Phase Equilibria for Aqueous Protein/Polyelectrolyte Gel Systems

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A molecular-thermodynamic analysis is directed toward predicting the partitioning of aqueous proteins into charged hydrogels. This analysis takes into account size exclusion by the network, electrostatic interactions, and the osmotic-pressure difference between a hydrogel and its surrounding solution. Electrostatic interactions in the polyelectrolyte gel can be described by Debye–Hückel theory, or the Mean Spherical Approximation, or Katchalsky's cell model for polyelectrolyte solutions. The cell model gives best agreement with experimental partition coefficients for cytochrome c. The quasi-electrostatic potential difference between a gel and its surrounding solution demonstrates how the electrostatic contribution to the protein partition coefficient depends on protein charge, gel-charge density, and solution ionic strength. Finally, a qualitative guide is presented for design of a polyelectrolyte gel such that it exhibits specified swelling and partitioning properties.

Introduction

Hydrogels can separate proteins in an aqueous mixture. To design an optimum gel, it is desirable to predict the distribution of solutes between the gel and its surrounding solution. If the hydrogel bears no electric charge, it is often assumed that the protein does not interact with the polymer matrix. In this case, the gel phase is like a porous net, and a size-exclusion model is used to predict the distribution of a protein between the bath and the gel. Many authors have presented models for steric effects in porous media, particularly for size-exclusion chromatography (Ogston, 1958; Laurent and Killander, 1964; Casassa, 1967, 1971a,b; Giddings et al., 1968; Ackers, 1970; Davidson et al., 1987; Schnitzer, 1988; Fanti and Glandt, 1989, 1990; Fanti et al., 1990; Hussain et al., 1991).

However, if the gel is charged (a polyelectrolyte gel), the distribution of a protein is also affected by electrostatic interactions. In ion-exchange chromatography, separation of proteins depends on electrostatic interactions between a protein and the charged matrix. The distribution of solutes between a charged gel and a solution is also important in the medical sciences. For example, proteins may contact charged biological membranes. An example is provided by the studies by Deen et al. of filtration by the kidney (Daniels et al., 1992; Drummond and Deen, 1994; Oliver III and Deen, 1994). Synthetic polyelectrolyte hydrogels can be used, for example, in studies of protein sorption by contact lenses (Refojo and Leong, 1979; Gachon et al., 1986; Cassiani-Ingoni et al., 1988; Minarik and Rapp, 1989; Mirejovsky et al., 1991; Sassi et al., 1996c).

In controlled release, the affinity of a drug for a polymer matrix affects release kinetics; hydrogels are popular candidates for drug carriers (Hoffman et al., 1986; DeRossi et al., 1991; Kim et al., 1994).

The distribution of ionic solutes between a charged gel and a solution has been studied in the context of ion-exchange chromatography (Marinsky, 1966). While ion-exchange chromatography is widely used for the separation of proteins, theoretical descriptions of ion exchange are usually directed at predicting or correlating distributions of low-molecular-weight solutes such as inorganic salts or organic acids and bases. For such low-molecular-weight solutes, steric exclusion of the solute from the gel matrix can be directly related to the water content of the gel matrix; no knowledge of the geometry of the matrix is necessary. However, because proteins are much larger than ions of typical inorganic salts, size-exclusion effects must be included in predictions of ion-exchange equilibria for aqueous protein solutions.

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Proteins may interact with the gel matrix through short-range interactions such as hydrogen-bonding and dispersion forces. Protein separation in hydrophobic-interaction chromatography is based on these short-range forces. Short-range interactions between a protein and a polymer are highly dependent on the nature of protein, polymer, and solvent. Unfortunately, the effect of these specific interactions cannot be predicted in the absence of appropriate experimental data such as osmotic-pressure measurements.

To our knowledge, there are no published predictions of the distribution of a charged protein between a solution and a polyelectrolyte hydrogel, taking into account size-exclusion, electrostatics, and short-range interactions. In this article, we present a method for estimating the partitioning of a protein into a charged hydrogel in the absence of protein adsorption. We apply this method to calculate the distribution of proteins into low-charge-density, weakly ionizable, pH- and temperature-sensitive hydrogels. We consider proteins whose molecular weights are in the range 12,000 to 45,000.

It is often difficult to discern experimentally the individual contributions of adsorption and partitioning. Adsorption of proteins onto polymer surfaces has been studied by many authors (Bull, 1956; Ratner and Miller, 1973; Ratner and Hoffman, 1975; Holly, 1979; Horbett and Weathersby, 1981; Andrade, 1985; Gachon et al., 1985; Brash and Horbett, 1987). In this article we consider only the distribution of a charged solute between a gel and a solution as a result of partitioning in the absence of adsorption.

The partition coefficient

For a solute that distributes between a hydrogel and the surrounding solution (bath), the distribution coefficient, K, is defined by

$$K = \frac{[\text{solute}]_{\text{gel}}}{[\text{solute}]_{\text{bath}}},$$

where the brackets denote some measure of concentration; in this work we use molarity. Following the work of Albertsson (1986) and Guggenheim (1959), we separate contributions to the partition coefficient from electrostatic and nonelectrostatic forces:

$$\ln(K) = \ln(K^{\text{nonelectrostatic}}) + \ln(K^{\text{electrostatic}}) \qquad (1)$$

For $K^{\text{nonelectrostatic}}$, we use free-volume arguments to describe the exclusion of a finite-sized solute by the network. For $K^{\text{electrostatic}}$, we use results from statistical mechanics of electrolyte or polyelectrolyte solutions to describe coulombic interactions. In the following two sections, we discuss our calculations of $K^{\text{nonelectrostatic}}$ and $K^{\text{electrostatic}}$.

Size Exclusion

Elsewhere (Sassi et al., 1996a), we discuss various methods to predict size exclusion by highly swollen hydrogels. Here, we use Schnitzer's uniform-pore model to calculate the contribution of size exclusion to the partition coefficient, because this model agrees well with experimental data for partitioning of polymeric solutes into the highly swollen hydrogels we are interested in (Sassi et al., 1996a). Schnitzer's model gives us the probability that a spherical solute can access any given volume element in the gel as compared to any given volume element in the bath (Schnitzer, 1988). This probability is the partition coefficient due solely to size exclusion, K^{SEC}, which we use for $K^{\text{nonelectrostatic}}$ in Eq. 1.

For spherical, noninteracting solutes partitioning into a matrix of uniformly distributed pores, Schnitzer's expression for the partition coefficient is

$$K^{\text{SEC}} = \upsilon_c^0(1-\vartheta)^2 \qquad (2)$$

where ϑ is the ratio of solute to pore radius, r_s/r_p, and υ_c^0 is the volume of pore space per unit volume (the porosity) (Schnitzer, 1988). The mean pore radius, r_p, was taken to be one-half the mesh size, ζ, calculated using the method of Peppas et al. (1985). The mesh size of a polymer matrix is related to ϕ_p, the volume fraction of polymer, and the square root of the mean square end-to-end distance of the chains of the network:

$$\zeta = \phi_p^{-1/3}\langle r_{e-e}^2\rangle^{1/2}, \qquad (3)$$

where $\langle r_{e-e}^2\rangle$ is the mean square end-to-end distance. Here, a "chain" of the network is considered to consist of the average number of segments between cross-links. The mean square end-to-end distance for a random-flight chain is related to the mean square radius of gyration, $\langle s^2\rangle$, which can be measured by light-scattering:

$$\langle r_{e-e}^2\rangle = 6\langle s^2\rangle \qquad (4)$$

Coulombic Interactions

To obtain $K^{\text{electrostatic}}$, we specify the composition of the bath and find that composition of the gel that satisfies the criterion for thermodynamic phase equilibrium, accounting for coulombic interactions between charges. For phase equilibrium, the general criterion is that the chemical potential of an *neutral* solute i must be the same in each phase containing that component:

$$\mu_i^b = \mu_i^g \qquad (5)$$

where μ_i is the chemical potential of diffusible solute i; superscripts b and g denote bath and gel, respectively.

However, if solute i is charged, the *electrochemical* potential must be the same in each phase containing solute i. This condition is also expressed by Eq. 5, except that μ_i now refers to the electrochemical potential of *charged* solute i.

The chemical potential for a neutral solute depends on temperature, pressure, and composition, whereas the electrochemical potential for a charged solute depends on temperature, pressure, composition, and the electrical state of the phase. Because both bath and gel are electrically neutral, ions must diffuse between the phases in neutral combinations. We call a neutral combination of ions a *component* of the system; the individual mobile ions are *species*. To determine the composition of the gel phase, we solve Eq. 5 for each independ-

ent *component* of the system. (The advantage of solving Eq. 5 for each component is that we can obtain the composition of the gel phase independent of how we define or characterize the electrical state of a phase, as discussed in the second section of the Appendix.) An example of a *component* is a protein and its associated counterions. In the first section of the Appendix, we discuss how to solve Eq. 5 for the composition of the gel phase.

In practice, we need only be concerned with differences in chemical potentials. Because we cannot measure the absolute value of a chemical potential, we define a chemical potential for component i with respect to that at a standard temperature, pressure, and composition:

$$\mu_i = \mu_i^o + RT \ln a_i = \mu_i^o + RT \ln \frac{\gamma_i m_i}{m_i^o}, \tag{6}$$

where μ_i^o is the chemical potential of component i in the standard state, a_i is the conventional activity of component i at concentration m_i, and γ_i is the activity coefficient of component i with respect to the standard state; in the standard state, the concentration of i is m_i^o. The standard state is a hypothetical ideal dilute solution at system temperature and pressure and at a fixed concentration m_i^o usually set at unity. In an ideal dilute solution, γ_i always equals one. At normal pressures, we neglect the pressure dependence of the activity coefficient.

In systems containing gels, however, we must also account for the osmotic-pressure difference between the gel and the bath. The osmotic-pressure difference is directly related to the elasticity of the network, which prevents the gel phase from dissolving. Because γ_i is independent of pressure by convention, we introduce a correction term, $\Delta\mu_i^{\Delta P}$, into chemical potential of a component i in the gel phase:

$$\mu_i^g = \mu_i^o + RT \ln \frac{\gamma_i^g m_i^g}{m_i^o} + \Delta\mu_i^{\Delta P}, \tag{7}$$

where $\Delta\mu_i^{\Delta P} = -(\Delta\Pi_{\text{elastic}})\bar{V}_i$. Here, $\Delta\Pi_{\text{elastic}}$ is the elastic contribution to the swelling pressure of the gel, and $\bar{V}_i$ is the partial molar volume of component i. (The elastic contribution to the swelling pressure of the gel counteracts the osmotic forces that cause the gel to swell. At equilibrium, the swelling pressure of the gel must be zero; that is, forces that act to expand and contract the network must balance. $\Delta\Pi_{\text{elastic}}$ prevents the network from expanding infinitely because the network is physically entangled and chemically cross-linked.) We calculate $\Delta\Pi_{\text{elastic}}$ from an expression for the Helmholtz elastic energy of a network. If we use the phantom network theory of elasticity (Mark and Erman, 1988; Baker, 1993; Baker et al., 1994), we obtain

$$\Delta\Pi_{\text{elastic}} = -\frac{1}{\bar{V}_{\text{solvent}}}\left(\frac{\partial \Delta A^{\text{elastic}}}{\partial n_{\text{solvent}}}\right)_{T,P} = -c_{XL}RT\left(\frac{\phi_{\text{polymer}}}{\phi_{\text{polymer, synthesis}}}\right)^{1/3}, \tag{8}$$

where $\bar{V}_{\text{solvent}}$ is the partial molar volume of the solvent; $\Delta A^{\text{elastic}}$ refers to the change in Helmholtz energy of the gel due to network tension that arises when solvent enters the gel; the volume fraction of polymer is denoted by ϕ_{polymer}, and c_{XL} is the concentration of cross-links at synthesis [mol/m^3] (Baker et al., 1994). In the ion-exchange literature, $\Delta\Pi_{\text{elastic}}$ is usually neglected, although $\Delta\Pi_{\text{elastic}}$ can easily be 0.5 bar. Because simple ions have small molar volumes, the pressure correction is negligible for simple salts such as sodium phosphate or sodium chloride. However, for macromolecular solutes such as proteins, the pressure correction may significantly influence the distribution coefficient.

For a component i that distributes between a bath and a gel, we rewrite Eq. 5 using Eqs. 6–8:

$$\ln(\gamma_i^b m_i^b) = \ln(\gamma_i^g m_i^g) + c_{XL}\bar{V}_i\left(\frac{\phi_{\text{polymer}}}{\phi_{\text{polymer, synthesis}}}\right)^{1/3}. \tag{9}$$

In our system, activity coefficients account for Coulombic interactions between charges. In practice, we calculate activity coefficients for individual ions; we call these activity coefficients *single-ion activity coefficients*. (Single-ion activity coefficients are also defined by Eq. 6, where μ_i is the electrochemical potential of ion i.) The single-ion activity coefficient for a cation is denoted by γ_+; that for an anion is denoted by γ_-. Using this notation, we can express Eq. 9 in terms of calculable quantities:

$$\ln\left[(\gamma_+^b m_+^b)^{\nu_+}(\gamma_-^b m_-^b)^{\nu_-}\right] = \ln\left[(\gamma_+^g m_+^g)^{\nu_+}(\gamma_-^g m_-^g)^{\nu_-}\right] + c_{XL}(\bar{V}_i)\left(\frac{\phi_{\text{polymer}}}{\phi_{\text{polymer, synthesis}}}\right)^{1/3}, \tag{10}$$

where ν is the stoichiometric coefficient of an ion in the neutral salt, and subscripts + and − denote cation and anion, respectively. In the second section of the Appendix, we explain how to obtain Eq. 10.

