Electrostatic Potentials and Protein Partitioning in Aqueous Two-Phase Systems

C. A. Haynes, J. Carson, H. W. Blanch, and J. M. Prausnitz
Dept. of Chemical Engineering, University of California, Berkeley, CA 94720

A thermodynamic analysis unambiguously relates interfacial-electrostatic-potential differences measured with Ag/AgCl capillary electrodes to the equilibrium properties of an aqueous two-phase system. Interfacial electrostatic potentials were measured as a function of total α-cyclodextrin concentration in an aqueous two-phase system containing 9.1 wt. % poly(ethylene glycol), 6.1 wt. % Dextran T-70, and 1-mM potassium iodide. An order-of-magnitude increase in the interfacial electrostatic potential was observed as the total concentration of α-cyclodextrin increased from 0 to 1 mM. Measured partition coefficients for α-chymotrypsin, lysozyme, and bovine serum albumin depend strongly on α-cyclodextrin concentration. For example, as the concentration of α-cyclodextrin rises from 0 to 1 mM, the partition coefficient of lysozyme decreases from 1.7 to 0.55. These measurements are in good agreement with theoretical expectations.

Introduction

A useful method for separating dilute to moderately concentrated aqueous mixtures of biomolecules is provided by aqueous two-phase extraction technology (Kula, 1982). When a mixture of proteins is introduced into an aqueous two-phase extraction system, each protein partitions uniquely between the phases. Introduced by Per-Åke Albertsson in 1956, aqueous two-phase systems have found increasing use in industrial protein-purification processes (Albertsson, 1986).

Recent experiments have shown that protein partitioning in aqueous two-phase systems is strongly influenced by the presence of strong electrolytes, with different electrolytes producing different effects (Johansson, 1974; Reitherman et al., 1973; King et al., 1988). Experiments on poly(ethylene glycol)/dextran two-phase systems indicate that salts with polyvalent anions, such as sulfate and phosphate, partition preferentially into the dextran-rich bottom phase, while salts of halides partition almost evenly (Bamberger et al., 1983; Brooks et al., 1984; Sharp et al., 1987). Through capillary-electrode experiments, Brooks et al. found that the presence of electrolyte in a two-phase system induces an interfacial-electrostatic-potential difference and that the magnitude of this electrostatic-potential difference rises with increasing valence on the anion. King et al. found that the magnitude of the interfacial-electrostatic potential difference is directly proportional to the partition coefficient of the added electrolyte. It is therefore widely believed that the effect of a salt on the partitioning of a protein is due in large part to the electrostatic-potential gradient which develops as a result of the salt's presence; in essence, a protein responds to the electric field in proportion to the sign and magnitude of the protein's surface charge density. This idea was suggested first by Albertsson (1986), who developed a theory based on simple thermodynamic arguments relating the interfacial-electrostatic-potential difference to the "chemical" activity coefficients of the ions of the added electrolyte.

The Albertsson model (1986) suggests that interfacial electrostatic-potential differences are created by a difference in the relative chemical affinities of the ions of the added electrolyte for the two liquid phases. Analysis of the Albertsson model raises two subtle questions:

1. What is the role of the electrostatic potential in thermodynamics?

2. How is the electrostatic-potential difference defined in Albertsson's model related to the electrostatic-potential difference measured by Brooks and others (King et al., 1988; Haynes et al., 1989)?

In this work, we advance the Albertsson model by applying the "quasi-electrostatic-potential theory" developed by Newman (1973) to the thermodynamic description of the capillary-electrode experiments reported by our group, by Brooks et al., and by Sharpe et al.; this analysis allows us to link unambiguously measured electrostatic-potential differences with the

equilibrium properties of the two-phase system under consideration.

Following our thermodynamic analysis, we consider compounds that are able to form inclusion complexes with guest molecules such as ions. For example, crown ethers (macrocyclic polyethers) are a class of compounds known to form stable complexes with cations. Another class of complex-forming compounds are the cyclodextrins. The cyclodextrins are cyclic oligosaccharides containing from 6 to 12 α-1,4-linked glycopyranose units. Cyclodextrins form inclusion complexes with a number of different molecules and ions; but contrary to crown ethers, they form complexes with anions (Szejtli, 1988). The cyclodextrin used in this work is α-cyclodextrin, a six-unit ring which is known to complex strongly with the iodide anion. For an aqueous solution at 25°C containing equimolar amounts of potassium iodide and α-cyclodextrin, the equilibrium constant for the iodide-cyclodextrin complexation reaction is $19.0 \pm 0.3\ M^{-1}$ (Gelb et al., 1983).

In this work, interfacial-electrostatic-potential-difference data are presented for aqueous two-phase systems at 25°C containing a single strong electolyte. Particular attention is given to a system containing 1.21-g Dextran T-70, 1.82-g PEG 3350, 16.97-g water, 1-mM KI, and varying amounts of α-cyclodextrin. These experiments are designed to examine the relationships among the interfacial-electrostatic-potential difference, the partition coefficient of the added electrolyte, and the relative affinities of the ions of the electrolyte for the two equilibrium phases.

