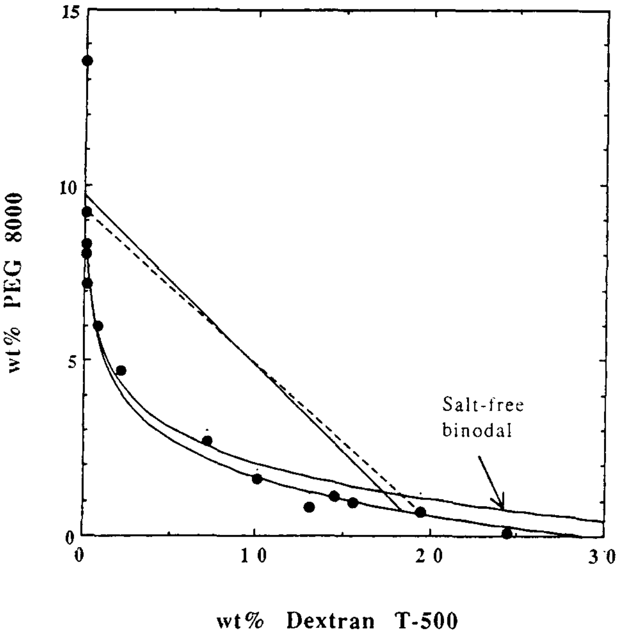

Figure 5. Experimental and calculated phase diagrams for the poly(ethylene glycol) 8000/dextran T-500 aqueous two-phase system at 25°C containing no salt (upper calculated curve) or 50-mM KH_2PO_4 (lower 'unmarked' calculated curve).

The dashed tie-line is an experimental tie-line in the salt containing system; the solid tie-line is the calculated tie-line for the same system.

As suggested by the scaling-thermodynamics results of Abbott et al. (1991), the predictive abilities of the model become progressively worse with increasing molecular weight of the PEG fraction. Abbott et al. postulates that a transition in the conformation of PEG in aqueous solution from a compact, random-coil structure to a loose, entangled polymer network occurs upon increasing the polymer's molecular weight above 10,000 D. The failure of our model, which treats the polymer as a random coil, at PEG molecular weights above 20,000 D provides indirect evidence for the existence of this transition.

The model has also been applied to the prediction of phase diagrams for polymer/salt aqueous two-phase systems. To illustrate, Figure 4 shows experimental and calculated phase diagrams tor the polyethylene glycol 3350/potassium-phosphate aqueous two-phase system at 25°C. Again, model predictions are in good agreement with the experiment over the entire range of tie-line lengths applicable to protein extraction systems. Here, however, penetration effects make only a small contribution to the calculated phase diagrams, presumably because ions are repelled from the volume occupied by the polymer and by the relatively low dielectric constant of the polymer space compared with that of the free aqueous solution. As shown in Table 12, salt-PEG σ_{ij}'s are similar to the corresponding d_{ij}'s, indicating that PEG chains strongly repel most salts.

Salt partition coefficients in polymer-polymer aqueous two-phase systems

As shown by King et al. (1988) and Albertsson (1986), addition of millimolar quantities of a strong electrolyte to polymer/polymer aqueous two-phase systems has only a small effect on the location of the coexistence curve. However, salts which partition unevenly between the phases of such systems are known to create interfacial electrostatic-potential differences (Brooks et al., 1985; Bamberger et al., 1984; Sharp et al., 1986; King et al., 1988), which, in turn, can influence dramatically the partitioning behavior of charged biomolecules (Johansson, 1974b; Reitherman et al., 1973; Haynes et al., 1991).

