Lawrence Berkeley National Laboratory
Recent Work

Title
Phase Equilibria for Aqueous Protein/Polyelectrolyte Gel Systems

Permalink
https://escholarship.org/uc/item/2rk796g4

Authors
Sassi, A.P.
Blanch, H.W.
Prausnitz, John M.

Publication Date
1995

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

CHEMICAL SCIENCES DIVISION

Phase Equilibria for Aqueous Protein/Polyelectrolyte Gel Systems

A.P. Sassi, H.W. Blanch, and J.M. Prausnitz

January 1995

DISCLAIMER

Phase Equilibria for Aqueous Protein/Polyelectrolyte Gel Systems

Alexander P. Sassi, Harvey W. Blanch, and John M. Prausnitz

Department of Chemical Engineering
University of California, Berkeley

and

Chemical Sciences Division
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

January 1995

PHASE EQUILIBRIA FOR AQUEOUS
PROTEIN/POLYELECTROLYTE GEL SYSTEMS

Alexander P. Sassi, Harvey W. Blanch and John M. Prausnitz*

Chemical Engineering Department, University of California, Berkeley
and
Chemical Sciences Division, Lawrence Berkeley Laboratory,
Berkeley, CA 94720

Key Words:

Phase Equilibria, Gel, Protein, Polyelectrolyte, Partitioning

* to whom correspondence should be addressed

PHASE EQUILIBRIA FOR AQUEOUS PROTEIN/POLYELECTROLYTE GEL SYSTEMS

ABSTRACT

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.

I. 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; Giddings et al., 1968; Ackers, 1970; Casassa, 1971a; Casassa, 1971b; Davidson et al., 1987; Schnitzer, 1988; Fanti and Glandt, 1989; Fanti and Glandt, 1990a; Fanti et al., 1990b; 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., 1994c).

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.

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 work, 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 work 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 (Albertsson, 1986) and Guggenheim (Guggenheim, 1959), we separate contributions to the partition coefficient from electrostatic and non-electrostatic forces:

$$\ln(K) = \ln\left(K^{\text{non-electrostatic}}\right) + \ln\left(K^{\text{electrostatic}}\right)$$

(I.1)

For $K^{\text{non-electrostatic}}$, 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 $K^{\text{non-electrostatic}}$ and $K^{\text{electrostatic}}$.

II. SIZE EXCLUSION

Elsewhere (Sassi et al., 1994a), 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., 1994a). 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 $K^{\text{non-electrostatic}}$ in equation I.1.

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

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

(II.1)

where ϑ is the ratio of solute to pore radius, r_s/r_p, and v_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 (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:

$$\zeta = \phi_p^{-1/3}\langle r_{e\text{-}e}^2 \rangle^{1/2}$$

(II.2)

where $\langle r_{e\text{-}e}^2 \rangle$ 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 crosslinks. The mean square end-to-end distance for a random-flight chain is related to the mean square radius of gyration, $<s^2>$, which can be measured by light-scattering :

$$\langle r_{e\text{-}e}^2 \rangle = 6\langle s^2 \rangle$$

(II.3)

III. COULOMBIC INTERACTIONS

To obtain $K^{electrostatic}$, we specify the composition of the bath and find that composition of the gel which 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 any <u>neutral</u> solute i must be the same in each phase containing that component:

$$\mu_i^b = \mu_i^g$$

(III.1)

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 equation III.1, except that μ_i now refers to the electrochemical potential of <u>charged</u> 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 equation III.1 for each independent *component* of the system[†] . An example of a *component* is a protein and its associated counterions. In Appendix A, we discuss how to solve equations III.1 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:

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

(III.2)

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 the chemical potential of a component i in the gel phase:

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

[†] The advantage of solving equation III.1 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 Appendix B.

where $\Delta\mu_i^{\Delta P} = -(\Delta\Pi_{elastic})\overline{V}_i$. Here, $\Delta\Pi_{elastic}$ is the elastic contribution to the swelling pressure of

the gel[†], and $\overline{V}_i$ is the partial molar volume of component i. We calculate $\Delta\Pi_{elastic}$ 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:

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

where $\overline{V}_{solvent}$ is the partial molar volume of the solvent. $\Delta A^{elastic}$ 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 $\phi_{polymer}$, and c_{XL} is the concentration of crosslinks at

synthesis $[mol/m^3]$ (Baker et al., 1994). In the ion-exchange literature, $\Delta\Pi_{elastic}$ is usually

neglected, although $\Delta\Pi_{elastic}$ 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 which distributes between a bath and a gel, we rewrite equation III.1

using equations III.2-4 :

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

In our system, activity coefficients account for coulombic interactions between charges.

In practice, we calculate activity coefficients for individual ions; we call these activity

---

[†] The elastic contribution to the swelling pressure of the gel counteracts the osmotic forces which cause the gel to

swell. At equilibrium, the swelling pressure of the gel must be zero; that is, forces which act to expand and contract

the network must balance. $\Delta\Pi_{elastic}$ prevents the network from expanding infinitely because the network is

physically entangled and chemically crosslinked.

coefficients *single-ion activity coefficients*[†]. 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 equation III.5 in terms of calculable quantities:

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

(III. 6)

where ν is the stoichiometric coefficient of an ion in the neutral salt, and subscripts + and – denote cation and anion, respectively. In Appendix B, we explain how to obtain equation III.6. In Appendices C, D, and E, we discuss how to calculate single-ion activity coefficients[†] from the

---

[†] Single-ion activity coefficients are also defined by equation III.2, where μ_i is the electrochemical potential of ion i.

[†]   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 (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 which corresponds to experiment), we add the term $-P^{ex}\overline{V}_i$, 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, most recently by Haynes (Haynes, 1992; Haynes et al., 1993). If single-ion activity coefficients are derived in the McMillan-Mayer framework, equation III.6 is written:

$$\ln\left[\left(\gamma_+^b m_+^b\right)^{\nu_+}\left(\gamma_-^b m_-^b\right)^{\nu_-}\right] - P^{ex,b}\overline{V}_i =$$

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

(III.7)

For our systems, including the excess pressure/volume terms in equation III.7 has a negligible effect on calculated partition coefficients. We discuss how to obtain P^{ex} in Appendices C, D, and E.

Helmholtz energy, A. The following section presents three models for single-ion activity coefficients.

IV. 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 uncrosslinked 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.

We could, as a first approximation, ignore the presence of charges on the polyelectrolyte and use a theory for dilute polymer solutions. However, this is 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

---

[†] Fortunately, our "polymer-rich" phase (the gel) is dilute in polymer because charged hydrogels tend to be highly swollen. This is advantageous because electrostatic theories for polyelectrolyte solutions usually neglect interactions between polyelectrolyte chains.

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 for polyelectrolyte solutions.

A. 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 0.1M for a 1-1 salt) (McQuarrie, 1975). Single-ion activity coefficients are given by:

$$\ln\left(\gamma_j^{single\ ion}\right) = -\frac{z_j^2 e^2 \beta \kappa}{8\pi\varepsilon_0\varepsilon_r(1+\kappa a)}$$

(IV.A.1)

where β is $(k_b T)^{-1}$, k_b is Boltzmann's constant, e is the charge on an electron, ε_0 is the permittivity of a vacuum, ε_r is the permittivity of the solvent (water) relative to a vacuum ($\varepsilon_0\varepsilon_r$ 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 3.04Å for a[†]; κ is given by:

[†] In the systems for which 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.

$$\kappa^2 = \frac{e^2\beta}{\varepsilon_o\varepsilon_r}\sum_j \eta_j z_j^2$$

(IV.A.2)

where η_j is the number density (N_j/V) of ions of type j. Equations IV.A.1 and IV.A.2 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[†]. Appendix C discusses in further detail the derivation of activity coefficients from the Poisson-Boltzmann equation using Debye-Hückel theory.

B. 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 reference (Sassi et al., 1994b). A simpler expression for the single-ion activity coefficient is obtained by calculating an effective

---

[†] 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.

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:

$$\ln\left(\gamma_j^{\text{single ion}}\right) = -\frac{\Gamma^3}{\pi}\left[\frac{(1+a_{mix}\Gamma)z_j^2}{\sum\limits_j n_j z_j^2} - \frac{\Gamma}{\eta}(a_j - a_{mix})\right]$$

(IV.B.1)

where

$$\eta a_{mix} = \sum_j n_j a_j \qquad\qquad \eta = \sum_j n_j$$

(IV.B.2, 3)

$$\Gamma = \frac{1}{2a_{mix}}\left[(1+2a_{mix}\kappa)^{1/2} - 1\right] \qquad\qquad \kappa^2 = \frac{e^2\beta}{\varepsilon_o\varepsilon_r}\sum_j n_j z_j^2$$

(IV.B.4, 5)

where 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. In Appendix D, we discuss briefly how the OZ equation is solved in the MSA. Details can be found in references (Waisman and Lebowitz, 1970), (Blum, 1975), and (Blum and Høye, 1977).

C. The 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. We discuss this integration in Appendix E. However, Guerón and Weisbuch have used the integration procedure to examine some general characteristics of the numerical solutions to the Poisson-Boltzmann equation (Guerón and Weisbuch, 1979). 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 which bears the fixed charges:

$$\gamma_{counterion} = \frac{0.7\aleph/\xi + 1}{\aleph + 1}$$

(IV.C.1)

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

$$\gamma_{coion} = \frac{0.7\aleph/\xi + 1}{0.53\aleph/\xi + 1}$$

(IV.C.2)

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[†] $\Im$, to the axial length per unit charge, b:

$$\xi = \frac{\Im}{b} = \frac{e^2\beta}{4\pi\varepsilon_o\varepsilon_r b}$$

(IV.C.3)

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.

V. DETERMINATION OF MODEL PARAMETERS

To apply the models described above, we must obtain values for parameters which 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 Bjerrum length is the length whose potential has a magnitude $k_b T$.

The solutes (salt and protein) are modelled 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 reference (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 tri-valent 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 stoichiometric sum of the partial molar volumes for each ion of the salt.

When Katchalsky's cell model is used, the polyelectrolyte gel is modelled as a network of cylindrical fibers. We set the length of a monomer at 2.52Å; 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 5Å. 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 crosslink density at synthesis was determined from the nominal %C[†] [(mole crosslinker/mole total monomer) • 100]. 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)[††].