Expressions for single-ion activity coefficients are usually derived in the McMillan–Mayer framework. In the McMillan–Mayer framework, the solvent is a dielectric medium, and the appropriate independent variables are temperature, volume, moles of solutes, and the solvent chemical potential (McQuarrie, 1975; Haynes, 1992). To convert the chemical potential of a species i calculated in the McMillan–Mayer framework to that in the Lewis–Randall framework (the framework that corresponds to experiment), we add the term $-P^{ex}\bar{V}_i$, where P^{ex} is the excess pressure. P^{ex} depends on the model used to calculate single ion activity coefficients; for a given phase, P^{ex} is obtained by taking the partial derivative of the contribution of Coulombic interactions to the Helmholtz energy of the phase with respect to volume, keeping constant temperature, the number of each solute, and the chemical potential of water. The need for the excess pressure/volume term has been discussed by several authors (Friedman, 1972a,b,c; De M. Cardoso and O'Connell, 1987; Cabezas Jr. et al., 1989; Cabezas and O'Connell, 1993), most recently by Haynes (Haynes, 1992; Haynes et al., 1993). If single-ion activity coefficients are derived in the McMillan–Mayer framework, Eq. 10 is written:

$$\ln\left[(\gamma_+^b m_+^b)^{\nu_+}(\gamma_-^b m_-^b)^{\nu_-}\right] - P^{ex,b}\bar{V}_i = \ln[(\gamma_+^g m_+^g)^{\nu_+}(\gamma_-^g m_-^g)^{\nu_-}] + c_{XL}(\bar{V}_i)\left(\frac{\phi_{\text{polymer}}}{\phi_{\text{polymer, synthesis}}}\right)^{1/3} - P^{ex,g}\bar{V}_i. \quad (11)$$

For our systems, Eq. (11) including the excess pressure/volume terms has a negligible effect on calculated partition coefficients. The following section presents three models for single-ion activity coefficients.

Single-Ion Activity Coefficients

In electrolyte solutions not containing polyelectrolytes, electrostatics can be described theoretically using the primitive model for electrolyte solutions: charged, hard spheres are subject to Coulombic interactions in a medium of constant dielectric. It is more difficult to describe electrostatics in a polyelectrolyte gel because in that case, some of the charges (those on the polymer chain) are fixed in space, while others (counterions and added salt) are mobile.

Our system is analogous to a salt-induced polymer aqueous two-phase system in that we have a "polymer-rich" phase (the gel) in equilibrium with a "polymer-poor" phase; in our case, the bath is polymer-free. Therefore, we imagine that the gel is an uncross-linked polyelectrolyte solution separated from the bath solution by a flexible membrane permeable to all species except the polyelectrolyte. We then use a model for polyelectrolyte solutions to obtain activity coefficients for mobile ions in the gel. Fortunately, our "polymer-rich" phase (the gel) is dilute in polymer because charged hydrogels tend to be highly swollen. High swelling is advantageous because electrostatic theories for polyelectrolyte solutions usually neglect interactions between polyelectrolyte chains.

We could, as a first approximation, neglect the presence of charges on the polyelectrolyte and use a theory for dilute polymer solutions. However, such neglect provides a poor approximation because the fixed charges on the polymer influence properties such as osmotic pressure. The literature gives two simple methods to calculate electrostatic contributions to partitioning. In the first method, we ignore the geometry of the polyelectrolyte by considering only the Coulombic interactions between the spherical, fully mobile ions. In other words, we assume that the electrostatic potentials set up by charges on the polymer are negligible compared to the electrostatic potentials set up by mobile ions. We can assume so only if the fixed charge density (fixed charges per unit volume) is sufficiently low relative to the concentration of mobile ions. In this method, we use single-ion activity coefficients obtained from the Debye–Hückel theory or from the Mean Spherical Approximation (McQuarrie, 1975).

In the second method, we assume that the potential set up by the polyelectrolyte is dominant (Lifson and Katchalsky, 1954). In other words, the mobile ions are influenced more by the polymer charges than by other mobile ions. Here, the polyelectrolyte is taken to be a cylinder of uniform surface charge density. This second method is commonly referred to as the cell model polyelectrolyte solutions.

Ionic activity coefficients from Debye – Hückel theory

The Debye–Hückel theory for electrolyte solutions accounts for electrostatic interactions between charged, hard spheres. It is particularly attractive for thermodynamic calculations because it is analytic and simple compared to other theories. However, its use for quantitative calculations is restricted to dilute electrolyte solutions (typically less than 0.1 M for a 1–1 salt) (McQuarrie, 1975). Single-ion activity coefficients are given by

$$\ln\left(\gamma_j^{\text{single ion}}\right) = -\frac{z_j^2 e^2 \beta \kappa}{8\pi\epsilon_o\epsilon_r(1+\kappa a)}, \quad (12)$$

where β is $(k_bT)^{-1}$, k_b is Boltzmann's constant, e is the charge on an electron, ϵ_o is the permittivity of a vacuum, ϵ_r is the permittivity of the solvent (water) relative to a vacuum ($\epsilon_o\epsilon_r$ is the dielectric constant), z_j is the valence of ionic species j, and a is the ion diameter, taken to be the same for all ions. In this work, we use 3.04 Å for a; for those systems for where we have calculated partition coefficients, the protein is extremely dilute when a *molar* scale is used. For example, the molarity of protein may be four orders of magnitude lower than the molarity of any other simple ion, even though the *mass* concentration of protein may be larger than that of a simple ion. κ is given by

$$\kappa^2 = \frac{e^2\beta}{\epsilon_o\epsilon_r}\sum_j \eta_j z_j^2, \quad (13)$$

where η_j is the number density (N_j/V) of ions of type j. Equations 12 and 13 are written for Systeme Internationale (SI) units, a convention we follow throughout this work. κ^{-1} is the Debye screening length; it provides a rough measure of the average screening of two ions from each other by the remainder of the ions. (In calculations of phase equilibria in electrolyte systems, polymer charges are always included in maintaining electroneutrality, but they are often not included in the calculation of the screening length, κ^{-1}. There is no theoretical support for this procedure, but, in some cases, better agreement with experiment is obtained.) McQuarrie (1975) discusses in further detail the derivation of activity coefficients from the Poisson–Boltzmann equations using Debye–Hückel theory.

Ionic activity coefficients from the mean spherical approximation

Activity coefficient expressions developed from Debye–Hückel theory, while simple, require that all ions in a system have the same diameter. If the diameters of the ions differ appreciably, Debye–Hückel theory is no longer useful except at very low ion concentrations where the influence of ion diameter vanishes (Zemaitis et al., 1986). Diameters of the smallest proteins are on the order of ten times larger than those of simple ions. We therefore turn to integral-equation theory for a suitable model for activity coefficients of ions in solutions where the ion diameters vary widely.

The Mean Spherical Approximation (MSA) is based on solving the Ornstein–Zernike (OZ) integral equation. Unlike

other integral-equation theories, such as the hypernetted-chain (HNC) theory, the solutions to the OZ equation using the MSA are analytical. Unfortunately, however, the exact MSA expression for the single-ion activity coefficient is too complex for use in phase equilibrium calculations, as discussed in Appendix C of Sassi et al. (1996b). A simpler expression for the single-ion activity coefficient is obtained by calculating an effective diameter for the ions in solution (the single-ion diameter or SID approximation) (Harvey et al., 1988). In the SID approximation, the excess chemical potential is given by

$$\ln\left(\gamma_j^{\text{single ion}}\right) = -\frac{\Gamma^3}{\pi}\left[\frac{(1+a_{\text{mix}}\Gamma)z_j^2}{\sum_j \eta_j z_j^2} - \frac{\Gamma}{\eta}(a_j - a_{\text{mix}})\right], \quad (14)$$

where

$$\eta a_{\text{mix}} = \sum_j \eta_j a_j \qquad \eta = \sum_j \eta_j \quad (15)$$

$$\Gamma = \frac{1}{2a_{\text{mix}}}\left[(1+2a_{\text{mix}}\kappa)^{1/2} - 1\right] \qquad \kappa^2 = \frac{e^2\beta}{\epsilon_o \epsilon_r}\sum_j \eta_j z_j^2, \quad (16)$$

where a_{mix} is the average diameter of all ions in solution (m), κ is the reciprocal Debye screening length, as before, and a_j is the diameter of ion j. The sums extend over all ionic species. The MSA screening parameter, Γ, is similar to the reciprocal Debye screening length, κ; Γ tends to $\kappa/2$ at infinite dilution. Waisman and Lebowitz (1970), Blum (1975), and Blum and Høye (1977) provide details regarding the solution of the OZ equation in the MSA.

Cell model for polyelectrolyte solutions

The cell model for polyelectrolyte solutions was originally proposed by Katchalsky (Lifson and Katchalsky, 1954). It differs from the MSA and the Debye–Hückel theory because, in the cell model, we assume the dominant electrostatic interactions in a polyelectrolyte solution are those between a mobile ion and the polyelectrolyte rather than between mobile ions.

To obtain ionic activity coefficients rigorously in the cell model whenever a mobile salt is present in the polyelectrolyte phase, we must solve the Poisson–Boltzmann equation numerically for the electrostatic potential and then perform numerical integrations involving the electrostatic potential. Because the spatial variation of the electrostatic potential depends on the composition of the system, we must solve repeatedly the Poisson–Boltzmann equation for each iteration in our algorithm where composition changes. (We discuss methods of solution in the Section titled "Comparison of Calculated Experimental Protein Partition Coefficients"; methods for solving multicomponent phase equilibria are also discussed in Prausnitz, 1986).

For a multicomponent system, the calculational effort decreases significantly if we use explicit expressions for ionic activity coefficients in lieu of solving the Poisson–Boltzmann equation repeatedly. Guerón and Weisbuch have used the integration procedure for obtaining activity coefficients to examine some general characteristics of the numerical solutions to the Poisson–Boltzmann equation (Guerón and Weisbuch, 1979), and have developed semiempirical activity-coefficient expressions. They model the polyelectrolyte solution as a collection of adjacent volumes (cells) containing a uniformly charged cylinder (the polyelectrolyte) surrounded by point ions in a dielectric. Boundary conditions for the potential are applied only at the surface of the polyelectrolyte cylinder, as the ions (point charges) have no surface. This model of the polyelectrolyte solution is widely used (Alfrey Jr. et al., 1951; Lifson and Katchalsky, 1954; Kotin and Nagasawa, 1962; Schellman and Stigter, 1977; Le Bret and Zimm, 1984; Fogolari et al., 1992; Guerón and Weisbuch, 1992) and represents experimental data for salt partition coefficients and osmotic coefficients well in many cases (Katchalsky and Michaeli, 1955; Alexandrowicz and Katchalsky, 1963; Marinsky, 1966; Katchalsky, 1971).

Several authors have studied the solution of Poisson's equation in a polyelectrolyte solution that is modeled more realistically (Kagawa and Gregor, 1957; Sparnaay, 1972; Gregor and Greff; 1977 Gregor and Gregor, 1977; Glendinning and Russel, 1983; Granot, 1983). For example, Gregor and Gregor consider the case where the charged cylinder is in a bath containing spherical ions of different diameter (Gregor and Greff, 1977; Gregor and Gregor, 1977). Glendinning and Russel apply boundary conditions for the potential on the surface of the charged cylinder and the spherical ions (Glendinning and Russel, 1983). These refinements improve the representation of the geometry of a polyelectrolyte solution; however, they are more complex mathematically than the "classical" cell model as proposed by Katchalsky.

For this work, our first effort in predicting protein partition coefficients, we use the analytical expressions developed by Guerón and Weisbuch. For our purposes, the potential improvement in calculations does not warrant the increase in mathematical complexity associated with a more exact approach. Guerón and Weisbuch suggest the following semiempirical expression for the activity coefficient of a univalent ion whose charge is opposite to that of the polymer cylinder that bears the fixed charges:

$$\gamma_{\text{counterion}} = \frac{0.7\,\aleph/\xi + 1}{\aleph + 1} \quad (17)$$

For a univalent ion whose charge is the same as that of the cylinder, they suggest the following expression:

$$\gamma_{\text{coion}} = \frac{0.7\,\aleph/\xi + 1}{0.53\,\aleph/\xi + 1} \quad (18)$$

where $\aleph$ is the ratio of concentration of charged monomers to the concentration of salt, and ξ is the dimensionless polyelectrolyte linear charge density. The linear charge density, ξ, is the ratio of the Bjerrum length $\Im$ (the Bjerrum length is the length whose potential has a magnitude k_bT), to the axial length per unit charge, b:

$$\xi = \frac{\Im}{b} = \frac{e^2\beta}{4\pi\epsilon_o\epsilon_r b}. \quad (19)$$

The Bjerrum length has an approximate value of 7.14 Å in water at room temperature. These activity-coefficient expressions should be most reliable at low $\aleph$, that is, when the concentration of mobile salt is much greater than the concentration of polymer charges. For an ion of valence z, Guerón and Weisbuch substitute $|z|\xi$ for ξ in the activity coefficients.

Determination of Model Parameters

To apply the models described previously, we must obtain values for parameters that characterize the physical chemistry of the pertinent gel/solvent/solute(s). Such parameters include information on the sizes and charges of the solutes and of the polymer backbone. The solvent (water) is not discretely considered and is taken to be a medium of constant dielectric: we used 78.3 for the relative permittivity, ϵ_r, of water at 25°C.

The solutes (salt and protein) are modeled as hard spheres with a characteristic diameter and net charge. Diameters for salt and buffer ions were taken from Haynes et al. (Haynes, 1992). Protein diameters were taken as twice the radius of gyration (measured by light-scattering techniques) from Tyn and Gusek (1990) or twice the Stokes radius (Shaw and Hartzell, 1976). The former reference contains extensive data on radii of gyration, diffusion coefficients, molecular weights, and experimental viscosities of proteins in aqueous solution. We determined the charge on simple ions from stoichiometry of the salt and, for multiprotic buffer salts, by the stoichiometry of chemical equilibria for the various ions. For phosphates, we calculated the relative concentration of uni-, bi-, and trivalent anions using equilibrium constants published in the *CRC Handbook of Chemistry and Physics* (Weast, 1988) and corrected with activity coefficients from the SID approximation to the MSA. The charge on a protein as a function of pH was determined from titration data at the appropriate ionic strength in the literature (Tanford, 1961). We assumed that the titration curve for a protein was not influenced by the presence of the (charged) polymer. The counterions of the protein and the charged monomers on the gel were taken to be the appropriate univalent ion of the added salt or buffer. Partial molar volumes for individual ions were calculated using the hard-sphere diameters obtained previously. Partial molar volumes for neutral salts were taken to be the stochiometric sum of the partial molar volumes for each ion of the salt.