Figure 5 shows experimental and calculated phase diagrams for the polyethylene glycol 8000/dextran T-500 aqueous two-phase system containing 50-mM K_2HPO_4. Comparison with the salt-free phase diagram measured by King et al. (1988) indicates that the salt decreases slightly the concentration of PEG in the dextran-rich phase. As shown in Figure 5, the model predictions capture this subtle change in the phase diagram. The curve which represents the predicted coexistence curve for the salt-free system lies just above the curve which represents the calculated coexistence curve for the salt-containing system. The difference in the two curves is largely due to the effect of mutual repulsion of PEG chains and small ions. In essence, PEG chains are salted out of the dextran-rich phase by the presence of the relatively high concentration of electrolyte. This strong exclusion force between PEG and salts provides some explanation for the effectiveness of mixtures of PEG and ammonium salts in precipitating globular proteins (Cohn and Edsall, 1944). Here, large fractions of the solvent are needed to solubilize PEG and salts as a result of their strong mutual repulsion; this water is then unavailable to solubilize the relatively insoluble (that is, relatively hydrophobic) proteins. This effect, combined with the well-known osmotic attraction between protein macroions due to local depletion of polymer and salts between nearby protein macroions, can be used to explain many of the observed trends in protein-precipitation systems (see, for example, Vlachy and Prausnitz, 1992). Of course, other

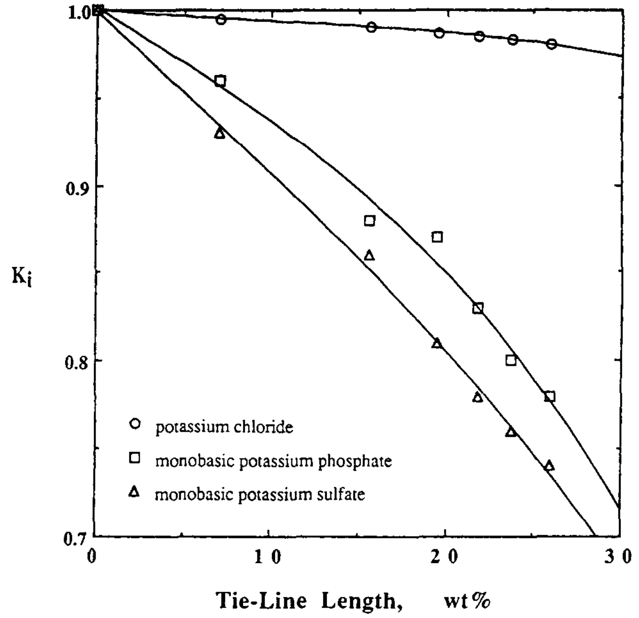

Figure 6. Experimental and calculated partition coefficients for KCl, KH_2PO_4, or $KHSO_4$ in the quaternary aqueous two-phase system containing poly(ethylene glycol) 8000 and dextran T-500 at 25°C.

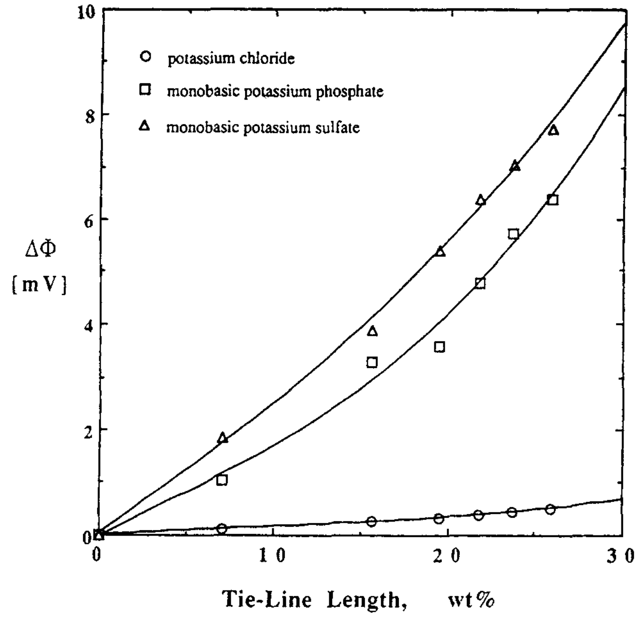

Figure 7. Measured and calculated ΔΦ's for the poly(ethylene glycol) 8000/dextran T-500 aqueous two-phase system at 25°C containing KCl, KH_2PO_4, or $KHSO_4$.

forces, such as hydrogen bonding, may also play a role in salt-induced protein precipitation.