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 which 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

---

[†] The effect of crosslinking 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 crosslinking applicable to partitioning and swelling calculations, we nevertheless use the nominal parameters.

[††] 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 0.1M 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 0.1M or more.

at the desired salt concentration and ionic strength. We estimated this average pK by the pH at which the experimental plot of swelling equilibria versus 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[†] :

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

(V.1)

We were able to predict better 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).

VI. RESULTS

A. Comparison of Calculated and Experimental Protein Partition Coefficients

We investigated three algorithms to calculate protein partition coefficients in polyelectrolyte hydrogels. We discuss these algorithms in Appendix F; here, we present calculations using the semi-rigorous 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 0.1M 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 reference (Sassi et al., 1994d). We calculated partition coefficients using the semi-rigorous algorithm with single-ion activity coefficients from Debye-Hückel theory, the MSA, the cell model, or assuming activity coefficients to be unity.

[†] Equation V.1 is written for an acid HA which dissociates into A⁻ and H⁺. If the ionizable group is an amine, the corresponding equation is:

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

where th amine (NR_3) is charged when protonated (NR_3H^+).

We obtained the best agreement between calculation and experiment when we used activity coefficients from Guerón and Weisbuch (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 < 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 0.1M-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 à 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 non-electrostatic interactions (other than size exclusion) between 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 Appendix G, 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., 1994a).

B. 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[†] ($K^{\Delta P} = \exp\left(\Delta\Pi_{elastic}\overline{V}_{protein}\right)$,

---

[†] Here, we calculate explicitly the contribution of the pressure difference to the partition coefficient. The contribution of the pressure difference to the partition coefficient is related to the overall partition coefficient :

where $\Delta\Pi_{elastic}$ is given by equation III.4 as a function of solute radius (see also Appendix F). 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, however, can be modelled (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

$$\ln(K) = \ln\left(K^{SEC}\right) + \ln\left(K^{\Delta P}\right) + \ln\left(K^{electrostatic}\right)$$

where K^{SEC} is calculated using Schnitzer's uniform-pore model, and $K^{electrostatic}$ includes contributions only from coulombic interactions. The contribution of the pressure difference to the partition coefficient, $K^{\Delta P}$, is calculated separately:

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

where $\Delta\Pi_{elastic}$ is given by equation III.4. The overall protein partition coefficient is calculated in this manner when the quasi-electrostatic potential algorithm is used, as described in Appendix F.

protein and the charge density of the gel, which, in turn, is influenced by the chemical composition of the gel and factors which 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 0.1M ionic-strength buffer at 22.2°C. 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 7g/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[†].

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 0.1M 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

---

[†] 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 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.

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 0.01M, 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 0.5mM 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 0.5mM (corresponding to Figure 4), the calculated partition coefficient for ovalbumin rises slightly. The partitioning of lysozyme at 0.5mM does not increase monotonically as in Figure 4 but shows an unexpected minimum and maximum. The unexpected trends at 0.5mM 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 0.05mM 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 0.05mM concentration in the bath solution and 0.01M 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.

We can decouple partially the complex relationship between the electrostatic contribution to the protein partition coefficient, gel composition, and ionic strength of the bath by using the quasi-electrostatic potential difference, $\Delta\Phi$, between the gel and bath . (The quasi-electrostatic potential is discussed in Appendices F and H.) 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:

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

(VI.B.1)

for a negatively charged gel:

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

(VI.B.2)

where $z_{protein}$ is the net electronic charge of the protein (Haynes et al., 1991; Newman, 1991; Haynes, 1992).

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

---

[†] To define the quasi-electrostatic potential, we must choose a reference ion (Eq. H.2a of Appendix H); the quasi-electrostatic potential difference depends on the sign of the reference ion (Eq. H.4 of Appendix H). If a mono-monovalent salt partitions into a 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. We discuss this in Appendix H. Equations VI.B.1 and VI.B.2 result because of 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 VI.B.1 is obtained using equations F.1 and the last term of F.3 in Appendix F. Equation VI.B.2 is obtained using equations F.2 and the last term of F.4 in Appendix F.

equation reported by Stigter for the cell model (Stigter, 1975)[†]. 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 1000 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 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 Appendix F. 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 millivolts. Here, the gel is positively charged. For negatively charged proteins, partition coefficients are not greater than two unless the potential difference exceeds one millivolt. For positively charged proteins, partition coefficients are not less than 0.1 unless the potential difference exceeds about 10 millivolts. For a protein of net charge -6, the potential difference must be approximately 10 millivolts 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

---

[†] We first use Stigter's results to calculate the partitioning of mono-monovalent salt using equation E.9. We then obtain the quasi-electrostatic potential difference using equation F.1 or F.2.

various salts in an aqueous two-phase polymer system (Haynes et al., 1991; Haynes, 1992; Haynes et al., 1993).

For "design" of a polyelectrolyte gel which 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:

$$\Delta\Pi_{swelling} = -\frac{\mu^{g}_{solvent} - \mu^{b}_{solvent}}{\overline{V}_{solvent}} = \Delta\Pi_{mixing} + \Delta\Pi_{elastic} + \Delta\Pi_{ion} = 0$$

(VI.B.3)

$$\Delta\Pi_{mixing} = -\frac{RT}{\overline{V}_{solvent}}\left(\ln\left(1 - \phi_{polymer}\right) + \phi_{polymer} + \chi\phi^{2}_{polymer}\right)$$

(VI.B.4)

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

(VI.B.5)

$$\Delta\Pi_{ion} = RT\sum_{j}(c^{g}_{j} - c^{b}_{j})$$

(VI.B.6)

where $\Delta\Pi$ is called the swelling pressure, $\phi_{polymer}$ is the volume fraction of polymer, $\overline{V}_{solvent}$ is the molar volume of the solvent, c_{XL} is theconcentration of crosslinks 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 VI.B.5 is obtained from the phantom network theory of elasticity. In the phantom network theory, the gel is assumed to be a perfect tetrafunctional network which swells isotropically and whose points of crosslinking fluctuate randomly in space due to thermal (Brownian) motion (Mark and Erman, 1988). In Equation VI.B.6, we consider only the entropic contribution of the ions to the swelling pressure. Although we could use a more exact expression for $\Delta\Pi_{ion}$, we would not change the qualitative features of Figures 9a and 9b. Baker et al have shown that equations VI.B.3-6 predict swelling equilibria satisfactorily for polyelectrolyte hydrogels in dilute aqueous solutions of simple salts.

As an example, we have applied equations II.1, III.6, and VI.B.3 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 ratio in 1.0 M salt. Figure 9b presents the similar results for 0.1 M salt. From Figure 9a, we see that, to obtain a potential difference greater than one millivolt 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 b 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.

VII. 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 semi-rigorous algorithm is simple to use and provides better agreement between calculated and experimental data than other algorithms. In the semi-rigorous 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 à 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 which has desirable swelling and partitioning properties in aqueous salt solutions.

ACKNOWLEDGMENTS

FIGURE CAPTIONS

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 0.1M 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.

Figure 2. Calculated and experimental partition coefficients as a function of pH for cytochrome c in poly-NIPA/10%SA at 36.4°C and in poly-NIPA/10%DMA at 22.2° and 36.4°C. The buffer was 0.1M ionic-strength sodium phosphates. Single-ion activity coefficients are from the cell model.

Figure 3. The 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.

Figure 4. The calculated effect of pH on the partitioning of ovalbumin and lysozyme into poly-NIPA/10%SA gels (15%T, 1%C) in 0.1M 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. The calculated effect of pH on the partitioning of ovalbumin and lysozyme into poly-NIPA/10%DMA gels (15%T, 1%C) in 0.1M 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.

Figure 6. The calculated effect of pH on the partitioning of ovalbumin and lysozyme into poly-NIPA/10%SA gels (15%T, 1%C) in 0.01M ionic-strength buffer at 22.2°C. Results are shown for protein concentrations in the bath solution of 0.5 and 0.05 mM. Activity coefficients are from the cell model.

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

Figure 9a. Relation between the swelling ratio for a hypothetical polylectrolyte 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 0.1M NaCl, $\Delta\Phi$, and χ. The gel is the same as that in Figure 9a.

NOMENCLATURE

Roman

a	uniform ion diameter (m)
a_j	diameter of ion j (m)
a_{mix}	average diameter of all ions in solution (m)
a_i	activity of component i
$\Delta A^{elastic}$	change in Helmholtz energy upon tension of the network (J)
b	axial length per unit charge (monomer length) (m)
c_j	concentration of mobile ionic species j (mol m^{-3})
c_{XL}	concentration of crosslinks at synthesis (mol m^{-3})
%C	percent crosslinking monomer
%CM	percent comonomer
e	electronic charge (1.6022×10^{-19} C)
F	Faraday's constant (96,500 C mol^{-1})
k_b	Boltzmann's constant (1.381×10^{-23} J K^{-1})
K	partition coefficient
m_i	measure of concentration of component i (for example, mol m^{-3})
m_i^o	measure of standard state concentration of component i (for example, mol m^{-3})
M	unit of concentration (mol L^{-1})
n_i	mole number of component i (mol)
N_j	number of species j
P^{ex}	excess pressure (Pa)
pH	negative the base ten logarithm of the activity of the hydrogen ion
pK	negative the base ten logarithm of the equilibrium dissociation constant
r_p	pore radius (m)
r_s	solute radius (m)

$\langle r_{c\text{-}e}^2 \rangle$	mean square end-to-end distance (m^2)
R	universal gas constant (8.314 J mol^{-1} K^{-1})
$<s^2>$	mean square radius of gyration (m^2)
T	temperature (K)
%T	ratio of monomer to diluent at synthesis (g mL^{-1})
V	volume (m^3)
$\overline{V}_i$	partial molar volume of component i (m^3 mol^{-1})
z_j	valence of species j
$z_{protein}$	net valence of protein