When Katchalsky's cell model is used, the polyelectrolyte gel is modeled as a network of cylindrical fibers. We set the length of a monomer at 2.52 Å; this value corresponds approximately to the distance between alternate carbons as used in our simulations (Sassi et al., 1992). Each ionized monomer contains only one charge. The fibers have an average diameter of 5 Å. The chemical composition of the gel was that at synthesis. We confirmed this assumption by subjecting our poly-NIPA-copolymer gels to elemental analysis; while the data are somewhat scattered because the monomers are similar in atomic composition, the data indicate that only a few percent of the comonomer was lost on polymerization. The volume fraction of polymer at synthesis was determined from the nominal $\%T$ [(mass of all monomers mass of diluent)·100]; likewise, the cross-link density at synthesis was determined from the nominal $\%C$ [(mole crosslinker/mole total monomer)·100]. (The effect of cross-linking and entanglements on swelling and partitioning behavior may not be well characterized by using nominal values of $\%T$ and $\%C$. Given the difficulty in determining the effective degree cross-linking applicable to partitioning and swelling calculations, we nevertheless use the nominal parameters.) The density of the polymer was taken to be the same as that for polyacrylamide. Radii of gyration for poly-NIPA chains as a function of molecular weight and temperature were taken from light-scattering data (Kubota et al., 1990). (Because of a lack of data we were forced to assume that charged hydrogels had the same pore sizes as those of uncharged gels; thus the influence of the polyelectrolyte nature of the gel on size exclusion entered solely through the increased swelling ratio as compared to a neutral gel. At external salt concentrations on the order of 0.1 M or above, the swelling ratios of the polyelectrolyte gels studied experimentally begin to approach the swelling ratio for the corresponding uncharged gel; therefore the assumption is least drastic at salt concentrations of 0.1 M or more.)

The charge density of the gel is especially crucial when calculating the distribution coefficient of a charged solute. If the charged monomers are strong electrolytes, the charge density of the gel is calculated from the nominal fraction of charged monomer present at polymerization and the resultant swelling ratio of the gel at equilibrium. If, however, the charged monomers are weak electrolytes, the fraction of these that are ionized is determined by the pK of the ionizable group and the pH of the surrounding solution. To a first approximation, we could assume that the pK is equal to its intrinsic value. However, because the pK of an ionizable group is influenced by its local charge environment, the pK depends on polymer conformation and charge, and the salt concentration surrounding the polymer charge. While we could calculate, in principle, the effect of polymer charge density and salt concentration inside the gel on the pK as a function of pH, this calculation would introduce added complexity to our set of equations, as we would have to recalculate the pK upon every iteration of the algorithm for simultaneous solution of all pertinent equations for phase equilibrium. We avoided this calculation by obtaining an average pK (different from the nominal pK) from experimental swelling equilibria at the desired salt concentration and ionic strength. We estimated this average pK by the pH at which the experimental plot of swelling equilibria vs. pH exhibited an inflection point. This average pK was then used to calculate the fraction of ionized monomers, α, by application of the Henderson–Hasselbalch equation:

$$pH - pK - \log_{10}\left(\frac{\alpha}{1-\alpha}\right) = 0. \tag{20}$$

Equation 20 is written for an acid HA that dissociates into A^- and H^+. If the ionizable group is an amine, the corresponding equation is

$$pH - pK + \log_{10}\left(\frac{\alpha}{1-\alpha}\right) = 0, \tag{21}$$

where the amine (NR_3) is charged when protonated (NR_3H^+). We were better able to predict the effect of pH

on protein partitioning if we used this average pK (obtained from experimental swelling data) than if we used the nominal pK values in the literature (Brandrup and Immergut, 1966).

Results

Comparison of calculated and experimental protein partition coefficients

We investigated three algorithms to calculate protein partition coefficients in polyelectrolyte hydrogels. In the first algorithm, the rigorous algorithm, we characterized the composition of the system at any given pH by two neutral components: (1) the protein and its counterions and (2) any one of the possible neutral combinations of buffer ions (phosphates). After specifying the composition of the bath solution, we solved five simultaneous equations: two equations of equal chemical potential in each phase (one for each neutral component), the two chemical equilibria for phosphate ions, and an equation of electroneutrality in the gel. The equations of chemical potential were expressed in the form of Eq. 11. After solving the five equations, we know the composition of the polyelectrolyte solution and can calculate a partition coefficient for the protein between the polyelectrolyte solution and the bath. Following Eq. 1, we multiplied this partition coefficient by K^{SEC} from Eq. 2 to obtain the overall partition coefficient between the polyelectrolyte gel and the bath.

We encountered convergence difficulties in the rigorous algorithm when activity coefficients were obtained from Debye–Hückel theory or the MSA. In this case, we obtained several sets of concentration of the ions in the gel, depending on our initial guess for the composition of the gel phase and on the restrictions we placed upon the possible calculated compositions (for example, that the ion concentrations had to be bounded by physically realistic values). Because the concentration values were of the same order of magnitude among the different sets of concentrations, we could not discard solutions based on purely physical grounds. We may have obtained multiple solutions because of some (unknown) feature inherent in the mathematics of the system of equations and its solution; alternatively, the multiple solutions could represent a metastable region of the system that is not observed experimentally. At present, a more conclusive analysis is prohibited because some experimental parameters we use in the calculations are not known exactly. These experimental parameters include the true charge state of the polyelectrolyte and the magnitude of nonelectrostatic, specific or nonspecific interactions between the protein and polyelectrolyte.

Because it was impossible to select the "correct" root, we simplified the algorithm so that we had only two simple ions (sodium and chloride), rather than the four ions of the phosphate buffer. (If we choose a simple salt other than NaCl, the results of the semi-rigorous algorithm will be affected only if we use ionic activity coefficients which are a function of ionic diameter and if the ionic diameters are much different from those of phosphate ions. Therefore, in most cases, the choice of simple salt does not affect the calculated protein partition coefficient significantly. Here we choose NaCl for simplicity. NaCl is highly soluble and tends to partition more evenly than other salts in aqueous two-phase systems (Haynes, 1992).) We fixed the salt concentration of the bath solution in the calculation to be 0.1 M, the ionic strength of the buffer. We then had to solve only three simultaneous equations for the composition of the polyelectrolyte solution: the two equations of equal chemical potential (one for the protein and its counterions and one for sodium chloride) and the equation of electroneutrality in the polyelectrolyte solution (gel). The partition coefficient thus obtained was multiplied, as before, by K^{SEC}. This second algorithm is the semirigorous algorithm.

In the third algorithm, the quasi-electrostatic potential algorithm, we determined the partitioning of a monovalent salt (NaCl) such that the concentration of salt in the bath solution in the calculation was equal to the experimental ionic strength of the bath solution containing buffer and protein. The quasi-electrostatic potential difference, $\Delta\Phi$, can be related to the monovalent salt partition coefficient, K_{salt}:

for a positively charged gel:

$$\Delta\Phi = \Phi^{gel} - \Phi^{bath} = -\frac{RT}{F}\ln(K_{salt}) \tag{22}$$

for a negatively charged gel:

$$\Delta\Phi = \Phi^{gel} - \Phi^{bath} = \frac{RT}{F}\ln(K_{salt}), \tag{23}$$

where $\Delta\Phi$ is the quasi-electrostatic potential difference (gel $-$ bath), R is the universal gas constant, T is temperature, and F is Faraday's constant (Haynes et al., 1991; Newman, 1991). To define the quasi-electrostatic potential, we must choose a reference ion, as discussed in the fifth section of the Appendix. For positively charged gels, we choose the *cation* of the mono-monovalent salt to be the reference ion; for negatively charged gels, we choose the *anion* of the mono-monovalent salt to be the reference ion. Because the partition coefficient for the mono-monovalent salt equals the partition coefficient for the reference ion, and using Eq. A16 of the Appendix, we obtain Eq. 22 for positively charged gels and Eq. 23 for negatively charged gels. The fifth section of the Appendix discusses the definition of the quasi-electrostatic potential. We then calculated the protein partition coefficient ($K_{protein}$) from:

for a positively charged gel:

$$\ln(K_{protein}) = \ln(K^{SEC}) + \ln(K^{\Delta P}) - \frac{z_{protein}F}{RT}\Delta\Phi \tag{24}$$

for a negatively charged gel:

$$\ln(K_{protein}) = \ln(K^{SEC}) + \ln(K^{\Delta P}) + \frac{z_{protein}F}{RT}\Delta\Phi, \tag{25}$$

where $K^{\Delta P}$ is the contribution to the partition coefficient from the pressure difference between the gel and the solution:

$$K^{\Delta P} = \exp\left(\Delta\Pi_{elastic}\overline{V}_{protein}\right) \tag{26}$$

and $\Delta\Pi_{elastic}$ is given by Eq. 8. We investigated the quasi-electrostatic potential algorithm because we can calculate partitioning of a protein without calculating its activity coeffi-

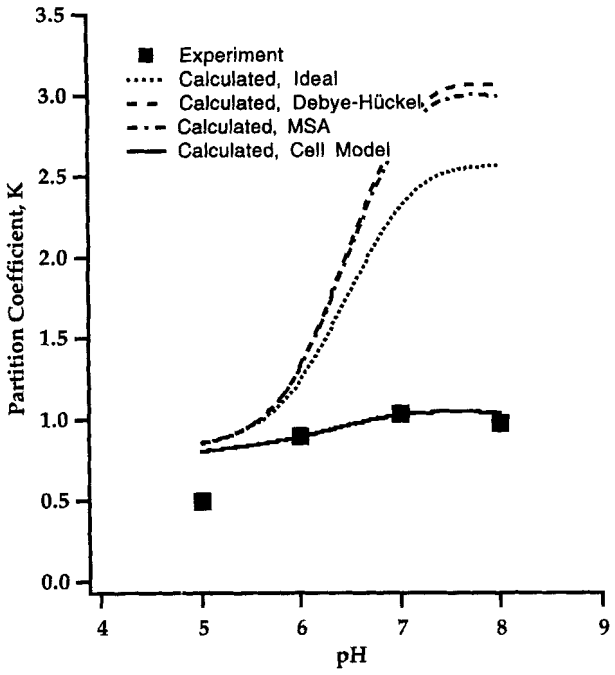

Figure 1. Calculated and experimental partition coefficients as a function of pH for cytochrome c in weakly acidic poly-NIPA/10%SA gels (15%T, 1%C) at 22.2°C.

The buffer was 0.1-M ionic-strength sodium phosphates. Single-ion activity coefficients were taken from Debye–Hückel theory, the Mean Spherical Approximation, the cell model, or assumed to be unity.

cient. While it is simple to calculate the partition coefficient of a protein using the quasi-electrostatic potential difference, this calculation gives poor results unless the salt concentration is large.

In the Appendix titled "Comparison of Calculations of Protein Partition Coefficients using Different Algorithms,"we compare results calculated using the three algorithms discussed earlier. Here, we present calculations using the semirigorous algorithm, which simplified calculations and agreed best with experimental data.

Figure 1 presents calculated and experimental partition coefficients as a function of pH for cytochrome c in poly-*N*-isopropylacrylamide (NIPA)/10% sodium acrylate (SA) gels (15%*T*, 1%*C*). The temperature was 22.2°C, and the buffer was 0.1-M ionic-strength sodium phosphates. In this range of pH, the protein is positively charged, and the gel is negatively charged. The experimental data are those shown in Figure 6a of Sassi et al. (1996d). We calculated partition coefficients using the semirigorous algorithm with single-ion activity coefficients from Debye–Hückel theory, the MSA, the cell model, or assuming activity coefficients to be unity.

We obtained the best agreement between calculation and experiment when we used activity coefficients from Guerón and Weisbuch (1979). Partition coefficients calculated using activity coefficients from either Debye–Hückel theory are identical to those using activity coefficients from the MSA. Deviation from experiment increases with rising gel charge density. The gel charge density increases with rising pH for the region $5 < \text{pH} < 8$.

Debye–Hückel theory or the MSA give calculated partition coefficients that are farther removed from experiment than they would be if the activity coefficients were assumed to be unity. We also calculated partition coefficients for the same system, but at 36.4°C, slightly above the collapse temperature for neutral poly-NIPA hydrogels. At this temperature, the partition coefficients calculated with Debye–Hückel theory or the MSA were one to two orders of magnitude greater than those calculated using the cell model for activity coefficients. The partition coefficients calculated using the cell model, while they did not agree quantitatively with experiment data, were at least of the same order of magnitude as experimental data. These and other calculations we performed suggest that the cell model is more appropriate to calculate protein partitioning in highly swollen hydrogels. In all cases, the partition coefficients calculated using the cell model were significantly closer to experiment than those calculated using other activity-coefficient models.

Figure 2 presents experimental and calculated partition coefficients for cytochrome c as a function of pH in poly-NIPA/10%SA gels at 36.4°C and in poly-NIPA/10% dimethylaminoethylmethacrylate (DMA) gels at 22.2 and 36.4°C. The buffer in each case was 0.1-M-ionic-strength sodium phosphate. The calculated partition coefficients are in fair agreement with experiment for the data at 22.2°C, recalling that these are *a-priori* calculations. At 36.4°C, the calculated partition coefficients do not agree quantitatively with experiment for either poly-NIPA/SA or poly-NIPA/DMA gels, but do predict the qualitative effect of pH for poly-NIPA/SA gels.

In the calculations, we have neglected the contribution of nonelectrostatic interactions (other than size exclusion) be-

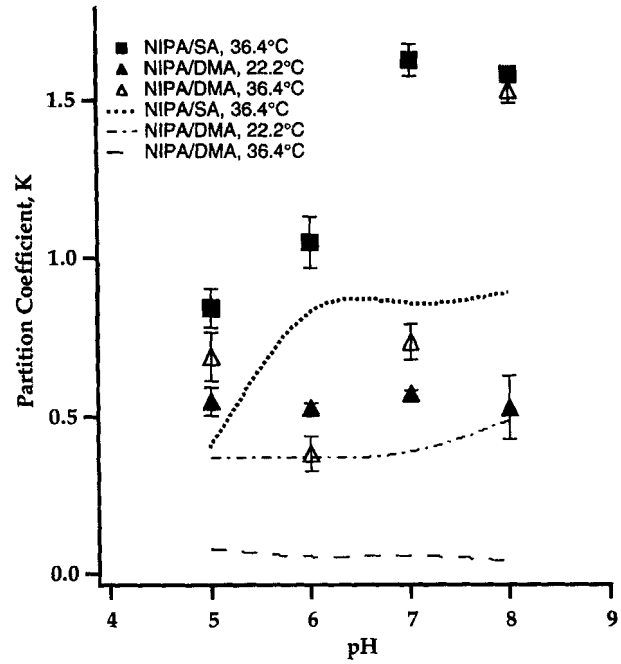

Figure 2. Calculated and experimental partition coefficients as a function of pH for cytochrome c poly-NIPA/10%SA at 36.4°C and inpoly-NIPA/10%DMA at 22.2 and 36.4°C.