Figure 6 shows experimental and calculated partition coefficients for three salts in quaternary PEG 8000/dextran T-500 aqueous two-phase systems. Here, the total concentration of the salt is 50-mM and the partition coefficient K_i of the salt is defined as

$$K_i = \frac{m_i^{\alpha}}{m_i^{\beta}} \tag{31}$$

where α represents the PEG-rich top phase. In this system, each salt partitions preferentially into the dextran-rich phase, with multivalent salts exhibiting the strongest preference for the dextran-rich phase. For each salt, calculated partition coefficients are in good agreement with the experiment. Once again, the model suggests that the large excluded-volume repulsion force between PEG and ions is largely responsible for the observed partitioning behavior.

Interfacial electrostatic-potential difference, ΔΦ

Albertsson (1986), Johansson (1974b), Brooks et al. (1984), King et al. (1988), and Haynes et al. (1991) have provided convincing experimental evidence for the existence of interfacial electrostatic-potential differences and their often dramatic influence on protein partitioning. In addition, Albertsson (1986) provided a thermodynamic argument for the formation of potential differences in aqueous two-phase systems which suggests that they are created by differences in the relative chemical affinities of the ions of an added electrolyte for the two liquid phases. Through application of the quasi-electrostatic potential theory of Newman (1973), Haynes et al. (1991) provided a direct thermodynamic relation between measured interfacial electrostatic-potential differences and the equilibrium composition of an aqueous two-phase system. For example, in a polymer/polymer aqueous two-phase system containing a 1:1 electrolyte, the partition coefficient of the electrolyte K_s and the ΔΦ for the system are related by (Haynes et al., 1991)

$$\Delta\Phi = (\Phi^{\beta} - \Phi^{\alpha}) = \frac{RT}{F}\ln\left(\frac{\gamma_{\pm}^{\beta}}{\gamma_{\pm}^{\alpha}}\right) = \frac{RT}{F}\ln(K_s) \tag{32}$$

Thus, ΔΦ's can be determined directly from experimental or calculated values of K_s. Conversely, ΔΦ's measured with a Ag/AgCl capillary-electrode apparatus (Brooks et al., 1984; King et al., 1988) can be used to verify calculated salt partition coefficients.

Figure 7 shows measured and calculated ΔΦ's as a function of tie-line length for three different strong electrolytes in the PEG 8000/dextran T-500 aqueous two-phase system. Equation 32 was used to calculate ΔΦ's from the predicted salt partition-coefficient curves shown in Figure 6. The apparatus and procedure used to measure ΔΦ's is described in recent articles (Haynes et al., 1991; King et al., 1988). Measured ΔΦ's range from near 0 for NaCl to 6.9 mV for Na_2SO_4 at a tie-line length of 25.0. These data are consistent with the measurements of Brooks et al. (1984) and King et al. (1988). For each salt, predicted ΔΦ's are in good agreement with the experiment.

Figure 8 shows calculated ΔΦ's for the PEG 3350/potassium phosphate aqueous two-phase system shown in Figure 4. Predicted ΔΦ's for salt/polymer two-phase systems are similar to those for polymer/polymer systems, although the maximum ΔΦ tends to be slightly higher in salt/polymer systems. Elec-