Greek, Hebrew, and Symbols

א	ratio of concentration of charged monomers to concentration of monovalent salt
ℶ	Bjerrum length (m)
α	fraction of ionizable monomers which are charged
β	$= (k_b T)^{-1}$ (mol J^{-1})
χ	Flory's interaction parameter
ε_o	vacuum permittivity (8.854 $\times$ 10^{-12} C^2 J^{-1} m^{-1})
ε_r	relative permittivity (78.3 for water at 25°C)
ϕ_p	polymer volume fraction
Φ	quasi-electrostatic potential (V) (defined in Appendix H)
$\Delta\Phi$	quasi-electrostatic potential difference (V)
υ_c^0	volume of pore space per unit volume (porosity)
ϑ	ratio of solute to pore radius
γ_i	activity coefficient of component i
γ_j	single-ion activity coefficient of ionic species j
γ_+	single-ion activity coefficient for a cation

γ_-	single-ion activity coefficient for an anion
Γ	screening parameter in the Mean Spherical Approximation (m^{-1})
η_j	number density of ionic species j (m^{-3})
η	total number density of solution, $\eta = \sum_j \eta_j$ (m^{-3})
κ	inverse Debye screening length (m^{-1})
μ_i	chemical potential of a neutral component i (J mol^{-1})
μ_i^0	standard state chemical potential of neutral component i (J mol^{-1})
$\Delta\mu_i^{\Delta P}$	contribution of pressure differential between bath and gel to chemical potential of component i (J mol^{-1})
$\Delta\Pi_{swelling}$	swelling pressure of a gel (Pa)
$\Delta\Pi_{elastic}$	elastic contribution to swelling pressure (Pa)
$\Delta\Pi_{mixing}$	mixing contribution to swelling pressure (Pa)
$\Delta\Pi_{ion}$	ionic contribution to swelling pressure (Pa)
ν_+	stoichiometric coefficient of cation in a neutral salt
ν_-	stoichiometric coefficient of anion in a neutral salt
ν	sum of stoichiometric coefficients ν_+ and ν_- for a neutral salt
ξ	dimensionless linear charge density
ζ	mesh size (m^{-1})

REFERENCES

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).

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*. Ph.D. thesis, University 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," *Macromolecules*, **27**, 1446 (1994).

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

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

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

Brash, J. L., and T. A. Horbett, Ed., *Proteins at Interfaces: Physicochemical and Biochemical Studies*, ACS Symposium Series, American Chemical Society, Washington, DC (1987).

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

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

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

Casassa, E. F., "Theoretical Models for Peak Migration in Gel Permeation Chromatography," *Journal of Physical Chemistry*, **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 Ophthalmic Hydrogels," *Makromolekuläre Chemie, 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," *American Journal of Physiology*, 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," *Macromolecules*, 20, 1141 (1987).

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

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

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

Fanti, L. A., and E. Glandt, "Partitioning of Spherical Particles into Fibrous Matrices 1. Density Functional Theory," *Journal of Colloid and Interfacial Science*, 135, 385 (1990a).

Fanti, L. A., E. D. Glandt, and W. G. Madden, "Fluids in Equilibrium with Disordered Porous Materials. Integral Equation Theory," *Journal of Chemical Physics*, 93, 5945 (1990b).

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

Gachon, A. M., T. Bilbaut, and B. Dastugue, "Adsorption of Tear Proteins on Soft Contact Lens," *Experimental Eye Research*, 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," *Journal of Physical Chemistry*, 72, 4397 (1968).

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).

Guggenheim, E. A., *Thermodynamics*, North Holland Publishing Company, 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," *Journal of Physical Chemistry*, 92, 6432 (1988).

Haynes, C. A., *Separation of Protein Mixtures by Extraction: Statistical-Mechanical Models of Aqueous Solutions Containing Polymers, Salts, and Globular Proteins*. Ph.D. thesis, University 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 Journal*, 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 Journal*, 37, 1401 (1991).

Hoffman, A. S., A. Afrassiabi, and L. Chang Dong, "Thermally Reversible Hydrogels: II. Delivery and Selective Removal of Substances from Aqueous Solutions," *Journal of Controlled Release*, 4, 213 (1986).

Holly, F. J., "Protein and Lipid Adsorption by Acrylic Hydrogels and Their Relation to Water Wettability," *Journal of Polymer Science, Polymer Symposium*, 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," *Journal of Biomedical Materials Research*, 15, 402 (1981).

Hussain, S., M. S. Mehta, J. I. Kaplan, and P. L. Dubin, "Experimental Evaluation of Conflicting Models for Size Exclusion Chromatography," *Analytical Chemistry*, **63**, 1132 (1991).

Kim, Y.-H., Y. H. Bae, and S. W. Kim, "pH/Temperature-sensitive Polymers for Macromolecular Drug Loading and Release," *Journal of Controlled Release*, **28**, 143 (1994).

Kubota, K., S. Fujishige, and I. Ando, "Single-Chain Transition of Poly(N-isopropylacrylamide) in Water," *Journal of Physical Chemistry*, **94**, 5154 (1990).

Laurent, T. C., and J. Killander, "A Theory of Gel Filtration and Its Experimental Verification," *Journal of Chromatography*, **14**, 317 (1964).

Lifson, S., and A. Katchalsky, "The Electrostatic Free Energy of Polyelectrolyte Solutions. II. Fully Stretched Macromolecules," *Journal of Polymer Science*, **13**, 43 (1954).

Marinsky, J. A., "Interpretation of Ion-Exchange Phenomena," in *Ion Exchange: A Series of Advances*, J. A. Marinsky, Ed., Marcel Dekker, Inc., New York, 353 (1966).

Mark, J. E., and B. Erman, *Rubberlike Elasticity, A Molecular Primer*, John Wiley and Sons, New York (1988).

McQuarrie, D. A., *Statistical Mechanics*, Harper and Row, New York (1975).

Minarik, L., and J. Rapp, "Protein Deposits on Individual Hydrophilic Contact Lenses: Effects of Water and Ionicity," *CLAO Journal*, **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," *Current Eye Research*, **10**, 187 (1991).

Newman, J. S., *Electrochemical Systems*, 2nd ed., Prentice Hall, Englewood Cliffs (1991).

Ogston, A. G., "The Spaces in a Uniform Random Suspension of Fibres," *Trans. Faraday Soc.*, **54**, 1754 (1958).

Oliver III, J. D., and W. D. Deen, "Random-Coil Model for Glomerular Sieving of Dextran," *Bulletin of Mathematical Biology*, **56**, 369 (1994).

Peppas, N. A., H. J. Moynihan, and L. M. Lucht, "The Structure of Highly Crosslinked Poly(2-hydroxyethylmethacrylate) Hydrogels," *Journal of Biomedical Materials Research*, **19**, 397 (1985).

Ratner, B. D., and A. S. Hoffman (1975). *Synthetic Hydrogels for Medical Applications*. American Chemical Society, Division of Polymer Chemistry, Chicago, American Chemical Society (1975).

Ratner, B. D., and I. F. Miller, "Transport Through Crosslinked Poly(2-Hydroxyethyl Methacrylate) Hydrogel Membranes," *Journal of Biomedical Materials Research*, **7**, 353 (1973).

Refojo, M., and F.-L. Leong, "Microscopic Determination of the Penetration of Proteins and Polysaccharides into Poly(hydroxyethylmethacrylate) and Similar Hydrogels," *Journal of Polymer Science, Polymer Symposium*, **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," *Journal of Chemical Physics*, **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," *submitted to Journal of Applied Polymer Science*, (1994a).

Sassi, A. P., D. Freed, H. W. Blanch, and J. M. Prausnitz, "Partitioning of Hexavalent Chromium in Temperature-Sensitive Hydrogels," *submitted to Journal of Applied Polymer Science*, (1994b).

Sassi, A. P., S.-H. Lee, Y. Park, H. W. Blanch, and J. M. Prausnitz, "Sorption of Lysozyme by HEMA Copolymer Hydrogels," *submitted to Journal of Applied Polymer Science*, (1994c).

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," *submitted to Journal of Applied Polymer Science*, (1994d).

Schnitzer, J., "Analysis of Steric Partition Behavior of Molecules in Membranes Using Statistical Physics," *Biophysical Journal*, **54**, 1065 (1988).

Shaw, R. W., and C. R. Hartzell, "Hydrogen Ion Titration of Horse Heart Ferricytochrome C," *Biochemistry*, **15**, 1909 (1976).

Stigter, D., "The Charged Colloidal Cylinder with a Gouy Double Layer," *Journal of Colloid and Interfacial Science*, **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," *Biotechnology and Bioengineering*, **35**, 327 (1990).

Waisman, E., and J. L. Lebowitz, "Exact Solution of an Integral Equation for the Structure of a Primitive Model of Electrolytes," *Journal of Chemical Physics*, **52**, 4307 (1970).

Weast, R. C., Ed., *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton (1988).

Zemaitis, J. F., D. M. Clark, M. Rafal, and N. C. Scrivner, *Handbook of Aqueous Electrolyte Thermodynamics*, AIChE Publishing, New York (1986).

SUPPLEMENTARY MATERIAL

for

PHASE EQUILIBRIA FOR AQUEOUS

PROTEIN/POLYELECTROLYTE GEL SYSTEMS

Alexander P. Sassi, Harvey W. Blanch and John M. Prausnitz*

Chemical Engineering Department, University of California, Berkeley

and

Chemical Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720

*to whom correspondence should be addressed

APPENDIX A

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 which satisfy equation III.1 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 non-dissociating 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 equation H.1[†]. 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:

[†] 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 (equation H.1), we obtain:

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

Thus, we do not need to calculate Ψ.

$$\mu^{b}_{water} = \mu^{g}_{water}$$

$$\mu^{b}_{MX} = \mu^{g}_{MX}$$

$$\mu^{b}_{PX_{z}} = \mu^{g}_{PX_{z}}$$

(A.1a-c)

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 the superscripts b and g denote the bath and gel, respectively. For a negatively-charged protein, we replace Equation A.1c with the following:

$$\mu^{b}_{M_{z}P} = \mu^{g}_{M_{z}P}$$

(A.1'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 equations (A.1a-c),:

for a positively-charged protein:

$$n^{g}_{M^{+}} + zn^{g}_{P^{z+}} - n^{g}_{X^{-}} \pm n^{g}_{ionized\ groups} = 0$$

$$n^{g}_{M^{+}} + n^{b}_{M^{+}} = n_{M^{+}}$$

$$n^{g}_{X^{-}} + n^{b}_{X^{-}} = n_{X^{-}}$$

$$n^{g}_{P^{z+}} + n^{b}_{P^{z+}} = n_{P^{z+}}$$

$$n^{g}_{water} + n^{b}_{water} = n_{water}$$

(A.2a-e)