The buffer was 0.1-M ionic-strength sodium phosphates. Single-ion activity coefficients are from the cell model.

tween protein and polymer, ion and polymer or ion and ion. It is possible that the discrepancy between calculated and experimental partition coefficients is due, in part, to short-range, net-attractive forces between the protein and the polymer. In the fourth section of the Appendix we discuss how we might incorporate these interactions into our calculations if we had fundamental data for the interaction between cytochrome and the poly-NIPA polymers. Another likely reason for the discrepancy between calculated and experimental partition coefficients is our inability quantitatively to predict size-exclusion effects in hydrogels as a function of $\%C$ and $\%T$, as discussed elsewhere (Sassi et al., 1996a).

Designing a polyelectrolyte gel

For most applications, we desire partition coefficients close to zero (the protein is excluded) or much greater than one (the protein distributes favorably into the gel). To obtain partition coefficients close to zero, we can synthesize a gel with high $\%T$ and $\%C$ to obtain a gel where the strands are highly entangled. In addition, we can synthesize a gel that incorporates a monomer of the same charge as that of the solute, resulting in repulsive electrostatic interactions between the solute and gel. Gels whose polymer chains are more entangled swell less; the partition coefficient decreases because entanglements prevent the solute from penetrating the gel.

For some applications (e.g., increasing protein concentration in the aqueous phase), we may want to reject a protein without decreasing gel swelling. If we alter the gel chemistry such that $\%T$ and $\%C$ are increased *at constant swelling*, we produce an increase in the pressure difference between the gel and the bath solution, hindering the protein from entering the gel. Figure 3 shows the effect of increasing $\%T$ and $\%C$ at constant swelling ratio on the partition coefficient for a protein. For gels of different swelling, $\%T$, and $\%C$, Figure 3 gives the contribution of the pressure difference to the partition coefficient ($K^{\Delta P} = \exp(\Delta\Pi_{\text{elastic}}\bar{V}_{\text{protein}})$; Eq. 26), where $\Delta\Pi_{\text{elastic}}$ is given by Eq. 8 as a function of solute radius. For example, raising $\%C$ from 1 to 5 significantly hinders macromolecules from partitioning into the gel at constant swelling ratio and $\%T$. Increasing $\%T$ from 15% to infinity (bulk polymerization) at constant swelling ratio also results in lower partition coefficients.

While the pressure difference between the gel and bath may be safely neglected when calculating the distribution of small molecules, Figure 3 shows that this pressure difference is significant for proteins and other macromolecules. The calculated results in Figure 3 also suggest a surprising feature: elastic media more efficiently exclude solutes than rigid media having the same solvent content. The effect of the pressure difference, present in elastic media but not in rigid media, hinders the solute from entering the gel.

Partition coefficients greater than unity are possible only if attractive interactions exist between the solute and gel. These attractive interactions may be long-range (i.e., electrostatic) or short-range (i.e., dispersion forces). We might obtain a measure of polymer–protein short-range interactions as a function of solution conditions from independent experimental techniques such as light scattering. However, because these data are unavailable, we do not include them in our predictions of the partition coefficient. Electrostatic interactions,

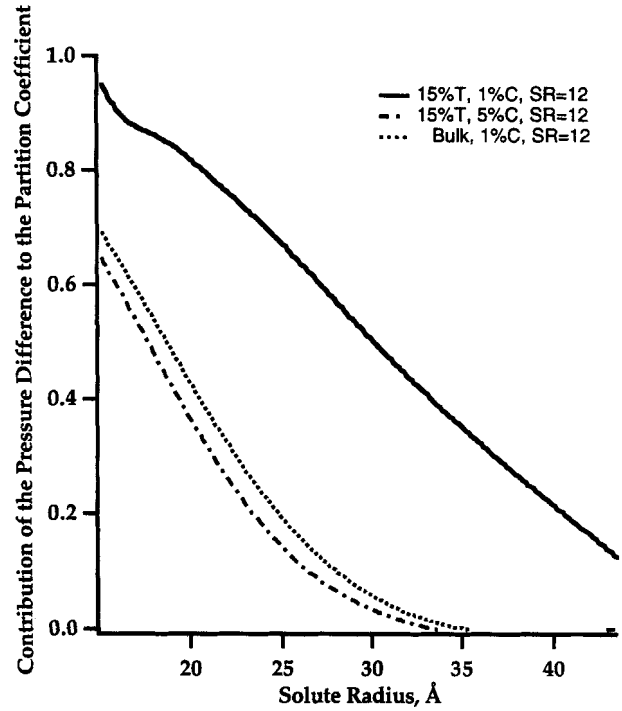

Figure 3. Elastic contribution to the partition coefficient as a function of solute radius for various %T, %C, and swelling ratio (SR).

The contribution to the partition coefficient was calculated using the phantom-network model.

however, can be modeled (to a first approximation) knowing the charges of all solute molecules in the system. Solutes charged oppositely to a polyelectrolyte gel distribute favorably into the gel if the favorable coulombic interactions overcome the tendency of a gel to exclude a macromolecular solute. The electrostatic contribution to the solute partition coefficient depends on the net charge of the protein and the charge density of the gel, which, in turn, is influenced by the chemical composition of the gel and factors that determine the swelling equilibrium of the gel (e.g., ionic strength and pH).

Figure 4 shows calculations of the effect of pH on partition coefficients for lysozyme and ovalbumin into weakly acidic poly-NIPA/SA gels ($15\%T$, $1\%C$, $10\%CM$) in 0.1-M ionic-strength buffer at 22.2°C where CM is comonomer. Ovalbumin is negatively charged, and lysozyme is positively charged at pH 5–8. The concentration of each protein in the bath solution was 0.0005 M, which corresponds to about 7 g/L lysozyme and 22.5 g/L ovalbumin. As we expect, lysozyme, which is oppositely charged with respect to the gel and smaller than ovalbumin, partitions more into the gels than ovalbumin. pH has a greater effect on partitioning of ovalbumin because the net charge of ovalbumin changes more rapidly with pH than that of lysozyme's net charge. Although electrostatic interactions between lysozyme and gel are attractive, the electrostatic interactions are not dominant. Hence partition coefficients for lysozyme are close to unity. (The size-exclusion contribution to the partition coefficient for lysozyme ranges from 0.856 to 0.873 between pH 5 and 8. The combined swelling-pressure and electrostatic contributions to the

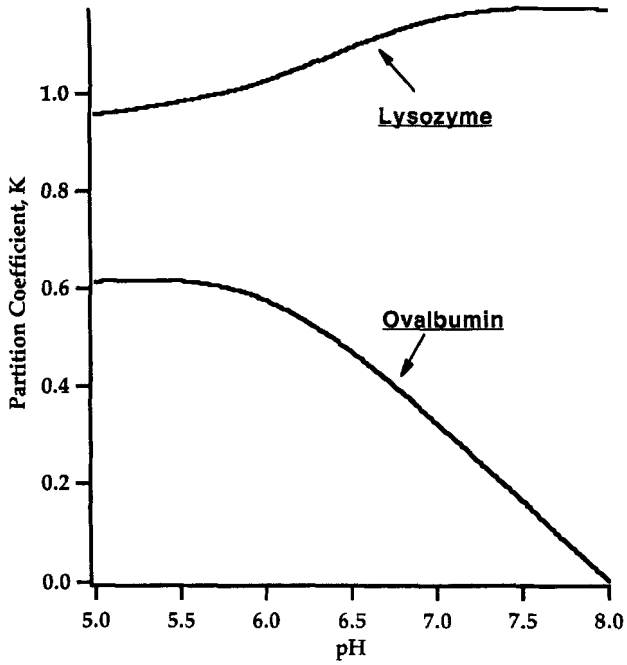

Figure 4. Calculated effect of pH on the partitioning of ovalbumin and lysozyme into poly-NIPA/10%SA gels (15%T, 1%C) in 0.1-M ionic strength buffer at 22.2°C.

The concentration of each protein in the bath solution is 0.5 mM. Ovalbumin is negatively charged, lysozyme is positively charged, and the gel is negatively charged for pH 5–8. Activity coefficients are from the cell model.

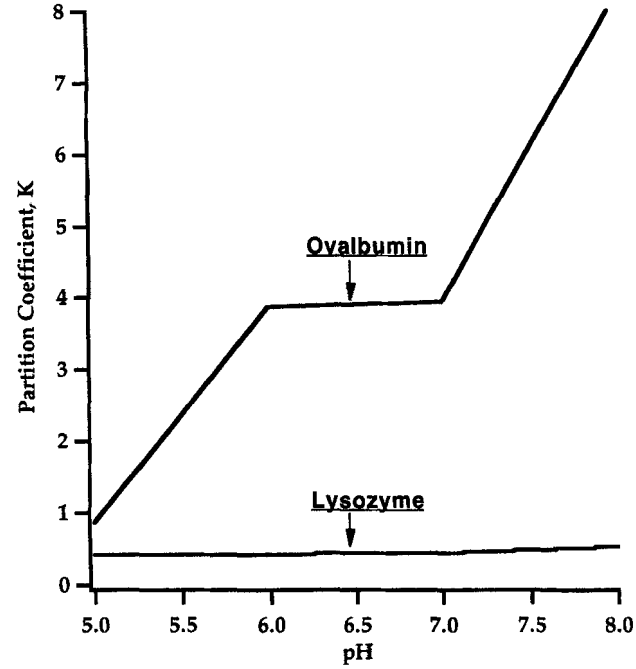

Figure 5. Calculated effect of pH on the partitioning of ovalbumin and lysozyme into poly-NIPA/10%DMA gels (15%T, 1%C) in 0.1-M ionic strength buffer at 22.2°C.

Protein concentrations and charges are the same as those in Figure 4, but the gel is positively charged. Activity coefficients are from the cell model.

partition coefficient for lysozyme range from 0.96 to 1.175 between pH 5 and 8. Thus, the overall partition coefficient ranges from 0.82 to 1.03 for lysozyme between pH 5 and 8).

Figure 5 shows calculations of the effect of pH on partition coefficients for lysozyme and ovalbumin into weakly basic poly-NIPA/DMA gels (15%*T*, 1%*C*, 10%*CM*) in 0.1-M ionic-strength buffer at 22.2°C. Here, the gel is positively charged. As we expect, the negatively charged ovalbumin partitions favorably into the gel, and the positively charged lysozyme is partially rejected by the gel. As pH increases, the magnitude of ovalbumin's net charge increases, but the gel charge density decreases. It appears that the increase in protein charge dominates the electrostatics, and the partition coefficient is higher at pH 8 than at pH 5. The partition coefficient of lysozyme is also slightly higher at pH 8 than at pH 5. In this case, the net positive charge on lysozyme and that on the gel decrease as pH rises. The partition coefficient rises slightly because the unfavorable electrostatic interactions are diminished, and the decrease in swelling is not great enough to counteract the trend in electrostatics.

For a given system, lowering salt concentration increases the magnitude of electrostatic interactions between the protein and the charged polymer because screening decreases. Figure 6 shows calculations for the same proteins and gel as those in Figure 4. Here the buffer ionic strength is only 0.01 M, and the protein concentration in the bath is 0.5 or 0.05 mM. The partition coefficient for lysozyme is significantly enhanced, and the partition coefficient for ovalbumin is significantly lowered, compared to those shown in Figure 4. The trends in the calculated results for protein concentrations of 0.5 mM in Figure 6 are different from those in Figure 4 because the molar ratio of the monovalent salt to the protein and its counterions is smaller. At a protein concentration in the bath solution of 0.5 mM (corresponding to Figure 4), the calculated partition coefficient for ovalbumin rises slightly. The partitioning of lysozyme at 0.5 mM does not increase monotonically as in Figure 4 but shows an unexpected minimum and maximum. The unexpected trends at 0.5-mM protein concentration are due to the ratio of buffer salt to protein and its counterions; these trends are demonstrated by examining the partition coefficients calculated at 0.05-mM protein in the bath solution. For this case, the ratio of buffer to protein and its counterions in the bath solution is the same as that in Figure 4. The general trends shown in Figure 4 are regained; the partition coefficient for ovalbumin falls and that for lysozyme rises with increasing pH. The calculated partition coefficient for lysozyme at 0.05-mM concentration in the bath solution and 0.01-M salt shows a slight maximum at pH 6. Figures 4–6 emphasize that the partitioning of a protein into a charged hydrogel is the result of a complex interplay of influences.

An alternate description of electrostatic contributions to the phase equilibria considered here is facilitated by the concept of the quasi-electrostatic potential difference, $\Delta\Phi$, between the gel and bath (Newman, 1991). To define the quasi-electrostatic potential, we must choose a reference ion (Eq. A14a of the Appendix); the quasi-electrostatic potential difference depends on the sign of the reference ion (Eq. A16 of the Appendix). If a mono-monovalent salt partitions into a

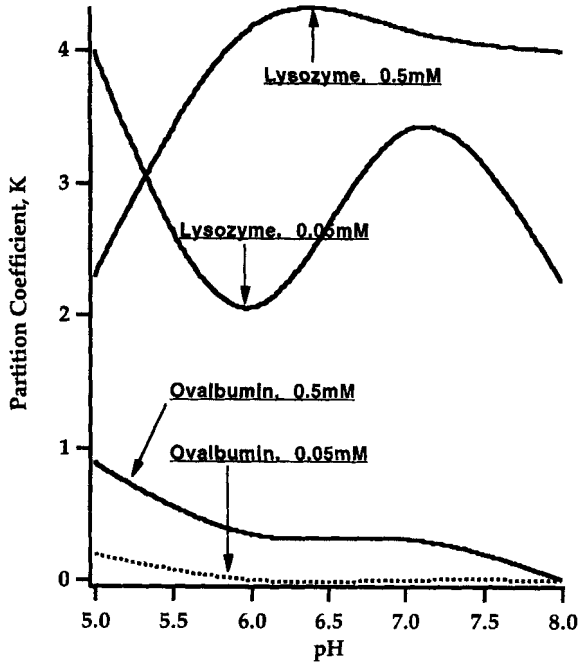

Figure 6. Calculated effect of pH on the partitioning of ovalbumin and lysozyme into poly-NIPA/10%SA gels (15%T, 1%C) in 0.01-M ionic-strength buffer at 22.2°C.