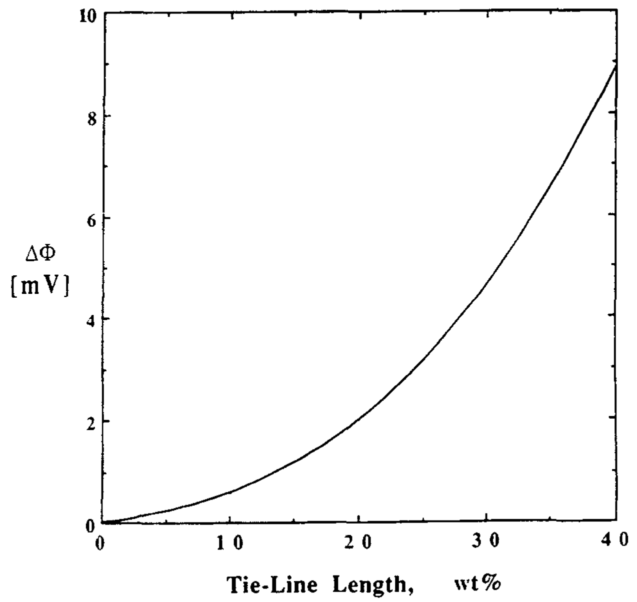

Figure 8. Calculated ΔΦ's for the poly(ethylene glycol) 8000/potassium phosphate aqueous two-phase system at 25°C.

trode leakage effects and poorly defined liquid junctions made it impossible to measure ΔΦ's for salt/polymer systems. Thus, it is not possible to determine the accuracy of the predicted ΔΦ's shown in Figure 8.

Protein partition coefficients

Figure 9 shows experimental and calculated partition coefficients for bovine serum albumin, α-chymotrypsin, and lysozyme in aqueous two-phase systems at pH 7.1 containing PEG 3350, dextran T-70, 50-mM potassium phosphate and 2 mg/mL of one of the three proteins. Calculated protein partition coefficients are in good agreement with experiment over a wide range of tie-line lengths. Indeed, the protein-partition-coefficient calculations shown in Figure 9 are noticeably superior to those obtained from a previously published model (Haynes et al., 1989b) which used a single distance of closest approach to characterize all ion-ion, ion-protein, and protein-protein interactions. For the system described in Figure 9, the model also predicts that the presence of the proteins (at a total concentration of 2 mg/mL or about 0.1 wt. %) forces the partition coefficient of the salt to increase by 3% to 7% relative to its protein-free value. In effect, the proteins exclude the salt and "push" it out of the dextran-rich phase. This subtle effect was confirmed by atomic absorption experiments, which showed that the phosphate partition coefficient increased by an average (over the entire range of tie-line lengths) of 6% in the presence of protein. Moreover, for the same system at a protein-free tie-line length of 16.1 (wt. %), the model predicts that the addition of 2 mg/mL BSA increases the PEG concentration in the dextran-rich phase by 4.7%; an increase of 3.6% was observed experimentally using size-exclusion HPLC.

Figure 10 shows experimental and calculated partition coefficients for a *mixture* of bovine serum albumin, α-chymotrypsin, and lysozyme in an aqueous two-phase system at pH

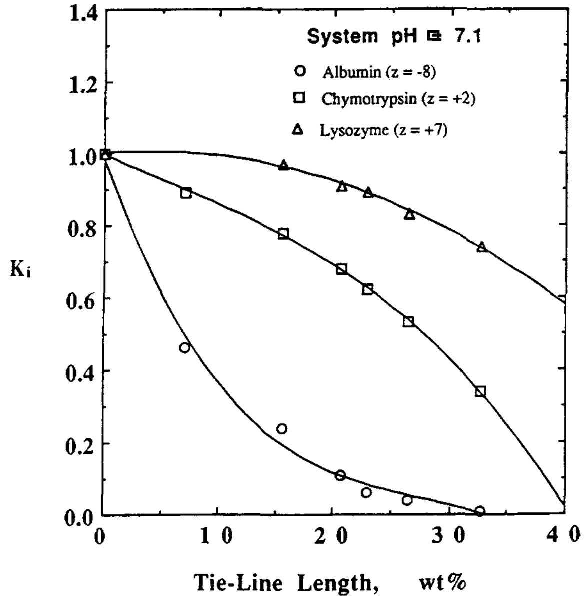

Figure 9. Experimental and calculated partition coefficients for (a) *bovine serum albumin*, (b) *α-chymotrypsin*, and (c) *lysozyme* in aqueous two-phase system at pH 7.1 containing poly(ethylene glycol) 3350, dextran T-70, 50-mM potassium phosphate, and 2 mg/mL of one of the three proteins.