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 $n^{g}_{ionized\ groups}$ means that $n^{g}_{ionized\ groups}$

is positive if the charges on the polymer are positive and negative otherwise. For a negatively-charged protein, Equation A.2a is replaced with:

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

(A.2'a)

Equations A.1a-c and A.2a-e provide a system of eight equations which we can solve for our eight unknown concentrations $\left(n_P^g, n_P^b, n_{X^-}^g, n_{X^-}^b, n_{M^+}^g, n_{M^+}^b, n_{\text{water}}^g, n_{\text{water}}^b\right)$. To simplify computations, we would like to reduce the number of equations we must solve simultaneously. 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 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 straight-forward.) As long as the contribution of the protein (the multivalent solute) to the ionic strength is not larger than that of univalent ions, we can use experimental data to determine the water content of each phase[†], thus eliminating equations A.1a and A.2e. For a positively-charged protein, the remaining equations are (equations A.1b, A.1c and A.2a):

[†] 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 use experimental swelling equilibria to determine the solvent content of each phase.

$$\mu^b_{MX} = \mu^g_{MX}$$

$$\mu^b_{PX_Z} = \mu^g_{PX_z}$$

$$n^g_{M^+} + zn^g_{P^{z+}} - n^g_{X^-} \pm n^g_{\text{ionized groups}} = 0$$

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 $\left(n^g_P, n^b_P, n^g_{X^-}, n^b_{X^-}, n^g_{M^+}, n^b_{M^+}, n^g_{\text{water}}, n^b_{\text{water}}\right)$. Expressing equations A.1b and A.1c in the form of equation III.6, we see that we must adopt a model to calculate single-ion activity coefficients.

APPENDIX B

Derivation of Equation III.6

Equation III.6 enables us to calculate the composition of a gel phase in equilibrium with a bath. To obtain equation III.6, we first express the chemical potential of a neutral component i as the sum of the electrochemical potentials of its constituent ions:

$$\mu_i^{chemical} = v_+ \mu_+^{electrochemical} + v_- \mu_-^{electrochemical}$$

(B.1)

where i denotes the neutral component (salt) which dissociates into v_+ cations and v_- anions, 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):

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

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

(B.2-3)

where ^{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 equations B.2 and B.3 into equation B.1 gives:

$$\mu_i^{chemical} = v_+ \mu_+^o + v_- \mu_-^o + v_+ RT\ln(\gamma_+ m_+) + v_- RT\ln(\gamma_- m_-) + v_+ z_+ F\Psi + v_- z_- F\Psi$$

(B.4)

The sum of the last two terms of equation B.4 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:

$$\mu_i^{chemical} = \nu_+\mu_+^o + \nu_-\mu_-^o + RT\ln\left[\left(\gamma_+ m_+\right)^{\nu_+}\left(\gamma_- m_-\right)^{\nu_-}\right]$$

(B.5)

Using equations B.5 and III.1 ($\mu_i^{bath} = \mu_i^{gel}$), 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:

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

(B.6)

where $\overline{V}_i$ is the partial molar volume of component i (salt), $\phi_{polymer}$ is the volume fraction of polymer, and c_{XL} is the concentration of crosslinks at synthesis [mol/m^3], and the superscripts b and g denote bath and gel, respectively. Equation B.6 has the same form as equation III.6.

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

$$\gamma_\pm^{(\nu_+ + \nu_-)} = \left(\gamma_+\right)^{\nu_+}\left(\gamma_-\right)^{\nu_-}$$

(B.7)

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 equation B.5 with this notation, for reference:

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

(B.8)

APPENDIX C

The Debye-Hückel (DH) Method

The foundation for the Debye-Hückel method is Poisson's Equation, which relates the electrostatic potential, ψ, to the charge density, ρ:

$$\nabla^2 \psi = -\frac{\rho}{\varepsilon_o \varepsilon_r}$$

(C.1)

where ε_o is the permittivity of vacuum, and ε_r is the permittivity of the solvent (water) relative to a vacuum ($\varepsilon_o \varepsilon_r$ is the dielectric constant). For simplicity, the relative permittivity of the solvent is assumed constant. Equation C.1 is written for standard units in the Systeme Internationale.

The electrostatic potential used here is related to the work required to move an ion from one phase to another; both phases have the same composition, mass density, and temperature, but they are in different electrical states. For example, consider two phases denoted by superscripts ' and " which have the same composition, mass density and temperature, but are not necessarily in equilibrium. The electrochemical potential of an ion j, μ_j, in each phase can be expressed by:

$$\mu_j' = \mu_j^{o'} + RT\ln\left(m_j'\gamma_j'\right) + z_j F\psi'$$

$$\mu_j'' = \mu_j^{o''} + RT\ln\left(m_j''\gamma_j''\right) + z_j F\psi''$$

(C.2-3)

where ^{o} denotes the standard state (taken to be the same in both phases), F is Faraday's constant, R is the gas constant, T is temperature, γ_j is the activity coefficient, and m_j is some measure of the concentration of ion j, often the molality. The work required to move an ion from phase ' to phase " ($\Delta\mu$) is related to the difference in electrostatic potentials of the two phases ($\Delta\psi = \psi'' - \psi'$) because both phases have the same composition, temperature and density, and thus the same chemical forces exist in both

phases (Newman, 1991). If these two phases were in equilibrium with each other, the difference in electrostatic potentials would be zero.

Formally, the electrostatic potential, ψ, is related to the electric field, $\mathbf{E}$, by:

$$\mathbf{E} = -\nabla \psi$$

(C.4)

If we can make a statement about ρ, we can solve Poisson's equation (Equation C.1) for ψ and obtain the electrostatic contribution to the Helmholtz energy (the electrostatic excess Helmholtz energy) through the relation:

$$\left(\frac{\partial A_{electro}^{ex}}{\partial q_j} \right)_{V,T} = \left\langle \tilde{\psi}_j \right\rangle$$

(C.5)

where q_j is the charge on a specific ion j and $\left\langle \tilde{\psi}_j \right\rangle$ is the canonical-ensemble-average electrostatic potential given by all possible configurations of ions acting on ion j at position j.

The electrostatic excess Helmholtz energy, $A_{electro}^{ex}$, is the difference in Helmholtz energy at constant temperature, volume and composition between a solution of charged species and a solution of uncharged species. Excess Helmholtz energy is defined by separating A into an ideal part and a remainder, called excess:

$$A = A^{ideal} + A^{ex}$$

(C.6)

A^{ex} is meaningful only with respect to A^{ideal}, the Helmholtz energy of some reference (ideal) system. A useful ideal system is a solution of uncharged, hard spheres; Appendix J provides A^{ideal} for reference. In our calculations, we do not use A^{ideal} because we assume separability of electrostatic and non-electrostatic contributions to protein partition coefficient (Eq. I.1) and use free volume arguments to describe steric exclusion of the solute by the network.

$\langle \tilde{\psi}_j \rangle$ can be obtained from ψ:

$$\langle \tilde{\psi}_j \rangle = \psi(\mathbf{r}) - \frac{q_j}{4\pi\varepsilon_r\varepsilon_o |\mathbf{r} - \mathbf{r}_j|}$$

(C.7)

where $\mathbf{r}$ denotes position, $\mathbf{r}_j$ is the position of ion j, and $\psi(\mathbf{r})$ is the potential (as a function of position) appearing in equation C.1. The derivation of equation C.7 is found in McQuarrie (1975).

To obtain the excess Helmholtz energy due to coulombic interactions between ions, $A^{ex}_{electro}$, using equation C.5, we integrate the electrostatic potential as ion j is charged from q=0 to q=q_j:

$$A^{ex}_{electro} = \sum_j q_j \int_0^1 \langle \tilde{\psi}_j \rangle d\lambda$$

(C.8)

$$q_j d\lambda = dq_j$$

(C.9)

where $A^{ex}_{electro}$ is in reference to an uncharged system of the same composition, temperature and volume.

The charge density, ρ, on the right-hand side of Poisson's equation can be expressed by a Boltzmann equation in terms of potentials of mean force, $w_{j,s}$, between an arbitrarily selected ion j and the other types of ions in solution:

$$\rho(\mathbf{r}) = \sum_{s=1}^{n} \eta_s q_s e^{-\beta w_{j,s}(\mathbf{r})}$$

(C.10)

where β is $1/(k_b T)$, k_b is Boltzmann's constant, η_s is the bulk number density (N_s/V) of ions of type s (sodium, chloride, etc...; for a solution containing two types of ions, n=2), and q_s is the charge on an ion of type s.

The first approximation in DH theory is to set the potential of mean force proportional to the electrostatic (Coulomb) potential:

$$w_{j,s}(r) = q_s \psi(r) = \frac{q_s q_j}{4\pi\varepsilon_o\varepsilon_r r}$$

(C.11)

Substituting into equation C.1 for the potential of mean force, we obtain the Poisson-Boltzmann Equation (PBE):

$$\nabla^2\psi(r) = \frac{-1}{\varepsilon_o\varepsilon_r}\sum_{s=1}^{n}\eta_s q_s e^{-\beta\psi(r)}$$

(C.12)

The second approximation in DH theory is to linearize the right-hand side of the PBE. Upon expanding the exponential and retaining only the second term, we obtain:

$$\sum_{s=1}^{n}\eta_s q_s e^{-\beta\psi(r)} \approx \sum_{s=1}^{n}\eta_s q_s - \beta\sum_{s=1}^{n}\eta_s q_s^2\psi(r) = 0 - \beta\sum_{s=1}^{n}\eta_s q_s^2\psi(r)$$

(C.13)

The first term in the linearization is zero because we consider an electroneutral system. We thus obtain the linear Poisson-Boltzmann equation:

$$\nabla^2\psi = \kappa^2\psi$$

(C.14)

$$\kappa^2 = \frac{e^2\beta}{\varepsilon_o\varepsilon_r}\sum_{k}\eta_k z_k^2$$

(C.15)

where z_k is the valence of species k, and e is the charge on an electron. The sum extends over all ions. 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[†] . The boundary conditions are (here r denotes radial distance from ion j): (1) $\psi(r)$ vanishes at infinity, (2) $\psi(r)$ is continuous at r=a, the diameter common to all ions, and (3) the product of the dielectric and the derivative of $\psi(r)$ with respect to r is continuous at r=a.