Result are shown for protein concentrations in the bath solution of 0.5 and 0.05 mM. Activity coefficients are from the cell model.

positively charged gel, we choose the reference ion to be the *cation* of the salt because the partition coefficient of the salt equals the partition coefficient of the *cation* of the salt. If a mono-monovalent salt partitions into a negatively charged gel, we choose the reference ion to be the *anion* of the salt because the partition coefficient of the salt equals the partition coefficient of the *anion* of the salt, as discussed in the fifth section of the Appendix.

The quasi-electrostatic potential difference allows us to use the partition coefficient for a mono-monovalent salt to estimate the electrostatic contribution to the partitioning of a z-valent solute:

for a positively charged gel:

$$\ln\left(K_{\text{protein}}^{\text{electrostatic}}\right) = \frac{-z_{\text{protein}}F}{RT}\Delta\Phi = z_{\text{protein}}\ln\left(K_{\text{1-1 salt}}^{\text{electrostatic}}\right) \quad (27)$$

for a negatively charged gel:

$$\ln\left(K_{\text{protein}}^{\text{electrostatic}}\right) = \frac{z_{\text{protein}}F}{RT}\Delta\Phi = -z_{\text{protein}}\ln\left(K_{\text{1-1 salt}}^{\text{electrostatic}}\right), \quad (28)$$

where z_{protein} is the net electronic charge of the protein (Haynes et al., 1991; Newman, 1991; Haynes, 1992). Equations 27 and 28 follow from the difference in sign of the reference ion for a positively charged gel and that of the reference ion for a negatively charged gel. Equation 27 is obtained using Eq. 22 and the last term of Eq. 24. Equation 28 is obtained using Eq. 23 and the last term of Eq. 25.

When gel and bath are in equilibrium, the magnitude of the quasi-electrostatic potential difference, $|\Delta\Phi|$, is a well-behaved function of the ratio of the gel charge density to the ionic strength of the bath solution. In a solution of mono-monovalent salt, the ionic strength is numerically equivalent to the salt concentration. Figure 7 presents a log-log plot of the dependence of $|\Delta\Phi|$ on the ratio of gel charge density to ionic strength of the bath. Calculations were performed for a $1\%C$ and $15\%T$ gel using numerical solutions to the Poisson–Boltzmann equation reported by Stigter for the cell model (Stigter, 1975). (We first use Stigter's results to calculate the partitioning of mono-monovalent salt using methods outlined in Stigter (1975). We then obtain the quasi-electrostatic potential difference using Eq. 22 or 23.) We fixed the monomer length at 2.52 Å, and each charged monomer has a single charged group. Molecular weights of the neutral and charged monomer were taken to be the molecular weights of NIPA and SA, respectively.

The effects of $\%C$ and $\%T$ on calculated $|\Delta\Phi|$ are small (data not shown). For example, the quasi-electrostatic potential changed approximately 0.07% as $\%T$ varied from 5 to 1,000 for $1\%C$ gels in equilibrium with 1.0-M salt solution (10% of monomers were charged, and the swelling ratio was held constant at 10 in the calculations). The quasi-electrostatic potential difference changed approximately 1.5% as $\%C$

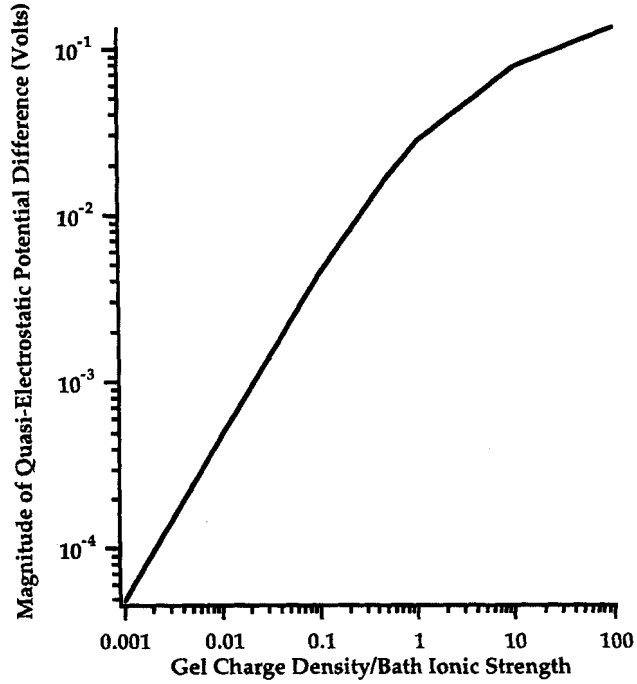

Figure 7. Dependence of the magnitude of the quasi-electrostatic potential difference, $|\Delta\Phi|$, on the ratio of gel charge density to ionic strength of the solution.

Calclation of the quasi-electrostatic potential was performed using the numerical solutions presented by Stigter (Stigter, 1975).

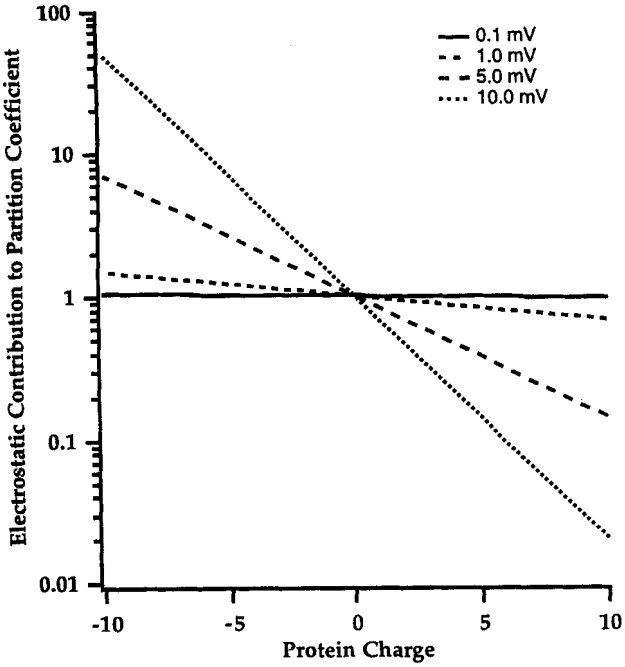

Figure 8. Dependence of the electrostatic contribution to the partition coefficient on protein charge for various values of the quasi-electrostatic potential difference.

Here, the gel is positively charged.

varied from 0.5 to 10 for 15%T gels in equilibrium with 0.01-M salt solution (10% of monomers were charged, and the swelling ratio was held constant at 10 in the calculations).

If we use quasi-electrostatic potential differences, we overpredict protein partition coefficients, as discussed in the third section of the Appendix. However, qualitative effects of Coulombic interactions are captured. To illustrate the qualitative utility of Figure 7, we consider how the electrostatic contribution to a partition coefficient depends on protein charge and the quasi-electrostatic potential difference. Figure 8 presents the partition coefficient (considering only Coulombic interactions) as a function of net protein charge for quasi-electrostatic potential differences of 0.1, 1, 5 and 10 mV. Here, the gel is positively charged. For negatively charged proteins, partition coefficients are not greater than two unless the potential difference exceeds 1 mV. For positively charged proteins, partition coefficients are not less than 0.1 unless the potential difference exceeds about 10 mV. For a protein of net charge -6, the potential difference must be approximately 10 mV to obtain a partition coefficient greater than 10 in a positively charged gel.

Thus, we see that potential differences must be of the magnitude of several millivolts or greater to obtain partition coefficients significantly greater than unity or less than 0.1 Similar conclusions were drawn by Haynes et al. in studies of protein partitioning in the presence of various salts in an aqueous two-phase polymer system (Haynes et al., 1991, 1993; Haynes, 1992).

For "design" of a polyelectrolyte gel that exhibits desired swelling and solute-partitioning properties, we would like to determine synthesis conditions (%T, %C, and monomer chemistry). Baker et al. used the following equations to calculate swelling equilibria for acrylamide and hydroxyethylmethacrylate copolymer hydrogels:

$$\Delta\Pi_{\text{swelling}} = -\frac{\mu^g_{\text{solvent}} - \mu^b_{\text{solvent}}}{\bar{V}_{\text{solvent}}} = \Delta\Pi_{\text{mixing}} + \Delta\Pi_{\text{elastic}} + \Delta\Pi_{\text{ion}} = 0 \quad (29)$$

$$\Delta\Pi_{\text{mixing}} = -\frac{RT}{\bar{V}_{\text{solvent}}}\left[\ln(1-\phi_{\text{polymer}}) + \phi_{\text{polymer}} + \chi\phi^2_{\text{polymer}}\right] \quad (30)$$

$$\Delta\Pi_{\text{elastic}} = -c_{XL}RT\left(\frac{\phi_{\text{polymer}}}{\phi_{\text{polymer, synthesis}}}\right)^{1/3} \quad (31)$$

$$\Delta\Pi_{\text{ion}} = RT\sum_j\left(c^g_j - c^b_j\right) \quad (32)$$

where $\Delta\Pi$ is called the swelling pressure, ϕ_{polymer} is the volume fraction of polymer, $\bar{V}_{\text{solvent}}$ is the molar volume of the solvent, c_{XL} is the concentration of cross-links at synthesis in mol/m^3, and c_j is the concentration of mobile ion j in mol/m^3 (Baker, 1993; Baker et al., 1994). [Equation 31 is obtained from the phantom network theory of elasticity. In the phantom network theory, the gel is assumed to be a perfect tetrafunctional network that swells isotropically and whose points of cross-linking fluctuate randomly in space due to thermal (Brownian) motion (Mark and Erman, 1988). In Eq. 32, we consider only the entropic contribution of the ions to the swelling pressure. Although we could use a more exact expression for $\Delta\Pi_{\text{ion}}$ (ionic contribution to swelling pressure in Pa), we would not change the qualitative features of Figures 9a and 9b. Baker et al. have shown that Eqs. 29–32 predict swelling equilibria satisfactorily for polyelectrolyte hydrogels in dilute aqueous solutions of simple salts.]

As an example, we have applied Eqs. 2, 10 and 29 to calculate the Flory parameter, χ, for hypothetical gels of different swelling ratios in 0.1-M or 1.0-M salt. We define the swelling ratio (SR) as:

$$\frac{\text{mass of swollen gel at equilibrium}}{\text{mass of dry gel}}.$$

The Flory parameter characterizes the polymer–solvent interaction energy and thus indicates in our calculations how hydrophobic the principal monomer should be for the gel to swell to a desired extent in a salt solution. By performing these calculations, we also obtain the partitioning of a mono-monovalent salt, which we can use to estimate the effect of electrostatics on the partition coefficient of a multivalent solute.

Synthesis conditions were fixed at 15%T and 1%C; in our experience, these parameters result experimentally in a poly-NIPA copolymer gel that is resilient to breakage. The percentage of strongly ionized, positively charged monomer was fixed at 10% for illustration.

Figure 9a presents the calculated quasi-electrostatic potential difference and Flory's parameter as a function of swelling

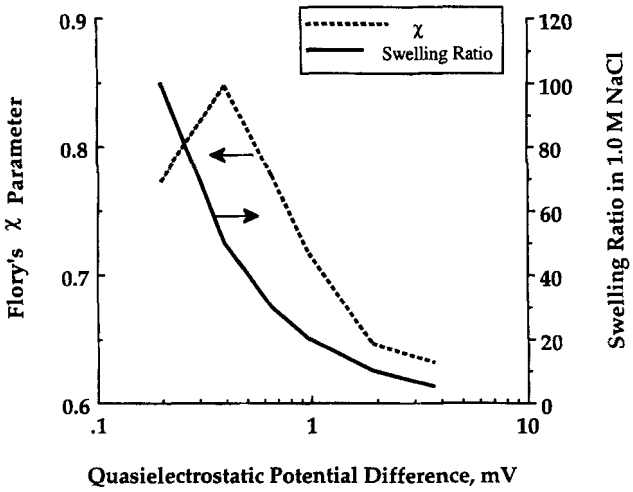

Figure 9a. Relation between the swelling ratio for a hypothetical polyelectrolyte gel (15%*T*, 1%*C*) in 1.0-M NaCl, the quasi-electrostatic potential difference, $\Delta\Phi$, and Flory's parameter, χ, for the polymer-solvent interaction.

Results are shown as a function of $\Delta\Phi$, which is determined by the gel charge density and the ionic strength of the bath. The percentage of positively charged monomers is 10%. χ is from model of gel swelling of Baker et al. (Baker, 1993; Baker et al., 1994).

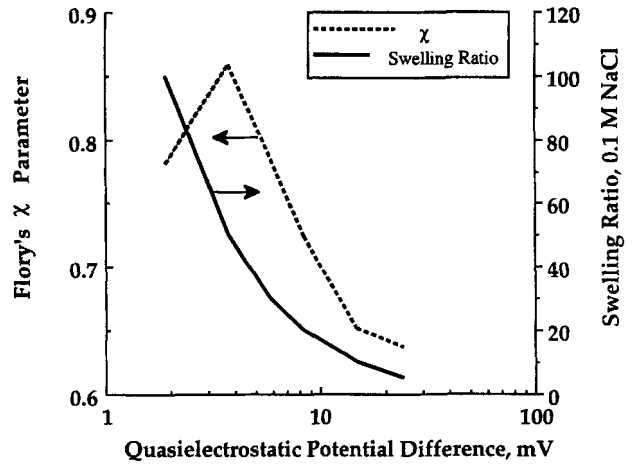

Figure 9b. Relation between the swelling ratio for a hypothetical polyelectrolyte gel in 0.1-M NaCl, $\Delta\Phi$, and χ.

The gel is the same as that in Figure 9a.

ratio in 1.0-M salt. Figure 9b presents the similar results for 0.1-M salt. Figure 9a shows that, to obtain a potential difference greater than 1 mV in 1.0-M salt, the swelling ratio must be less than 20 for a gel containing 10% charged monomer. Further, parameter χ must be less than about 0.72. For comparison, Baker et al. regressed values of χ ranging from 0.63 to 0.82 for poly-hydroxyethylmethacrylate copolymer gels of varying charge density swelling in water (Baker, 1993). From Figure 9b, we see that potential differences greater than 1 mV are easily obtained for a wide range of swelling ratios. We also performed calculations at higher swelling ratios than those shown in Figures 9a and 9b to confirm that χ continues to decline as the swelling ratio rises above 50. While there are many inherent assumptions in these calculations, they nevertheless can be used as a guide in designing a gel to have specified partitioning and swelling properties.