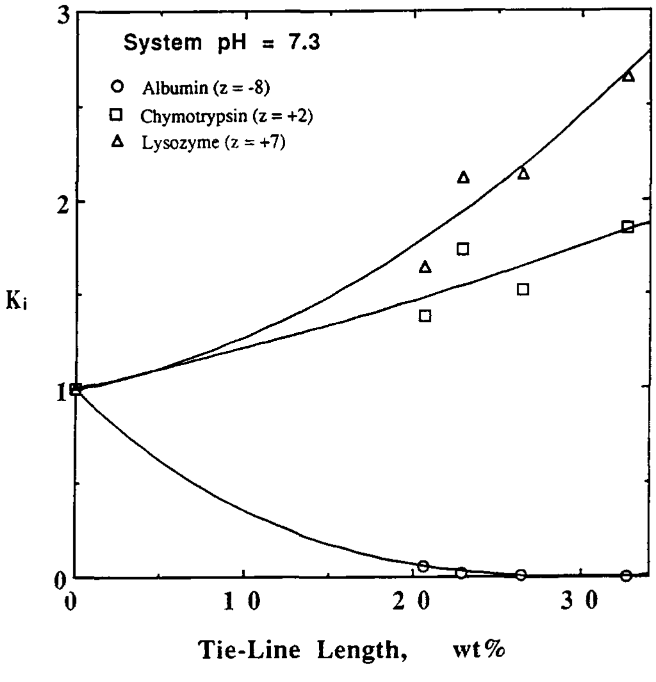

Figure 10. Experimental and calculated partition coefficients for *bovine serum albumin*, *α-chymotrypsin*, and *lysozyme* in an aqueous two-phase system at pH 7.3 containing poly(ethylene glycol) 3350, dextran T-70, 50-mM potassium chloride, and 2 mg/mL of *each* of the three proteins.

Table 13. Comparison of Experimental and Calculated Partition Coefficients for (2 mg/mL) *α-chymotrypsin* in the Poly(ethylene Glycol) 3,350/Potassium Phosphate Two-Phase System at pH 6.5 and 25°C

Tie-Line Length	Experimental K_p	Calculated K_p
17.0	1.3	1.2
20.4	1.7	1.4
25.3	2.4	1.8
28.1	2.9	2.0

7.3 containing PEG 3350, dextran T-70, 50-mM potassium chloride, and 2 mg/mL of each of the three proteins. Again, calculated protein partition coefficients are in good agreement with experiment over a wide range of tie-line lengths. Moreover, comparison of Figures 9 and 10 shows that the model captures the important effects of the added strong electrolyte on protein partitioning; the model appears to account properly for the influence of the interfacial electrostatic-potential difference, salt-protein electrostatic forces, and salt-protein excluded volume forces. For example, α-chymotrypsin and lysozyme carry a net positive charge at pH 7. In the chloride-containing system, where no interfacial electrostatic-potential difference is created, both proteins partition preferentially into the PEG-rich phase. In the phosphate containing system, where an interfacial potential difference is created with positive pole in the PEG-rich phase, the positively charged proteins partition into the dextran-rich phase. As shown in Figures 9 and 10, the model accurately predicts this important electrostatic effect.

Finally, the model also qualitatively predicts protein partition coefficients in PEG/potassium phosphate aqueous two-phase systems. For example, Table 13 compares calculated and experimental partition coefficients as a function of TLL for chymotrypsin in the PEG 3350/potassium phosphate two-phase system at pH 6.5. The reason for the model's mild divergence from experiment is unclear; it may be due to the tendency for chymotrypsin to partially dimerize at elevated salt concentrations (Birktoft and Blow, 1972).