[†] In calculations of phase equilibria in electrolyte systems (such as solving for the distribution between two phases), polymer charges 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.

Details of the solution of the Poisson-Boltzmann equation subject to these boundary conditions are given in McQuarrie (1975). $\langle \tilde{\psi}_j \rangle$ is given by:

$$\langle \tilde{\psi}_j \rangle = -\frac{q_j \kappa}{4\pi\varepsilon_o\varepsilon_r(1+\kappa a)}$$

(C.16)

By integration as per equation C.8 to obtain the excess electrostatic Helmholtz energy, we obtain the Debye-Hückel activity coefficient for an ion:

$$\ln(\gamma_j) = \beta\left(\frac{\partial A_{electro}^{ex}}{\partial n_j}\right)_{T,V,n_k,k\neq j} = -\frac{z_j^2 e^2 \beta \kappa}{8\pi\varepsilon_o\varepsilon_r(1+\kappa a)}$$

(C.17)

The Debye-Hückel activity coefficient can be used in equation III.6 to solve for the distribution of an ion between a charged gel and bath.

The excess pressure, P^{ex}, is also derived from the excess electrostatic Helmholtz energy:

$$P^{ex} = -\left(\frac{\partial A_{electro}^{ex}}{\partial V}\right)_{T,\mu_{solvent},n_j} = -RT\frac{\kappa^3}{24\pi}\tau(\kappa a)$$

(C.18)[†]

where

$$A_{electro}^{ex} = \frac{-RTV}{12\pi}\kappa^3\tau'(\kappa a)$$

(C.19)

$$\tau'(\kappa a) = \frac{3}{\kappa^3 a^3}\left[\ln(1+\kappa a) - \kappa a + \frac{\kappa^2 a^2}{2}\right]$$

(C.20)

$$\tau(\kappa a) = \frac{3}{(\kappa a)^3}\left[\kappa a - 2\ln(1+\kappa a) + 1 - \frac{1}{1+\kappa a}\right]$$

(C.21)

The functions $\tau(\kappa a)$ and $\tau'(\kappa a)$ account for effects of finite ion size and finite concentration; as κa goes to zero, $\tau(\kappa a)$ and $\tau'(\kappa a)$ approach unity. The excess pressure P^{ex} is used in equation III.7; however, the inclusion of the terms involving P^{ex} has a

_____

[†] Notice that P^{ex} is negative.

negligible effect on calculated partition coefficients for our systems. Derivation of equations C.18 and C.19 appears in reference McQuarrie (1975).

APPENDIX D

The Mean Spherical Approximation (MSA)

The Mean Spherical Approximation is based on solving the Ornstein-Zernike integral equation:

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

(D.1)

where η_k is the number density of species k, $C_{ij}(r)$ is the direct correlation function between species i and species j, and $h_{ij}(r)$ is the total correlation function between species i and j, and r denotes spatial position (Waisman and Lebowitz, 1970; Blum, 1975; McQuarrie, 1975; Blum and Høye, 1977). The total correlation function, $h_{ij}(r)$, is a measure of the total influence of a particle of species i on a particle of species j. The pair correlation function, $g_{ij}(r)$, from which thermodynamic properties can be obtained, is related to the total correlation function:

$$h_{ij}(r) = g_{ij}(r) - 1$$

(D.2)

The Ornstein-Zernike equation cannot be solved directly because both $h_{ij}(r)$ and $C_{ij}(r)$ are unknown. To solve equation D.1 for $h_{ij}(r)$, we make use of approximate relations, called closure relations because their application results in a closed expression for $h_{ij}(r)$. In the MSA, the closure relations are the following:

$$C_{ij}(r) = -\beta u_{ij}(r) = -\beta \frac{q_i q_j}{\varepsilon_o \varepsilon_r r}; \quad r > \sigma_{ij}$$

(D.3)

$$g_{ij}(r) = 0; \quad r < \sigma_{ij}$$

(D.4)

where β is $1/(k_b T)$, k_b is Boltzmann's constant, T is temperature, q_j is the charge on ion j, ε_o is the permittivity of vacuum, ε_r is the permittivity of the solvent (water) relative to a

vacuum ($\varepsilon_0\varepsilon_r$ is the dielectric constant), u_{ij} is the pair potential between ion i and ion j, r is the distance between ion i and ion j, and σ_{ij} is the distance of closest approach between ion i and ion j. These closure relations stipulate that any two ions are directly correlated via the Coulomb potential at distances greater than the distance of closest approach between two ions ($r > \sigma_{ij}$), and that any two ions cannot be closer than the distance of closest approach, σ_{ij}.

Equations D.1, D.3, and D.4 are solved via Fourier transforms for the total correlation function, from which the pair correlation function is directly obtainable. Thermodynamic properties, such as the excess electrostatic Helmholtz energy, are then obtained from the pair correlation function as described by McQuarrie (1975).

The excess pressure, P^{ex}, is obtained from the excess Helmholtz energy. For the Single Ion Diameter approximation to the MSA (Harvey et al., 1988), P^{ex} is given by:

$$P^{ex} = -\left(\frac{\partial A^{ex}_{electro}}{\partial V}\right)_{T,\mu_{solvent},n_j} = -RT\frac{\Gamma^3}{3\pi}$$

$$(D.5)^\dagger$$

where

$$A^{ex}_{electro} = \frac{-2\Gamma^3 RTV}{3\pi}\left(1 + \frac{3}{2}a_{mix}\Gamma\right)$$

$$(D.6)$$

The parameter Γ is the MSA screening parameter defined by equation IV.B.4, a_{mix} is the effective ion diameter of all species defined by equation IV.B.2, R is the universal gas constant, T is temperature, and V is volume (Harvey et al., 1988).

---

† Notice that P^{ex} is negative.

APPENDIX E

The Cell Model for Polyelectrolyte Solutions

Figure E-1 presents a schematic of the cell model for polyelectrolyte solutions (Katchalsky, 1971). The polyelectrolyte solution is divided into cylindrical cells of equal volume and radius $\Re$. At the center of each cell is the polyelectrolyte, represented by a cylindrical rod of radius d and uniform surface charge distribution. The radius $\Re$ can be related to the polymer volume fraction, ϕ_p, of the gel:

$$\phi_p = \frac{d^2}{\Re^2}$$

$$(E.1)$$

The cell volume must contain counterions to the polyelectrolyte to assure electroneutrality in the cell. The electrostatic potential, ψ, is set to zero at the boundary of the cell, $r = \Re$.

In the cell model, we solve the Poisson-Boltzmann equation [Eq. C.12 in Appendix C] in cylindrical coordinates for ψ, subject to two boundary conditions. The first is that the potential is continuous at the cell boundary:

$$\left.\frac{d\psi}{dr}\right|_{r=\Re} = 0$$

$$(E.2)$$

The second is that Gauss' law [†] is satisfied at the surface of the cylinder (r=d):

[†] Gauss' law states that the surface integral of εE over a closed volume is equal to the net charge enclosed in that volume:

$$\oint \varepsilon \mathbf{E} \cdot \mathbf{dS} = \int \rho \, dV$$

Poisson's Equation [Eq.C.1] can be derived directly from Gauss' law by using the divergence theorem and the relation between the electrostatic potential, ψ, and the electrical field, $\mathbf{E}$.

$$\frac{d\psi}{dr}\bigg|_{r=d} = -\frac{e}{2\pi\varepsilon_o\varepsilon_r db}$$

(E.3)

where e is the charge on an electron, ε_o is the permittivity of vacuum, ε_r is the permittivity of the solvent (water) relative to a vacuum ($\varepsilon_o\varepsilon_r$ is the dielectric constant), and b is the axial length per unit charge on the charged cylinder.

We account for electroneutrality when we determine the reference concentrations in the Boltzmann relation which relates the concentration of species j at any radial distance, $c_j(r)$, to the potential, $\psi(r)$, at that same distance. Because the electrostatic potential is zero at the cell boundary, the concentrations at the cell boundary, $c_j(\Re)$, are the reference concentrations in the Boltzmann relation. The Boltzmann relation has the following form:

$$c_j(r) = c_j(\Re)e^{-\beta q_j \psi(r)}$$

(E.4)

where β is $1/(k_b T)$, k_b is Boltzmann's constant, T is temperature, and q_j is the charge on ion j. The equation of electroneutrality of the cell can be expressed as the following:

$$z_{seg}c_{seg} + \sum_j z_j \int_V c_j(r)dV = 0$$

(E.5)

where z_{seg} is the valence of the total charge on a polymer segment (monomer), c_{seg} is the concentration of charged polymer segments in the cell, z_j is the valence of ion j, and V is the volume of the cell.

We can obtain an analytical solution to the Poisson-Boltzmann equation for $\psi(r)$ in the cell model if the only mobile ions present are counterions necessary to neutralize the polyelectrolyte. If we are interested in a polyelectrolyte solution with added mobile ions (a polyelectrolyte with added salt), the Poisson-Boltzmann equation must be solved numerically for the specific ionic composition and polymer charge density of interest. Then, to obtain ionic activity coefficients, we integrate the electrostatic potential

numerically to obtain the excess electrostatic Helmholtz energy (using equation C.8 of Appendix C) and apply equation C.17 to obtain the activity coefficient. These numerical procedures, while not difficult in principle, are tedious and impractical for multicomponent, phase-equilibrium calculations. To circumvent the numerical procedures outlined above, Guerón and Weisbuch have suggested the semiempirical, algebraic activity coefficient expressions given by equations IV.C.1 and IV.C.2.

Similarly, to evaluate the excess pressure, we would have to differentiate numerically the excess electrostatic Helmholtz energy with respect to volume. Because we found that inclusion of the P^{ex}-containing terms in equation III.7 had a negligible effect on calculated partition coefficients using the MSA or Debye-Hückel, we neglected these terms when calculating partition coefficient using the cell model.