Conclusions

We have discussed the distribution of a protein between a charged gel and a surrounding bath. We have accounted for size exclusion, Coulombic interactions, and the osmotic pressure difference between the gel and bath. We have neglected short-range polymer–protein interactions because we lack independent experimental data to determine these interactions for systems where we have experimental partition coefficients. The cell model of polyelectrolyte solutions is most appropriate to describe Coulombic interactions between the mobile ions and the charged polymer.

The semirigorous algorithm is simple to use and provides better agreement between calculated and experimental data than other algorithms. In the semirigorous algorithm, we assume that the concentrations of simple ions in the experimental bath can be replaced in calculations by the concentration of a 1:1 salt, which equals the ionic strength of the simple ions in the experimental bath. In other words, we reduce the number of types of mobile simple ions to two: the cation and anion of the 1:1 salt.

We emphasize that our calculations are *a priori*; that is, we did not use data from a partitioning experiment to predict partition coefficients. Given that we cannot describe rigorously the topography of the polymer network nor the charge density of the gel as a function of system parameters, calculated partition coefficients lie surprisingly close to experimental data for some protein-gel systems. The method we have used also allows us to understand some puzzling effects in the experimental pH-dependence of protein partition coefficients in weakly ionizable polyelectrolyte gels, where spurious maxima and minima have been observed. It appears that these extrema may be real, not due to experimental uncertainties. We also demonstrated that the osmotic-pressure difference between the gel and bath can result in significant exclusion of a macromolecule from a hydrogel.

We have shown that the magnitude of the quasi-electrostatic potential difference between a charged gel of fixed $\%T$ and $\%C$ and its bath is related to the ratio of charge density of the gel (defined by the charges on the polymer network per unit volume) to ionic strength of the bath. In turn, because the log of the electrostatic contribution to the partition coefficient depends linearly on the net charge of the protein for a given quasi-electrostatic potential difference, we can estimate the electrostatic contribution to the partition coefficient using experimental data for the ratio of charge density to ionic strength derived from swelling equilibria of charged hydrogels in salt solutions. The effect of $\%T$ and $\%C$ has a negligible effect on the relationship between the partitioning of a 1:1 salt and the ratio of gel charge density to salt concentration. Finally, we have outlined a method to guide determination of the nominal composition of a gel that has desirable swelling and partitioning properties in aqueous salt solutions.

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Literature Cited

Ackers, G. K., "Analytical Gel Chromatography of Proteins," *Adv. Prot. Chem.*, **24**, 343 (1970).

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

Alexandrowicz, Z., and A. Katchalsky, "Colligative Properties of Polyelectrolyte Solutions in Excess of Salt," *J. Poly. Sci.: Part A*, **1**, 3231 (1963).

Alfrey, T., Jr., P. W. Berg, and H. Morawetz, "The Counterion Distribution in Solutions of Rod-Like Polyelectrolytes," *J. Poly. Sci.*, **7**, 543 (1951).

Andrade, J. D., ed., *Protein Adsorption, Surface and Interfacial Aspects of Biomedical Polymers*, Plenum, New York (1985).

Baker, J. P., "Equilibrium Swelling Properties of Ionized Hydrogels," PhD Thesis, Univ. of California, Berkeley (1993).

Baker, J. P., L. H. Hong, H. W. Blanch, and J. M. Prausnitz, "Effect of Total Initial Monomer Concentration on the Swelling Behavior of Cationic Acrylamide-Based Hydrogels," *Macromol.*, **27**, 1446 (1994).

Beltrán, S., H. H. Hooper, H. W. Blanch, and J. M. Prausnitz, "Swelling Equilibria for Ionized Temperature-Sensitive Gels in Water and in Aqueous Solution," *J. Chem. Phys.*, **92**, 2061 (1990a).

Beltrán, S., J. P. Baker, H. H. Hooper, H. W. Blanch, and J. M. Prausnitz, "Swelling Equilibria for Weakly-Ionizable, Temperature-Sensitive Hydrogels," *Macromolecules*, **24**, 549 (1990b).

Blum, L., "Mean Spherical Model for Asymmetric Electrolytes I. Method of Solution," *Mol. Phys.*, **1975**, 1529 (1975).

Blum, L., and J. S. Høye, "Mean Spherical Model for Asymmetric Electrolytes. 2. Thermodynamic Properties of the Pair Correlation Function," *J. Phys. Chem.*, **81**, 1311 (1977).

Brandrup, J., and E. H. Immergut, *Polymer Handbook*, Wiley, New York (1966).

Brash, J. L., and T. A. Horbett, eds., *Proteins at Interfaces: Physicochemical and Biochemical Studies*, ACS Symp. Ser. (1987).

Bull, H. B., "Adsorption of Bovine Serum Albumin on Glass," *Biochim. Biophys. Acta*, **19**, 464 (1956).

Cabezas, H., Jr., J. D. Evans, and D. C. Szlag, "A Statistical Mechanical Model of Aqueous Two-Phase Systems," *Fluid Phase Equilibria*, **53**, 453 (1989).

Cabezas, H., Jr., and J. P. O'Connell, "Some Uses and Misuses of Thermodynamic Models for Dilute Liquid Solutions," *Ind. Eng. Chem. Res.*, **32**, 2892 (1993).

Casassa, E. F., "Equilibrium Distribution of Flexible Polymer Chains Between a Macroscopic Solution Phase and Small Voids," *J. Poly. Sci., Part B*, **5**, 773 (1967).

Casassa, E. F., "Gel Permeation Chromatography and Thermodynamic Equilibrium," *Sep. Sci.*, **6**, 305 (1971a).

Casassa, E. F., "Theoretical Models for Peak Migration in Gel Permeation Chromatography," *J. Phys. Chem.*, **75**, 3929 (1971b).

Cassiani-Ingoni, L., F. Subira, C. Bunel, J.-P. Vairon, and J.-L. Halary, "Enzymatic Release of Drug from Polymer Matrices: Diffusion and Activity of Lysozyme within Opthalmic Hydrogels," *Makromol. Chem. Macromol. Symp.*, **19**, 287 (1988).

Daniels, B. S., E. B. Hauser, W. M. Deen, and T. H. Hostetter, "Glomerular Basement Membrane: *In Vitro* Studies of Water and Protein Permeability," *Amer. J. Physiol.*, **262** (Renal Fluid Electrolyte Physiol. **31**), F919 (1992).

Davidson, M. G., U. W. Suter, and W. M. Deen, "Equilibrium Partitioning of Flexible Macromolecules between Bulk Solution and Cylindrical Pores," *Macromol.*, **20**, 1141 (1987).

De M. Cardoso, M. J. E., and J. P. O'Connell, "Activity Coefficients in Mixed Solvent Electrolyte Solutions," *Fluid Phase Equil.*, **33**, 315 (1987).

DeRossi, D., K. Kajiwara, Y. Osada, and A. Yamauchi, eds., *Polymer Gels: Fundamentals and Biomedical Applications*, Plenum Press, New York (1991).

Drummond, M. C., and W. M. Deen, "Structural Determinants of Glomerular Hydraulic Permeability," *Amer. J. Physiol.*, **266** (Renal Fluid Electrolyte Physiol. **35**), F1 (1994).

Fanti, L. A., and E. Glandt, "Partitioning of Spherical Solutes into Sponge-Type Materials," *AIChE J.*, **35**, 1883 (1989).

Fanti, L. A., and E. Glandt, "Partitioning of Spherical Particles in Fibrous Matrices 1. Density Functional Theory," *J. Colloid Interfacial Sci.*, **135**, 385 (1990).

Fanti, L. A., E. D. Glandt, and W. G. Madden, "Fluids in Equilibrium with Disordered Porous Materials. Integral Equations Theory," *J. Chem. Phys.*, **93**, 5945 (1990).

Fogolari, F., G. Manzini, and F. Quadrifoglio, "Polyelectrolytes in Mixed Salts: Scatchard Plots Obtained by Means of Poisson-Boltzmann Calculations," *Biophys. Chem.*, **43**, 213 (1992).

Friedman, H. L., "Lewis-Randall to McMillan-Mayer Conversion for the Thermodynamic Excess Functions of Solutions: III. Common-Ion Mixtures of Two Electrolytes," *J. Solut. Chem.*, **1**, 419 (1972a).

Friedman, H. L., "Lewis-Randall to McMillan-Mayer Conversion for the Thermodynamic Excess Functions of Solutions: I. Partial Free Energy Coefficients," *J. Solut. Chem.*, **1**, 387 (1972b).

Friedman, H. L., "Lewis-Randall to McMillan-Mayer Conversion for the Thermodynamic Excess Functions of Solutions," *J. Solut. Chem.*, **1**, 413 (1972c).

Gachon, A. M., T. Bilbault, and B. Dastugue, "Protein Migration through Hydrogels: A Tool for Measuring Porosity—Application to Hydrogels Used as Contact Lenses," *Anal. Biochem.*, **157**, 249 (1986).

Gachon, A. M., T. Bilbault, and B. Dastugue, "Adsorption of Tear Proteins on Soft Contact Lens," *Exp. Eye Res.*, **40**, 105 (1985).

Giddings, J. C., E. Kucera, C. P. Russell, and M. N. Myers, "Statistical Theory for the Equilibrium Distribution of Rigid Molecules in Inert Porous Networks. Exclusion Chromatography," *J. Phys. Chem.*, **72**, 4397 (1968).

Glendinning, A. B., and W. B. Russel, "The Electrostatic Repulsion between Charged Spheres from Exact Solutions to the Linearized Poisson-Boltzmann Equation," *J. Colloid Interface Sci.*, **93**, 95 (1983).

Granot, J., "Effect of Finite Ionic Size on the Solution of the Poisson-Boltzmann Equation: Application to the Binding of Divalent Metal Ions to DNA," *Biopolymers*, **22**, 1831 (1983).

Gregor, H. P., and R. J. Greff, "Selective Uptake of Counterions of Different Size by Ion-Exchange Gels: Effect of Swelling Pressure and Coulombic Interactions," *J. Chem. Phys.*, **67**, 5742 (1977).

Gregor, H. P., and J. M. Gregor, "Coulombic Reactions of Polyelectrolytes with Counterions of Different Sizes," *J. Chem. Phys.*, **66**, 1934 (1977).

Guerón, M., and G. Weisbuch, "Polyelectrolyte Theory. 2. Activity Coefficients in Poisson-Boltzmann and in Condensation Theory. The Polarizability of the Counterion Sheath," *J. Phys. Chem.*, **83**, 1991 (1979).

Guerón, M., and G. Weisbuch, "Polyelectrolyte Theory. 4. Algebraic Approximation for the Poisson-Boltzmann Free Energy of a Cylinder," *J. Phys. Chem.*, **96**, 7816 (1992).

Guggenheim, E. A., *Thermodynamics*, North-Holland, Amsterdam (1959).

Harvey, A. H., T. W. Copeman, and J. M. Prausnitz, "Explicit Approximations to the Mean Spherical Approximation for Electrolyte Systems with Unequal Ion Sizes," *J. Phys. Chem.*, **92**, 6432 (1988).

Haynes, C. A., "Separation of Protein Mixtures by Extraction: Statistical-Mechanical Models of Aqueous Solutions Containing Polymers, Salts, and Globular Proteins," PhD Thesis, Univ. of California, Berkeley (1992).

Haynes, C. A., F. J. Benitez, H. W. Blanch, and J. M. Prausnitz, "Application of Integral-Equation Theory to Aqueous Two-Phase Partitioning Systems," *AIChE J.*, **39**, 1539 (1993).

Haynes, C. A., J. Carson, H. W. Blanch, and J. M. Prausnitz, "Electrostatic Potentials and Protein Partitioning in Aqueous Two-Phase Systems," *AIChE J.*, **37**, 1401 (1991).

Hoffman, A. S., A. Afrassiabi, and L. Chang Dong, "Thermally Reversible Hydrogels: II. Delivery and Selective Removal of Sub-