Conclusions

Integral-equation theory coupled with the McMillan-Mayer dilute-solution theory provides a molecular-thermodynamic framework useful for describing dilute and semidilute aqueous solutions containing random-coil polymers, salts and globular proteins. When coupled with light-scattering and osmotic-pressure data, a molecular-thermodynamic model derived from this framework properly describes the equilibrium properties of aqueous two-phase extraction systems.

Unlike most previous work in this area, the thermodynamic model shown here takes full account of electrostatic forces which cannot be neglected when describing real systems that are used in current biotechnology.

Acknowledgments

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Notation

a = distance of closest approach parameter, Å
a_i = $\mu_i - P\overline{V}_i^\theta$, J/mol; or, activity of species *i*
A = Helmholtz energy, J
A' = modified Helmholtz energy, J
A'_{Ex} = excess modified Helmholtz energy, J
B_{ij} = osmotic second virial coefficient, mL mol/g^2
B^*_{ij} = molecular osmotic second virial coefficient, L
$\hat{c}_i$ = molar concentration of species *i*, moles/L
c_i = mass concentration of species *i*, g/mL
$C_{ij}(r)$ = direct correlation function for solute *i*-solute *j* interactions
d_{ij} = hard-sphere distance of closest approach, Å
e = electronic charge, 1.60210×10^{-19} C
f = integration constant
F = Faraday's constant, 96,487 C/equiv.
$g_{ij}(r)$ = pair distribution function for solute *i*-solute *j* interactions
h = Planck's constant
$h_{ij}(r)$ = total correlation function for solute *i*-solute *j* interactions
k = Boltzmann's constant
K = optical constant, cm^2 mol/g^2
K_i = partition coefficient of solute *i*, (mol/kg)/(mol/kg)
K_i^* = partition coefficient of solute *i* in the absence of an interfacial potential difference, (mol/kg)/(mol/kg)
m_i = molality of solute *i*, mol/kg solvent
M_w = weight-average molecular weight, g/mol
M_n = number-average molecular weight, g/mol
n_i = number of moles of species *i*
n_o = solvent refractive index
N_{Av} = Avogadro's number, mol^{-1}
N_i = number of molecules of species *i*
P = pressure, Pa or mm Hg
P_{Ex} = excess pressure, Pa or mm Hg
P_o = pure-solvent vapor pressure, Pa or mm Hg
r = radial distance, Å
R = universal gas constant, J/mol K or *L* Pa/mol K
$\overline{R}_\theta$ = reduced Rayleigh ratio, cm^2/ml
T = absolute temperature, K
$u_{ij}(r)$ = interionic pair potential function, J/mol
V = volume, L
$\underline{V}_o$ = molar volume of pure solvent, L/mol
$\overline{V}_i^\theta$ = partial molar volume of species *i*, L/mol
$W_{ij}(r)$ = potential of mean force, J
w_i = weight fraction of species *i*
z_i = charge number of species *i*

Greek letters

β^*_{ij} = residual osmotic second virial coefficient, L
β_{ij} = solute *i*-solute *j* specific-interaction coefficient, kg/mol
γ_i = (molar) activity coefficient of species *i*
$\gamma_\pm$ = molar mean-ionic activity coefficient
Γ = MSA screening parameter, Å^{-1}
$\Delta\Phi$ = interfacial electrostatic-potential difference, mV
ϵ = permittivity, farad/cm
κ = reciprocal Debye length, Å^{-1}
λ_i = thermal wavelength of species *i*, m
μ_i = chemical potential or electrochemical potential of species *i*, J/mol
ν_i = refractive-index increment (of solute *i*) at infinite dilution, mL/g
ν_+, ν_- = number of cations and anions into which a molecule of electrolyte dissociates
ξ_m = reduced density-type parameter in the Boublik hard-sphere expansion
Π = osmotic pressure, Pa or mm Hg
ρ = solution density, g/L
ρ_i = number density of solute *i*, L^{-1}
σ_{ij} = true distance of closest approach between solutes *i* and *j*, Å
ϕ_i = volume fraction of species *i* (based on $\overline{V}_i^\theta$)

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