If we are interested in the partitioning of a single salt, we can avoid calculating ionic activity coefficients rigorously because we know the radial distribution of ions with respect to the polyelectrolyte; this distribution is given by the Boltzmann relation [Eq. C.10 in Appendix C] we used to solve Poisson's equation. Consider the cells of the polyelectrolyte-containing solution which are at the interface between the two solutions. Because the potential due to the polyelectrolyte is zero in the polyelectrolyte-free solution, we can equate the bulk concentration of species j in the polyelectrolyte-free solution with the concentration of species j at the cell boundary, $c_j(\Re)$, where the electrostatic potential (due to the polyelectrolyte) is also zero.

This can also be understood by considering that the gradient of the electrostatic potential due to the polyelectrolyte is zero at the boundary between the two solutions. Because the chemical potential of an ion must be equivalent throughout both solutions, the concentration of any ion i must be the same in the polyelectrolyte-free solution and at the outer boundary of the cells along the interface of the two solutions. That is, $c_j(\Re)^{PE\ solution} = \bar{c_j}^{PE-free\ solution}$, where the overbar denotes a spatial average property, and PE stands for polyelectrolyte.

We define partition coefficient, K_j for species j:

$$K_j = \frac{\overline{c_j}^{PE\ solution}}{\overline{c_j}^{PE-free\ solution}} = \frac{\overline{c_j}^{PE\ solution}}{c_j(\mathfrak{R})^{PE\ solution}}$$

(E.6)

Therefore, we can calculate the partition coefficient, K_j, from the solution of the

Poisson-Boltzmann Equation for the polyelectrolyte-containing phase:

For the mobile negative ions in a cell whose cylinder has a positive surface

charge:

$$\frac{\overline{c_j}}{c_j(\mathfrak{R})} = \frac{\int_{r=d}^{r=\mathfrak{R}} \exp\left(|z_j|e\beta\psi(r)\right)r\,dr}{\int_{r=d}^{r=\mathfrak{R}} r\,dr}$$

(E.7)

For the mobile positive ions in a cell whose cylinder has a positive surface

charge:

$$\frac{\overline{c_j}}{c_j(\mathfrak{R})} = \frac{\int_{r=d}^{r=\mathfrak{R}} \exp\left(-|z_j|e\beta\psi(r)\right)r\,dr}{\int_{r=d}^{r=\mathfrak{R}} r\,dr}$$

(E.8)

Both Stigter (Stigter, 1975) and Le Bret (Le Bret and Zimm, 1984) present

expressions for the partition coefficient, $\overline{c_{salt}}/c_{salt}(\mathfrak{R})$, of a monovalent salt. Stigter's

expression is :

$$\frac{\overline{c_{salt}}^{PE\ solution}}{c_{salt}(\mathfrak{R})} = 1 - b\eta_{seg}\left(\pi d^2 + \frac{1}{2\varsigma\Theta\eta_{salt}(\mathfrak{R})}\right) + O\left(\eta_{seg}^2\right)$$

(E.9)

where the last term is a correction of order 2 in η_{seg}. The coefficients ς and Θ are

correction factors tabulated by Stigter as a function of ionic strength and surface charge

density. The correction factors allow expression of properties of the numerical solution

to the Poisson-Boltzmann equation in cylindrical geometry in terms of the analytical solution of the Poisson-Boltzmann equation in planar (Cartesian) geometry.

We can use the partition coefficient of the salt calculated in equation E.9 to calculate (using equation F.1 for a positively charged gel or equation F.2 for a negatively charged gel; equations F.1 and F.2 appear in Appendix F) the quasi-electrostatic potential difference between a charged gel and bath. In the main text, we discuss how to use the quasi-electrostatic potential difference to gain qualitative insight into coulombic effects in protein partitioning.
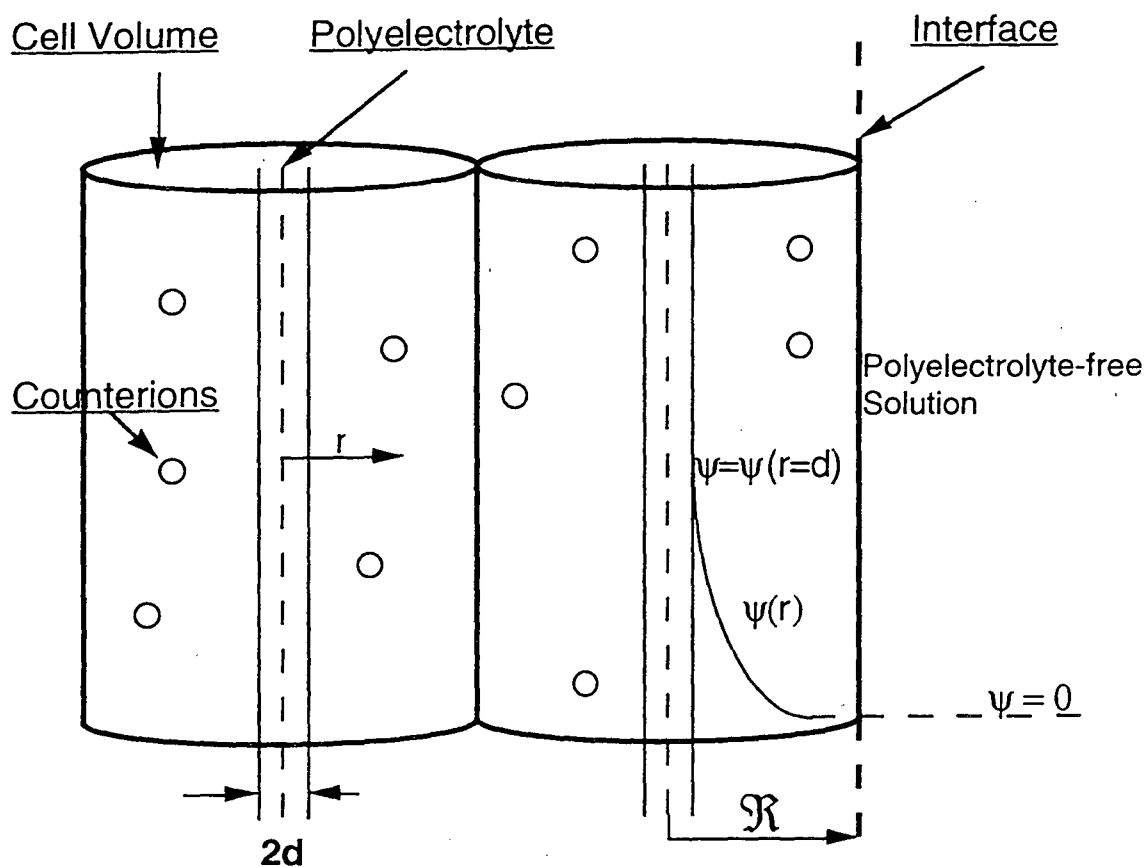
Figure E-1. Schematic of the cell model for polyelectrolyte solutions. The polyelectrolyte solution is divided into cylindrical cells of radius $\mathfrak{R}$. The polyelectrolyte is represented by a uniformly-charged cylinder of radius d located at the center of each cell. The cell volume must contain counterions to assure electroneutrality.

APPENDIX F

Algorithms for the Calculation of 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 equation III.7. After solving the five equations simultaneously, we know the composition of the polyelectrolyte solution and can therefore calculate a partition coefficient for the protein between the polyelectrolyte solution and the bath. Following equation I.1, we multiplied this partition coefficient by K^{SEC} from equation II.1 to obtain the overall partition coefficient between the polyelectrolyte gel and the bath. We then compared the calculated partition coefficient to experimental results.

We encountered convergence difficulties in the rigorous algorithm when activity coefficients were obtained from Debye-Hückel theory or the MSA. We obtained several sets of concentrations 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. For example, the calculated partition coefficient for cytochrome c in poly-NIPA/10% SA gels (pH 6, 22.2°C, 0.1M sodium phosphates) varied from 0.795 to 22.7 for five different, realistic sets of boundary conditions on the

partition coefficient. Four of these five calculated partition coefficients were in the range of 0.795 to 1.189 and were thus in the same order of magnitude as the experimental value, 1.045. Debye-Hückel theory was used for activity coefficients in the aforementioned calculations.

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. We fixed the salt concentration of the bath solution to be 0.1M, 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} and compared to experimental results. This second algorithm is the semi-rigorous 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:

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

(F.1)

for a negatively charged gel:

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

(F.2)

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)[†] . Appendix H discusses the definition of the quasi-electrostatic potential. We then calculated the protein partition coefficient ($K_{protein}$) from:

for a positively charged gel:

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

(F.3)

for a negatively charged gel:

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

(F.4)

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

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

(F.5)

and $\Delta\Pi_{elastic}$ is given by equation III.4. We investigated the quasi-electrostatic potential algorithm because we can calculate partitioning of a protein without calculating its activity coefficient.

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 F-1 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 0.1M ionic-strength sodium phosphates. In this range of pH, the protein is positively

---

[†] To define the quasi-electrostatic potential, we must choose a reference ion, as discussed in Appendix H. 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 equation H.4 of Appendix H, we obtain equation F.1 for positively charged gels and equation F.2 for negatively charged gels.