stances from Aqueous Solutions," *J. Controlled Release*, **4**, 213 (1986).
Holly, F. J., "Protein and Lipid Adsorption by Acrylic Hydrogels and Their Relation to Water Wettability," *J. Poly. Sci., Poly. Symp.*, **66**, 409 (1979).
Horbett, T. A., and P. K. Weathersby, "Adsorption of Proteins from Plasma to a Series of Hydrophilic-Hydrophobic Copolymers: I. Analysis with the *in-situ* Radioiodination Technique," *J. Biomed. Mat. Res.*, **15**, 402 (1981).
Hussain, S., M. S. Mehta, J. I. Kaplan, and P. L. Dubin, "Experimental Evaluation of Conflicting Models for Size Exclusion Chromatography," *Anal. Chem.*, **63**, 1132 (1991).
Kagawa, I., and H. P. Gregor, "Theory of the Effect of Counterion Size upon Titration Behavior of Polycarboxylic Acids," *J. Poly. Sci.*, **23**, 477 (1957).
Katchalsky, A., "Polyelectrolytes," *Pure Appl. Chem.*, **26**, 327 (1971).
Katchalsky, A., and I. Michaeli, "Polyelectrolyte Gels in Salt Solutions," *J. Poly. Sci.*, **15**, 69 (1955).
Kim, Y.-H., Y. H. Bae, and S. W. Kim, "pH/Temperature-sensitive Polymers for Macromolecular Drug Loading and Release," *J. Controlled Release*, **28**, 143 (1994).
Kotin, L., and M. Nagasawa, "Chain Model for Polyelectrolytes: VII. Potentiometric Titration and Ion Binding in Solutions of Linear Polyelectrolytes," *J. Chem. Phys.*, **36**, 873 (1962).
Kubota, K., S. Fujishige, and I. Ando, "Single-Chain Transition of Poly(*N*-Isopropylacrylamide) in Water," *J. Phys. Chem.*, **94**, 5154 (1990).
Laurent, T. C., and J. Killander, "A Theory of Gel Filtration and Its Experimental Verification," *J. Chromatogr.*, **14**, 317 (1964).
Le Bret, M., and B. H. Zimm, "Distribution of Counterions Around a Cylindrical Polyelectrolyte and Manning's Condensation Theory," *Biopolymers*, **23**, 287 (1984).
Lifson, S., and A. Katchalsky, "The Electrostatic Free Energy of Polyelectrolyte Solutions: II. Fully Stretched Macromolecules," *J. Poly. Sci.*, **13**, 43 (1954).
Marinsky, J. A., "Interpretation of Ion-Exchange Phenomena," *Ion Exchange: A Series of Advances*, J. A. Marinsky, ed., Marcel Dekker, New York, p. 353 (1966).
Mark, J. E., and B. Erman, *Rubberlike Elasticity, A Molecular Primer*, Wiley, New York (1988).
McQuarrie, D. A., *Statistical Mechanics*, Harper & Row, New York (1975).
Minarik, L., and J. Rapp, "Protein Deposits on Individual Hydrophilic Contact Lenses: Effects of Water and Ionicity," *CLAO J.*, **15**, 185 (1989).
Mirejovsky, D., A. S. Patel, and D. D. Rodriguez, "Effect of Proteins on Water and Transport Properties of Various Hydrogel Contact Lens Materials," *Curr. Eye Res.*, **10**, 187 (1991).
Newman, J. S., *Electrochemical Systems*, 2nd ed., Prentice-Hall, Englewood Cliffs, NJ (1991).
Ogston, A. G., "The Spaces in a Uniform Random Suspension of Fibres," *Trans. Faraday Soc.*, **54**, 1754 (1958).
Oliver, J. D., III, and W. D. Deen, "Random-Coil Model for Glomerular Sieving of Dextran," *Bull. Math. Bio.*, **56**, 369 (1994).
Peppas, N. A., H. J. Moynihan, and L. M. Lucht, "The Structure of Highly Crosslinked Poly(2-hydroxyethylmethacrylate) Hydrogels," *J. Biomed. Mater. Res.*, **10**, 397 (1985).
Prausnitz, J. M., R. N. Lichtenthaler, and E. Gomes de Azevedo, *Molecular Thermodynamics of Fluid-Phase Equilibria*, 2nd ed., Prentice-Hall, Englewood Cliffs, NJ (1986).
Ratner, B. D., and A. S. Hoffman, *Synthetic Hydrogels for Medical Applications*, American Chemical Society, Division of Polymer Chemistry, Chicago (1975),
Ratner, B. D., and I. F. Miller, "Transport Through Crosslinked Poly(2-Hydroxyethyl Methacrylate) Hydrogel Membranes," *J. Biomed. Mat. Res.*, **7**, 353 (1973).
Refojo, M., and F.-L. Leong, "Microscopic Determination of the Penetration of Proteins and Polysaccharides into Poly-(hydroxyethylmethacrylate) and Similar Hydrogels," *J. Poly. Sci. Poly. Symp.*, **66**, 227 (1979).
Sassi, A. P., S. Beltrán, H. H. Hooper, H. W. Blanch, and J. M. Prausnitz, "Monte Carlo Simulations of Hydrophobic Weak Polyelectrolytes: Titration Properties and pH-Induced Structural Transitions for Polymers Containing Weak Electrolytes," *J. Chem. Phys.*, **97**, 8767 (1992).
Sassi, A. P., H. W. Blanch, and J. M. Prausnitz, "Characterization of Size-Exclusion Effects in Highly Swollen Hydrogels: Correlation and Prediction," *J. Appl. Poly. Sci.*, **59**, 1337 (1996a).
Sassi, A. P., D. Freed, H. W. Blanch, and J. M. Prausnitz, "Partitioning of Hexavalent Chromium in Temperature-Sensitive Hydrogels," *Polymer Geisand Networks*, accepted (1996b).
Sassi, A. P., S.-H. Lee, Y. Park, H. W. Blanch, and J. M. Prausnitz, "Sorption of Lysozyme by HEMA Copolymer Hydrogels," *J. Appl. Poly. Sci.*, **60**, 225 (1996c).
Sassi, A. P., A. Shaw, S.-M. Han, H. W. Blanch, and J. M. Prausnitz, "Partitioning of Proteins and Small Biomolecules in Temperature- and pH-Sensitive Hydrogels," *Polymer*, in press (1996d).
Schellman, J. A., and D. Stigter, "Electrical Double Layer, Zeta Potential, and Electrophoretic Charge of Double-Stranded DNA," *Biopolymers*, **16**, 1415 (1977).
Schnitzer, J., "Analysis of Steric Partition Behavior of Molecules in Membranes Using Statistical Physics," *Biophys. J.*, **54**, 1065 (1988).
Shaw, R. W., and C. R. Hartzell, "Hydrogen Ion Titration of Horse Heart Ferricytochrome C," *Biochemistry*, **15**, 1909 (1976).
Sparnaay, M. J., "Ion-Size Corrections of the Poisson-Boltzmann Equation," *Electroanal. Chem. Interfacial Electrochem.*, **37**, 65 (1972).
Stigter, D., "The Charged Colloidal Cylinder with a Gouy Double Layer," *J. Colloid Interfacial Sci.*, **53**, 296 (1975).
Tanford, C., *Physical Chemistry of Macromolecules*, Wiley, New York (1961).
Tyn, M. T., and T. W. Gusek, "Prediction of Diffusion Coefficients of Proteins," *Biotechnol. Bioeng.*, **35**, 327 (1990).
Waisman, E., and J. L. Lebowitz, "Exact Solution of an Integral Equation for the Structure of a Primitive Model of Electrolytes," *J. Chem. Phys.*, **52**, 4307 (1970).
Weast, R. C., ed., *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL (1988).
Zemaitis, J. F., D. M. Clark, M. Rafal, and N. C. Scrivner, *Handbook of Aqueous Electrolyte Thermodynamics*, AIChE, New York (1986).

Appendix

Phase equilibria in a system containing solvent, gel, salt, and protein

To calculate the electrostatic contribution to the partition coefficient, we determine the compositions of each phase that satisfy Eq. 5 for each component. To illustrate, consider a system containing water, a 1:1 salt, a protein, and a charged hydrogel. The protein has a net positive charge; for simplicity we take the counterions to the net charge to be the oppositely charged ion of the 1:1 salt. Similarly, we take the counterions of the gel charges to be one of the ions of the 1:1 salt. Thus, we have reduced the unknown-species concentrations to four (water, protein, and the two ions of the 1:1 salt) in each phase.

Experimentally, we prepare electrolyte solutions by adding neutral combinations of ions (salts) to water. Each of the neutral compounds is a *component* of the system; the ions and nondissociating neutral components are *species*. If we characterize our gel/solution system as a mixture of neutral components, we do not have to define and calculate explicitly Ψ, the electrostatic potential in Eq. A13. For example, consider a neutral salt with composition M_2X_3. The chemical potential of one mole of salt can be written as a combination of the chemical potentials of a mole of each ion:

$$\mu_{M_2X_3} = 2\mu_{M^{3+}} + 3\mu_{X^{2-}}.$$

Substituting the electrochemical potentials (Eq. A13), we obtain:

$$\mu_{M_2X_3} = 2\mu_{M^{3+}}^{\text{chemical}} + 2(3)F\Psi + 3\mu_{X^{2-}}^{\text{chemical}} + 3(-2)F\Psi$$
$$= 2\mu_{M^{3+}}^{\text{chemical}} + 3\mu_{X^{2-}}^{\text{chemical}}.$$

Thus, we do not need to calculate Ψ. The three independent, diffusible components of our system are water, the 1-1 salt, and the protein salt; we write three equations of equal chemical potential:

$$\mu_{\text{water}}^b = \mu_{\text{water}}^g \tag{A1a}$$

$$\mu_{MX}^b = \mu_{MX}^g \tag{A1b}$$

$$\mu_{PX_z}^b = \mu_{PX_z}^g, \tag{A1c}$$

where MX denotes the 1:1 salt, PX_z denotes the protein with a positive net charge of z and its z associated counterions, and superscripts b and g denote the bath and gel, respectively. For a negatively charged protein, we replace Eq. A1c with:

$$\mu_{M_zP}^b = \mu_{M_zP}^g. \tag{A1'c}$$

To obtain the composition of each phase, we solve these three equations simultaneously subject to electroneutrality and mass-balance constraints. We write one mass-balance equation for each species. We require one phase to be electroneutral; because the charges on the gel cannot diffuse between the phases, we write our equation of electroneutrality for the gel. By requiring electroneutrality in the gel, the bath is automatically electroneutral. We then have the following equations, in addition to Eqs. A1a–A1c:

For a positively charged protein:

$$n_{M^+}^g + zn_{P^{z+}}^g - n_{X^-}^g \pm n_{\text{ionized groups}}^g = 0 \tag{A2a}$$

$$n_{M^+}^g + n_{M^+}^b = n_{M^+} \tag{A2b}$$

$$n_{X^-}^g + n_{X^-}^b = n_{X^-} \tag{A2c}$$

$$n_{P^{z+}}^g + n_{P^{z+}}^b = n_{P^{z+}} \tag{A2d}$$

$$n_{\text{water}}^g + n_{\text{water}}^b = n_{\text{water}}. \tag{A2e}$$

Here n_j denotes the number of moles of j. The absence of superscript denotes combined total quantities in both phases. The $\pm$ symbol preceding $n_{\text{ionized groups}}^g$ means that $n_{\text{ionized groups}}^g$ is positive if the charges on the polymer are positive and negative otherwise. For a negatively charged protein, Eq. A2a is replaced with:

$$n_{M^+}^g - |z| n_{P^{z-}}^g - n_{X^-}^g \pm n_{\text{ionized groups}}^g = 0. \tag{A2'a}$$

Equations A1a–A1c and A2a–A2e provide a system of eight equations that we solve for eight unknown concentrations (n_P^g, n_P^b, $n_{X^-}^g$, $n_{X^-}^b$, $n_{M^+}^g$, $n_{M^+}^b$, n_{water}^g, n_{water}^b). To simplify computations, we reduce the number of simultaneous equations. To assess whether a particular model is useful for predicting solute distributions, it is sufficient to fix the composition of one phase. Because it is easier experimentally to determine solute concentrations in the bath, we fix the composition of the bath. Furthermore, we do not need to use the equality of chemical potential of the solvent (water) if we know how gel swelling depends on the ionic strength of a bath containing 1:1 electrolyte. (The measurement of swelling equilibria in salt solutions, while time-consuming, is straightforward; for example, see Baker et al., 1993, and Beltrán et al., 1990a,b). As long as the contribution of the protein (the multivalent solute) to the ionic strength is not larger than those of univalent ions, we can use experimental data to determine the water content of each phase, thus eliminating Eqs. A1a and A2e. (It is advantageous to eliminate the equality of chemical potential of the solvent also for another reason. As we have seen, expressions for the electrostatic Helmholtz energy of a solution are usually developed for the primitive model of electrolyte solutions in the McMillan–Mayer framework, where the solvent is "smeared out" and taken to be a dielectric medium. In the McMillan–Mayer framework, we cannot obtain the chemical potential for the solvent independently. The chemical potential is instead obtained via the Gibbs–Duhem equation as a function of the chemical potentials of the salt. While this, in principle, does not present a serious problem, it is an unnecessary calculation if we used experimental swelling equilibria to determine the solvent content of the gel phase. If the protein is dilute, we use experimental swelling equilibria determined in a protein-free salt solution at the ionic strength of interest.) For a positively charged protein, the remaining equations are (Eqs. A1b, A1c and A2a):

$$\mu_{MX}^b = \mu_{MX}^g$$

$$\mu_{PX_z}^b = \mu_{PX_z}^g$$

$$n_{M^+}^g + zn_{P^{z+}}^g - n_{X^-}^g \pm g_{\text{ionized groups}}^g = 0.$$

To solve for the gel-phase concentrations of the protein and the two ions of the simple salt, we express the chemical potentials in terms of the eight unknowns (n_P^g, n_P^b, $n_{X^-}^g$, $n_{X^-}^b$, $n_{M^+}^g$, $n_{M^+}^b$, n_{water}^g, n_{water}^b). Expressing Eqs. A1b and A1c in the form of Eq. 10, we see that we must adopt a model to calculate single-ion activity coefficients.

Derivation of Eq. 10

Equation 10 enables us to calculate the composition of a gel phase in equilibrium with a bath. To obtain Eq. 10 we first express the chemical potential of a neutral component i as the sum of the electrochemical potentials of its constituent ions:

$$\mu_i^{\text{chemical}} = \nu_+ \mu_+^{\text{electrochemical}} + \nu_- \mu_-^{\text{electrochemical}} \tag{A3}$$

where i denotes the neutral component (salt) that dissociates into ν_+ cations and ν_- anions, m is measure of concentrations, subscripts + and − denote the cation and anion, respectively, and the superscripts are included for clarity. We define the electrochemical potential of the cation and anion as (Guggenheim, 1959):

$$\mu_+^{\text{electrochemical}} = \mu_+^o + RT \ln(\gamma_+ m_+) + z_+ F\Psi \tag{A4}$$

$$\mu_-^{\text{electrochemical}} = \mu_-^o + RT \ln(\gamma_- m_-) + z_- F\Psi \tag{A5}$$

where the superscript o denotes the standard state, R is the universal gas constant, T is temperature, z_+ and z_- are the valences of the cation and anion, respectively, F is Faraday's constant, Ψ is the electrostatic potential, and γ is the activity coefficient. We use the symbol Ψ for the electrostatic potential because we have not yet related Ψ unambiguously to the electrochemical potential. For the purpose of this Appendix, it is unnecessary to define Ψ and γ precisely.

Substituting Eqs. A4 and A5 into Eq. A3 gives

$$\mu_i^{\text{chemical}} = \nu_+ \mu_+^o + \nu_- \mu_-^o + \nu_+ RT \ln(\gamma_+ m_+) + \nu_- RT \ln(\gamma_- m_-) + \nu_+ z_+ F\Psi + \nu_- z_- F\Psi. \quad \text{(A6)}$$

The sum of the last two terms of Eq. A6 is zero because the salt is a neutral combination of ions. Thus, we do not need to calculate Ψ explicitly to obtain the chemical potential of a neutral component. Combining logarithmic terms, we have

$$\mu_i^{\text{chemical}} = \nu_+ \mu_+^o + \nu_- \mu_-^o + RT \ln[(\gamma_+ m_+)^{\nu_+}(\gamma_- m_-)^{\nu_-}]. \quad \text{(A7)}$$

Using Eqs. A7 and 5 ($\mu_i^{\text{bath}} = \mu_i^{\text{gel}}$), accounting for the pressure difference between the gel and bath, and taking the standard-state chemical potentials of the ions to be the same in each phase, we obtain

$$\ln\left[(\gamma_+^b m_+^b)^{\nu_+}(\gamma_-^b m_-^b)^{\nu_-}\right] = \ln[(\gamma_+^g m_+^g)^{\nu_+}(\gamma_-^g m_-^g)^{\nu_-}] + c_{XL}(\bar{V}_i)\left(\frac{\phi_{\text{polymer}}}{\phi_{\text{polymer, synthesis}}}\right)^{1/3}, \quad \text{(A8)}$$

where $\bar{V}_i$ is the partial molar volume of component i (salt), ϕ_{polymer} is the volume fraction of polymer, and c_{XL} is the concentration of cross-links at synthesis [mol/m^3], and superscripts b and g denote bath and gel, respectively. Equation A8 has the same form as Eq. 10.

In the literature, the activity coefficient for a salt is expressed as the mean ionic activity coefficients, $\gamma_\pm$, where

$$\gamma_\pm^{(\nu_+ + \nu_-)} = (\gamma_+)^{\nu_+}(\gamma_-)^{\nu_-}. \quad \text{(A9)}$$

To calculate partition coefficients, it is not necessary to use mean ionic activity coefficients. However, expressions for mean ionic activity coefficients are common in the literature, and thus we present Eq. A7 with this notation, for reference:

$$\mu_i^{\text{chemical}} = \nu_+ \mu_+^o + \nu_- \mu_-^o + RT \ln(\nu_+^{\nu_+}\nu_-^{\nu_-}) + (\nu_+ + \nu_-)RT \ln(\gamma_\pm m_i) \quad \text{(A10)}$$

where m_i refers to the concentration of component i (mol·m^{-3})

Comparison of calculations of protein partition coefficients using different algorithms

Figure A1 presents a comparison between experimental partition coefficients and those calculated using the rigorous, semirigorous and quasi-electrostatic-potential algorithms with the activity coefficient expressions of Guerón and Weisbuch (1979). The system is the same as that in Figure 1. The calculations based on the quasi-electrostatic potential algorithm lie much farther from the experimental data than those based on the other two algorithms. The partition coefficients calculated using the semirigorous algorithm agree best with experiment.

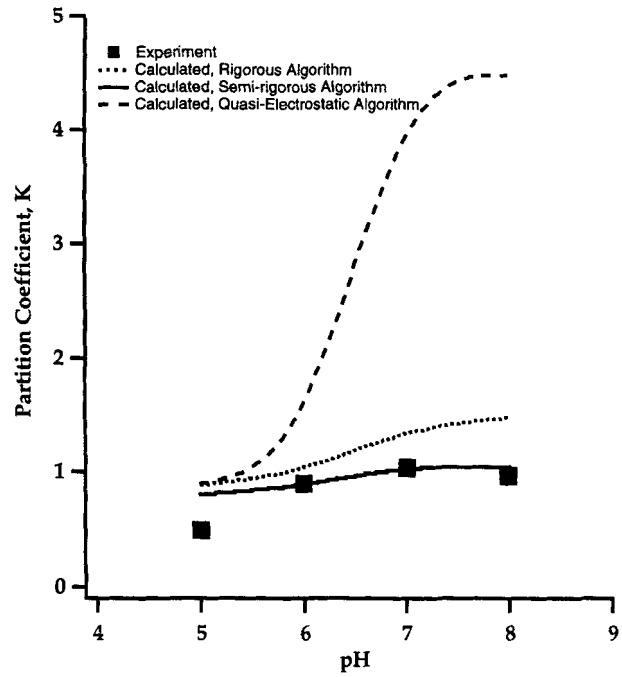

Figure A1. Calculated and experimental partition coefficients as a function of pH for cytochrome c in poly-NIPA/10%SA gels.

Experimental conditions are the same as those in Figure A2. Calculations are compared for the rigorous, semirigorous, and quasi-electrostatic potential algorithms, using ionic activity coefficients from the cell model.

It is simple to calculate the partition coefficient of a protein using the quasi-electrostatic potential difference calculated from the distribution of a mono-monovalent salt between bath and gel. However, this calculation gives poor results unless the salt concentration is large. Figure A2 presents calculated and experimental partition coefficients as a function of pH for cytochrome c in poly-*N*-isopropylacrylamide/10% sodium acrylate gels (15%*T*, 1%*C*). The temperature was 22.2°C, and the buffer was 0.1-M ionic-strength sodium phosphates. In this range of pH, the protein is positively charged and the gel is negatively charged. The results are analogous to those in Figure 1, except that in Figure A2, partition coefficients were calculated using the quasi-electrostatic potential algorithm. Experimental data are shown for comparison. With this algorithm, the partition coefficients calculated using Guerón and Weisbuch's activity-coefficient expressions lie farther from the experimental data than those partition coefficients calculated assuming activity coefficients to be unity. In this algorithm, the partition coefficients calculated with Debye–Hückel-theory (not shown) or MSA ionic activity coefficients differ little from the partition coefficients calculated assuming ionic activity coefficients are unity.

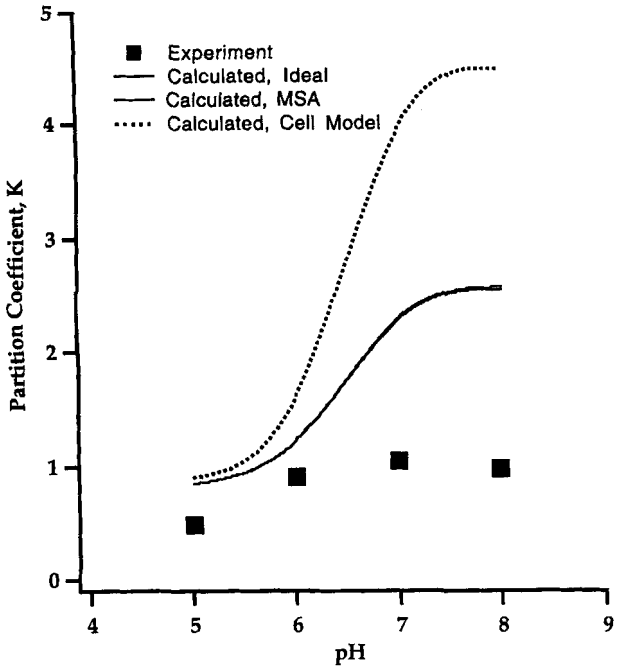

Figure A2. Calculated and experimental partition coefficients as a function of pH for cytochrome c in poly-NIPA/10%SA gels.

The experimental conditions are the same as those in Figure 1. Calculations were performed using the quasi-electrostatic potential algorithm. Ionic activity coefficients were taken from the Mean Spherical Approximation, the cell model, or assumed to be unity. When the MSA is used for activity coefficients, the results overlap with those obtained where the activity coefficients are unity.

Incorporation of short-range, nonelectrostatic forces into the calculation of partition coefficients

To incorporate nonelectrostatic interactions other than size-exclusion, we can follow the recent work of Haynes et al. (1993), who derived the excess Helmholtz energy due to nonelectrostatic interactions in:

$$A^{ex}_{\text{nonelectrostatic}} = RTN_{AV}\sum_i \sum_j n_i \frac{n_j}{V}\Lambda_{ij}(\mu_{\text{solvent}}, T), \quad \text{(A11)}$$

where N_{AV} is Avogadro's number, V is volume, R is the universal gas constant, T is temperature, n_i is the number of moles of i, and Λ_{ij} is a binary interaction parameter that characterizes the nonelectrostatic, nonexcluded-volume interaction of species i and species j in solvent. The excess Helmholtz energy is the contribution of short-range interactions (other than excluded volume) to the Helmholtz energy of a mixture of hard spheres. If we were to include only a binary protein–polymer interaction term into our partitioning model, we could calculate an activity coefficient for this interaction by assuming a one-parameter expansion in polymer concentration:

$$\ln\left(\gamma^{\text{nonelectrostatic}}_{\text{protein}}\right) = m_{\text{polymer}}\Lambda_{\text{polymer-protein}}, \quad \text{(A12)}$$

where m_{polymer} is some measure of the concentration of polymer in the gel and $\Lambda_{\text{polymer-protein}}$ is a binary interaction parameter characterizing the interaction between polymer and protein other than Coulombic forces or excluded volume.

The activity coefficient thus calculated for the protein in the gel ($\gamma^{\text{nonelectrostatic}}_{\text{protein}}$) would be multiplied by the single-ion activity coefficient for the protein in the gel ($\gamma^{\text{single-ion}}_{\text{protein}}$). The new activity coefficient (the product $\gamma^{\text{single-ion}}_{\text{protein}}\gamma^{\text{nonelectrostatic}}_{\text{protein}}$) would be used in place of the single-ion activity coefficient in Eq. 10. However, because we lack fundamental data for the interaction between cytochrome c and the poly-NIPA polymers, we cannot obtain $\Lambda_{\text{polymer-protein}}$ independently.

Quasi-electrostatic potential and the electrochemical potential

The electrochemical potential is sometimes separated into a chemical and an electrical term (Newman, 1991):

$$\mu_j = \mu_j^{\text{chemical}} + z_j F\Psi = RT\ln(m_j\Omega_j\delta_j^o) + z_j F\Psi, \quad \text{(A13)}$$

where z_j is the electronic charge of ion j, F is Faraday's constant, Ψ is the electrostatic potential, R is the universal gas constant, T is temperature, m_j is a measure of the concentration of ion j, Ω_j is the activity coefficient of ion j, and δ_j^o is a system-specific constant that depends on temperature and pressure but is independent of composition or electrical state.

Equation A13 has two undefined quantities, Ω_j and Ψ. As discussed, for example, by Newman (1991) and Haynes (Haynes et al., 1991; Haynes, 1992), either Ψ or Ω_j must be related unambiguously to the electrochemical potential. Without such a relation, calculations based on Ψ or Ω_j are ambiguous. Using quasi-electrostatic potentials, we can relate the electrochemical potential μ_j unambiguously to the electrostatic potential appearing in Eq. A13. We designate the quasi-electrostatic potential Φ to distinguish it from Ψ, which is not well-defined. Other means to relate μ unambiguously to the electrostatic potential are discussed by Newman (Newman, 1991).

To define the quasi-electrostatic potential, we select a reference species arbitrarily (Haynes et al., 1991; Newman, 1991) and define the electrochemical potential for the reference ion, r:

$$\mu_r = RT\ln(m_r) + z_r F\Phi. \quad \text{(A14a)}$$

We can also express the electrochemical potential for the reference ion in more standard notation:

$$\mu_r = \mu_r^o + RT\ln(m_r\gamma_r). \quad \text{(A14b)}$$

In Eqs. A14a and A14b, $z_r \neq 0$, and μ_r^o is the standard-state chemical potential. Faraday's constant is denoted by F, γ_r is the activity coefficient of the reference ion, and Φ is the quasi-electrostatic potential. γ_r includes all contributions from electrostatic and nonelectrostatic forces. Equating Eqs. A14a and A14b and solving for Φ, we obtain

$$\Phi = \frac{\mu_r^o}{z_r F} + \frac{RT\ln(\gamma_r)}{z_r F}. \quad \text{(A15)}$$

Consider two phases (′ and ″) at equilibrium. Using $\mu_r' = \mu_r''$ and Eq. A14a, we can relate the partitioning of the reference ion between liquid phases ′ and ″ directly to the difference in quasi-electrostatic potentials:

$$\ln K_r = \ln\left(\frac{m_r'}{m_r''}\right) = \frac{z_r F}{RT}(\Phi'' - \Phi'), \qquad \text{(A16)}$$

where K_r is the partition coefficient of the reference ion and z_r is the valence of reference ionic species r. We define the electrochemical potential for any other ionic species j as

$$\mu_j = \mu_j^o + RT\ln(m_j\gamma_j) = RT\ln(m_j\Omega_j\delta_j^o) + z_jF\Phi, \quad \text{(A17)}$$

where $m_j\Omega_j\delta_j^o$ is the chemical contribution to the electrochemical potential of ion j and is independent of the electrical state of the system. Rearranging this equation and substituting Eq. A15 for Φ, we obtain

$$RT\ln(m_j\Omega_j\delta_j^o) = \mu_j^o + RT\ln(m_j\gamma_j) - \frac{z_j}{z_r}\mu_r^o - \frac{z_j}{z_r}RT\ln(\gamma_r). \qquad \text{(A18)}$$

Substituting Eq. A18 into Eq. A17, the electrochemical potential for species j is

$$\mu_j = \mu_j^o + RT\ln(m_j\gamma_j) - \frac{z_j}{z_r}\mu_r^o - \frac{z_j}{z_r}RT\ln(\gamma_r) + z_jF\Phi \qquad \text{(A19)}$$

and the partition coefficient for species j between phases ′ and ″ is

$$\ln K_j = \frac{z_jF}{RT}(\Phi'' - \Phi') + \ln\left(\frac{\gamma_j''}{\gamma_j'}\right) - \frac{z_j}{z_r}\ln\left(\frac{\gamma_r''}{\gamma_r'}\right). \quad \text{(A20)}$$

To illustrate, consider the distribution of NaCl between an uncharged gel and a bath. Because each phase is electrically neutral, the partition coefficients of NaCl, Na^+, and Cl^- are equivalent. If we choose Na^+ to be the reference ion and ignore the osmotic-pressure difference between the gel and bath, we can equate Eqs. A16 and A20 to obtain

$$\Phi^g - \Phi^b = \frac{RT}{(z_r - z_-)F}\ln\left[\frac{(\gamma_-^g/\gamma_-^b)}{(\gamma_r^g/\gamma_r^b)^{z_-/z_r}}\right] = \frac{RT}{2F}\ln\left(\frac{\gamma_-^g\,\gamma_r^g}{\gamma_-^b\,\gamma_r^b}\right) = \frac{RT}{F}\ln\left(\frac{\gamma_\pm^g}{\gamma_\pm^b}\right) = -\frac{RT}{F}\ln(K_{\text{salt}}), \quad \text{(A21)}$$

where subscripts r and $-$ denote the reference cation (Na^+) and the anion (Cl^-), respectively, and the mean activity coefficient, $\gamma_\pm$, for a 1:1 salt is

$$\gamma_\pm^2 = \gamma_+\,\gamma_-, \qquad \text{(A22)}$$

where $\gamma_+ = \gamma_r$ in this example. The partition coefficient for the salt is the ratio of the concentration of the salt in the gel to that in the bath.

Equation A21 relates the quasi-electrostatic potential difference directly to the salt partitioning. If the gel is charged, it is advantageous to choose the reference ion to be the mobile ion whose charge is the same as that of the polymer. By doing so, we retain the direct relationship between the quasi-electrostatic potential difference and the partition coefficient of a salt (Eq. A21).

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