charged and the gel is negatively charged. The results are analogous to those in Figure 1, except that in Figure F-1, 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 F-2 presents a comparison between experimental partition coefficients and those calculated using the rigorous, semi-rigorous and quasi-electrostatic-potential algorithms with the activity coefficient expressions of Guerón and Weisbuch (Guerón and Weisbuch, 1979). The system is the same as that in Figures 1 and F-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 semi-rigorous algorithm agree best with experiment.
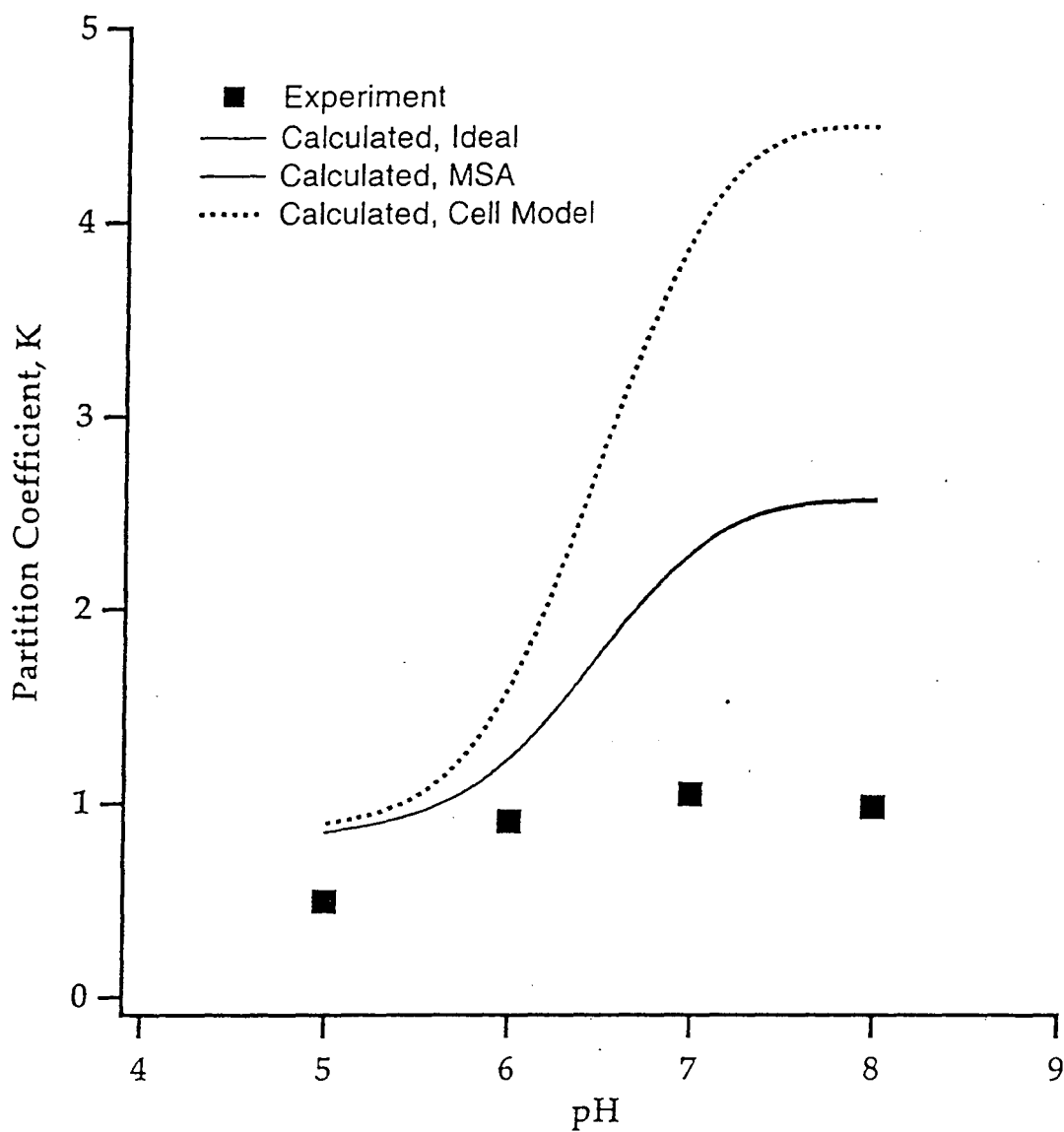
Figure F-1. Calculated and experimental partition coefficients as a function of pH for cytochrome c in poly-NIPA/10% SA gels. The experimental conditions are the same as those in Figure 1. Calculations were peformed using the quasi-electrostatic potential algorithm. Ionic activity coefficients were taken 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.
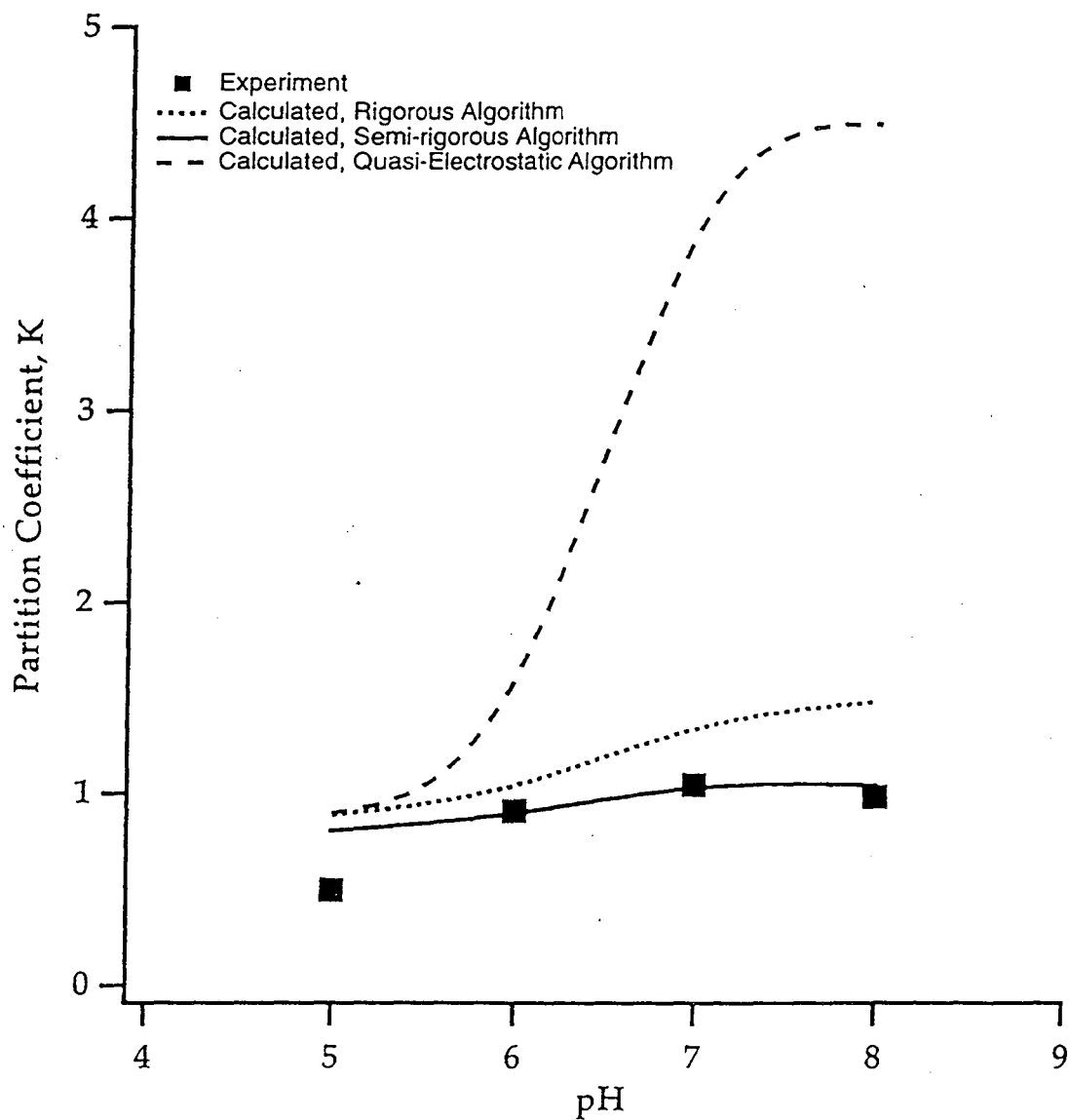
Figure F-2. Calculated and experimental partition coefficients as a function of pH for cytochrome c in poly-NIPA/10%SA gels. Experimental conditions are the same as those in Figure F-1. Calculations are compared for the rigorous, semi-rigorous and quasi-electrostatic potential algorithms, using ionic activity coefficients from the cell model.

APPENDIX G

Incorporation of Short-range, Non-Electrostatic Forces

into the Calculation of Partition Coefficients

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

$$A^{ex}_{non-electrostatic} = RTN_{AV} \sum_i \sum_j n_i \frac{n_j}{V} \Lambda_{ij}(\mu_{solvent}, T)$$

$$(G.1)$$

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 i, and Λ_{ij} is a binary interaction parameter which characterizes the non-electrostatic, non-excluded-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:

$$\ln\left(\gamma^{non-electrostatic}_{protein}\right) = m_{polymer}\Lambda_{polymer-protein}$$

$$(G.2)$$

where $m_{polymer}$ is some measure of the concentration of polymer in the gel and $L_{polymer-protein}$ 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 would be multiplied by single-ion activity coefficient for the protein in the gel. The new activity coefficient would be used in place of the single-ion activity coefficient in equation III.6. However, because we lack fundamental data for the interaction between cytochrome c and the poly-NIPA polymers, we cannot obtain $\Lambda_{polymer-protein}$ independently.

APPENDIX H

The Quasi-Electrostatic Potential and the Electrochemical Potential

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

$$\mu_j = \mu_j^{chemical} + z_j F\Psi = RT\ln(m_j\Omega_j\delta_j^\circ) + z_j F\Psi$$

$$(H.1)$$

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° is a system-specific constant that depends on temperature and pressure but is independent of composition or electrical state.

Equation H.1 has two undefined quantities, Ω_j and Ψ. As discussed, for example, by Newman (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 equation H.1. We give the quasi-electrostatic potential the symbol 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 :

$$\mu_r = RT\ln(m_r) + z_r F\Phi$$

$$(H.2a)$$

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

$$\mu_r = \mu_r^o + RT\ln(m_r\gamma_r)$$

(H.2b)

In equations H.2a and H.2b, $z_r \neq 0$, 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 equations H.2a and H.2b and solving for Φ, we obtain:

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

(H.3)

Consider two phases (' and ") at equilibrium. Using $\mu_r' = \mu_r''$ and equation H.2a, we can relate the partitioning of the reference ion between liquid phases ' and " directly to the difference in quasi-electrostatic potentials:

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

(H.4)

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

$$\mu_j = \mu_j^o + RT\ln(m_j\gamma_j) = RT\ln(m_j\Omega_j\delta_j^o) + z_j F\Phi$$

(H.5)

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 equation H.3 for Φ, we obtain:

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

(H.6)

Substituting equation H.6 into equation H.5, the electrochemical potential for species j is:

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

(H.7)

and the partition coefficient for species j between phases ' and " is:

$$\ln K_j = \frac{z_jF}{RT}\left(\Phi^" - \Phi'\right) + \ln\left(\frac{\gamma_j'}{\gamma_j"}\right) - \frac{z_j}{z_r}\ln\left(\frac{\gamma_r'}{\gamma_r"}\right)$$

(H.8)

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 chöose Na^+ to be the reference ion and ignore the osmotic-pressure difference between the gel and bath, we can equate equations H.4 and H.8 to obtain:

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

(H.9)

where the 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:

$$\gamma_\pm^2 = \gamma_+\gamma_-$$

(H.10)

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 H.9 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. H.9).

APPENDIX J

Expression for A^{ideal}

One possible expression for A^{ideal} is that suggested by Haynes et al (Haynes et al., 1993) following the work of Boublik (Boublik, 1970) and Mansoori (Mansoori et al., 1971) for a solution containing water, simple ions, proteins, and uncharged polymers in the McMillan-Mayer framework. However, in our calculations of protein partition coefficients, we do not use an expression for A^{ideal} because we separate the non-electrostatic and electrostatic contributions to the partition coefficient and use free-volume arguments to describe the non-electrostatic contributions to the protein partition coefficient (Eq. I.1).

Haynes' expression for A^{ideal} follows:

$$A^{ideal} = f\left(T, n_{i, i \neq water}, \mu_{water}\right) - P_o V_{total}$$

$$-\frac{6V_{total}}{\beta\pi}\left[\varpi_o \ln\left[V_{total}(1-\varpi_3)\right] - \frac{3\varpi_1\varpi_2}{1-\varpi_3} - \frac{3(\varpi_2)^3}{2(1-\varpi_3)^2} + \frac{(\varpi_2)^3(1-2\varpi_3)^2}{2(\varpi_3)^2(1-\varpi_3)^2} - \frac{(\varpi_2)^3}{(\varpi_3)^2}\ln\left(\frac{1}{1-\varpi_3}\right)\right]$$

$$(J.1)$$

where

$$f\left(T, n_{i, i \neq water}, \mu_{water}\right) = P_o V_{total} \sum_{i, i \neq water} \phi_i + \sum_{i, i \neq water} n_i\left(\mu_i^o - P\overline{V_i^o}\right) + RT \sum_{i, i \neq water} n_i \ln(\phi_i)$$

$$-\frac{RT}{\overline{V}_{water}} \sum_{i, i \neq water} n_i \overline{V_i} + \frac{6V_{total}}{\beta\pi}\left[\varpi_o \ln\left(V_{total}\right) + \frac{(\varpi_2)^3}{2(\varpi_3)^2}\right],$$

$$P_o = \frac{\left(\mu_{water} - a_{water}\right)}{\overline{V}_{water}}; \quad \varpi_m = \frac{\pi N_{AV} \sum_{i, i \neq water} n_i\left(a_i\right)^m}{6V_{total}}; \quad \phi_i \text{ is the volume fraction of species i, while}$$

a_i is the diameter of species i.

NOMENCLATURE

Roman

a_j	diameter of ion j (m)
a_{mix}	average diameter of all ions in solution (m)
a_i	activity of component i
A	Helmholtz energy (J)
A^{ex}	excess Helmholtz energy (J)
A^{ideal}	ideal Helmholtz energy (J)
$A^{ex}_{electro}$	excess Helmholtz energy resulting from electrostatic interactions (J)
$A^{ex}_{non-electrostatic}$	excess Helmholtz energy resulting from short-range non-electrostatic interactions (J)
b	axial length per unit charge (monomer length) (m)
c_j	concentration of mobile ionic species j (mol m^{-3})
$\bar{c}_j$	spatial average concentration of mobile ionic species j (bulk concentration) (mol m^{-3})
c_{salt}	concentration of salt (mol m^{-3})
c_{seg}	concentration of charged polymer segments (mol m^{-3})
c_{XL}	concentration of crosslinks at synthesis (mol m^{-3})
$C_{ij}(r)$	direct correlation function between ionic species i and j
%C	percent crosslinking monomer
e	electronic charge (1.6022×10^{-19} C)
d	radius of rod representing polyelectrolyte (m)
E	electric field (V m^{-1})
F	Faraday's constant (96,500 C mol^{-1})
$g_{ij}(r)$	pair correlation function between ionic species i and j
$h_{ij}(r)$	total correlation function between ionic species i and j
k_b	Boltzmann's constant (1.381×10^{-23} J K^{-1})

K	partition coefficient
m_j	measure of concentration of ionic species j (for example, mol m^{-3})
m_r	measure of concentration of reference ionic species r (for example, mol m^{-3})
m_+	measure of concentration of a cation (for example, mol m^{-3})
m_-	measure of concentration of an anion (for example, mol m^{-3})
n_i	mole number of component i (mol)
n_j	mole number of ionic species j (mol)
N_{AV}	Avogadro's number (6.02×10^{23} mol^{-1})
N_j	number of species j
P^{ex}	excess pressure (Pa)
P_o	solvent contribution to pressure (Pa) (defined in Appendix J)
pH	negative the base ten logarithm of the activity of the hydrogen ion
q_j	charge on ion j (C)
$\mathbf{r}$	position vector
r	center-to-center distance between two ions (m)
R	universal gas constant (8.314 J mol^{-1} K^{-1})
$\mathfrak{R}$	radius of cylinder in cell model for polyelectrolyte solution (m)
T	temperature (K)
%T	ratio of monomer to diluent at synthesis (g mL^{-1})
$u_{ij}(\mathbf{r})$	pair potential between ion i and j (J)
V	volume (m^3)
$\overline{V}_i$	partial molar volume of component i (m^3 mol^{-1})
$w_{j,s}$	potential of mean force between ion j and ion of type s
z_j	valence of species j
$z_{protein}$	net valence of protein
z_r	valence of reference ionic species r

z_{seg}	valence of a charged polymer segment
z_+	valence of a cation
z_-	valence of an anion

Greek, Hebrew, and Symbols

β	$= (k_b T)^{-1}$ (mol J^{-1})
χ	Flory's interaction parameter
δ_j°	system-specific constant for ionic species j (defined in Appendix H)
ε_o	vacuum permittivity (8.854×10^{-12} C^2 J^{-1} m^{-1})
ε_r	relative permittivity (78.3 for water at 25°C)
ϕ_p	polymer volume fraction
ϕ_i	volume fraction of species i
Φ	quasi-electrostatic potential (V) (defined in Appendix A)
$\Delta\Phi$	quasi-electrostatic potential difference (V)
γ_j	single-ion activity coefficient of ionic species j
γ_+	single-ion activity coefficient for a cation
γ_-	single-ion activity coefficient for an anion
$\gamma_\pm$	mean ionic activity coefficient for a salt
Γ	screening parameter in the Mean Spherical Approximation (m^{-1})
η_j	number density of ionic species j (m^{-3})
η_{salt}	number density of salt (m^{-3})
η_{seg}	number density of charged segments (m^{-3})
κ	inverse Debye screening length (m^{-1})
λ	fractional charge (defined in Appendix C)
Λ_{ij}	binary, non-electrostatic interaction parameter between molecules i and j (m^3 mol^{-1})
μ_j	electrochemical potential of ionic species j (J mol^{-1})

μ_r	electrochemical potential of reference ionic species r (J mol^{-1})
μ_+	electrochemical potential of a cation (J mol^{-1})
μ_-	electrochemical potential of an anion (J mol^{-1})
μ_i^o	standard state chemical potential of neutral component i (J mol^{-1})
μ_j^o	standard state chemical potential of ionic species j (J mol^{-1})
μ_r^o	standard state chemical potential of reference ionic species r (J mol^{-1})
μ_+^o	standard state chemical potential of a cation (J mol^{-1})
μ_-^o	standard state chemical potential of an anion (J mol^{-1})
$\Delta\Pi_{elastic}$	elastic contribution to swelling pressure (Pa)
ϖ_m	reduced density (ϖ_1 has units m^{-2}, ϖ_2 has units m^{-1}, ϖ_3 has no units)
Θ	empirical coefficient in Stigter's expression for the partitioning of a salt (Eq.E.9)
ρ	charge density (C m^{-3})
σ_{ij}	distance of closest approach between ion i and ion j (m)
ς	empirical coefficient in Stigter's expression for the partitioning of a salt (Eq.E.9)
ν_+	stoichiometric coefficient of cation in a neutral salt
ν_-	stoichiometric coefficient of anion in a neutral salt
$\tau(\kappa a)$	function in expression for Debye-Hückel excess pressure (Eq. C.18, C.21)
$\tau'(\kappa a)$	function in expression for Debye-Hückel $A_{electro}^{ex}$ (Eq. C.19, C.20)
Ω_j	general activity coefficient of ionic species j
ψ	electrostatic potential of Poisson's equation (V)
$\langle \tilde{\psi}_j \rangle$	canonical-ensemble-average electrostatic potential acting on a specific ion j at position j (V)
Ψ	general electrostatic potential in electrochemical potential

REFERENCES

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

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

Boublik, T., "Hard-Sphere Equation of State," *Journal of Chemical Physics*, **53**, 471 (1970).

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).

Guggenheim, E. A., *Thermodynamics*, North Holland Publishing Company, 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," *Journal of Physical Chemistry*, **92**, 6432 (1988).

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

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 Journal*, **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 Journal*, **37**, 1401 (1991).

Katchalsky, A., "Polyelectrolytes," *Pure and Applied Chemistry*, **26**, 327 (1971).

Le Bret, M., and B. H. Zimm, "Distribution of Counterions Around a Cylindrical Polyelectrolyte and Manning's Condensation Theory," *Biopolymers*, **23**, 287 (1984).

Mansoori, G. A., N. F. Carnahan, K. E. Starling, and T. W. Leland, "Equilibrium Thermodynamic Properties of the Mixture of Hard Spheres," *Journal of Chemical Physics*, **54**, 1523 (1971).

McQuarrie, D. A., *Statistical Mechanics*, Harper and Row, New York (1975).

Newman, J. S., *Electrochemical Systems*, 2nd ed., Prentice Hall, Englewood Cliffs (1991).

Stigter, D., "The Charged Colloidal Cylinder with a Gouy Double Layer," *Journal of Colloid and Interfacial Science*, **53**, 296 (1975).

Waisman, E., and J. L. Lebowitz, "Exact Solution of an Integral Equation for the Structure of a Primitive Model of Electrolytes," *Journal of Chemical Physics*, **52**, 4307 (1970).

LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
TECHNICAL INFORMATION DEPARTMENT
BERKELEY, CALIFORNIA 94720