To illustrate the importance of the interfacial-electrostatic-potential difference in aqueous two-phase partitioning systems, we present partition-coefficient data for α-chymotrypsin, lysozyme, and bovine serum albumin in PEG 3350/Dextran T-70 aqueous two-phase systems at 25°C containing 1-mM KI and varying amounts of α-cyclodextrin.

Electrostatic Potential in Thermodynamics

Investigation of the electrical states of equilibrium phases raises a number of questions:

Can an electrostatic-potential difference arise between two phases in equilibrium?

If so, what is its physical origin?

How does one use thermodynamics to calculate an electrostatic-potential difference between two equilibrium phases?

Unlike the large energy requirement for macroscopic charge separation, the energy required to separate charge in solution over microscopic distances may not be prohibitive. Therefore, microscopic charge separation within the volume of a liquid-liquid interface may be considered. Microscopic charge separation in solution gives rise to an electrostatic-potential gradient across the region of charge separation. Copious experimental data confirm that the partitioning of ions between two equilibrium phases can result in the development of an interfacial-electrostatic-potential difference (see, for example, Walter et al., 1985).

Nichols and Pratt (1984) used well-established theories for salt effects on the surface tension of dilute electrolytic solutions to determine the relationship between ion partitioning and electrostatic-potential differences. They concluded that the electrostatic-potential difference, which develops between the bulk phases, is caused by a microscopic charge separation in the interfacial region. Unfortunately, Nichols and Pratt provide no explanation of how the interfacial-electrostatic-potential difference is related to the compositions of the two equilibrium phases; to determine this relation, we must turn to thermodynamics.

For thermodynamic calculations, we require the reversible work required to transfer ions (or molecules) from one phase to another. This reversible work provides the electrochemical potential μ_i of the ionic species. The interfacial electrostatic-potential difference $\Delta\Phi$ between two equilibrium phases is not related to reversible work. Indeed, as discussed in an earlier publication (Haynes et al., 1989), knowledge of $\Delta\Phi$ is not necessary for the determination of equilibrium compositions or any other thermodynamic property of the two-phase system (Guggenheim, 1967; Newman, 1973). However, as shown later, knowledge of $\Delta\Phi$ is often useful in design calculations and in understanding the relative magnitudes of the intermolecular forces acting on the charged species in the system.

It is customary to calculate a difference in the electrical state of two equilibrium phases by dividing arbitrarily the electrochemical potential of the ionic species into a "chemical" term and an electrical term:

$$\mu_i = \mu_i^\theta + \mathrm{RT}\ \ln(m_i\gamma_i) = \mu_i^{\mathrm{chem}} + z_i F\Phi$$
$$= \mu_i^\theta + \mathrm{RT}\ \ln(m_i\Gamma_i) + z_i F\Phi \qquad (1)$$

where γ_i is the activity coefficient of ion i, Φ is the "thermodynamic" electrostatic potential, and Γ_i is the "chemical" activity coefficient for ion i which is assumed to be independent of the electrical state of the phase; μ_i^θ is the standard-state chemical potential of component i, m_i is the molality of ion i, and F is Faraday's constant. Equation 1 has two undefined quantities, Φ and Γ_i. Thus, application of Eq. 1 to the determination of Φ and Γ_i requires a second relation that relates unambiguously either Φ or Γ_i to the electro-mechanical potential of ion i. If such a relation is not established, ambiguity will be present in subsequent calculations involving Φ or Γ_i.

An alternative relation between Φ and the electrochemical potential of ion i can be obtained through application of the quasi-electrostatic-potential theory developed by Newman. Here, we select an arbitrary reference ion r (e.g., Cl^-) and define the electrochemical potential of that species (Newman, 1973)

$$\mu_r = \mu_r^\theta + \mathrm{RT}\ \ln(m_r\gamma_r) = \mathrm{RT}\ \ln(m_r) + z_r F\Phi. \qquad (2)$$

All nonidealities in the electrochemical potential of reference ion r are therefore assumed to be electrostatic in nature. The electrostatic potentials in Eqs. 1 and 2 are given the same symbol since both of them are a measure of the contribution that the electrical state of the system makes to the electrochemical potentials of the ions in the system; however, Φ in Eq. 2 differs from that in Eq. 1 because Φ in Eq. 2 is unambiguously related to the electrochemical potential of the reference ion. From Eq. 2, we obtain for the electrochemical potential of any other ionic species i:

$$\mu_i = \mu_i^\theta - \frac{z_i}{z_r}\mu_r^\theta + \mathrm{RT}\ \ln(m_i\gamma_i) - \mathrm{RT}\frac{z_i}{z_r}\ln(\gamma_r) + z_i F\Phi. \qquad (3)$$

Thus, although not explicitly present in Eq. 2, ionic activity coefficients appear in Eq. 3 in a manner that compensates for the arbitrary choice of the reference species r. Smyrl and Newman use the term "quasi-electrostatic" potential for a Φ defined in this manner (Smyrl et al., 1968). A short derivation of Eq. 3 is given in the Appendix.

For two liquid phases ($'$ and $''$) at temperature T and pressure P_o, the condition of phase equilibrium for any ion i present in both phases is:

$$\mu_i' = \mu_i'' \tag{4}$$

Substitution of Eq. 2 into Eq. 4, subsequent cancellation of the standard-state electrochemical potentials, and some rearrangement give,

$$\ln K_r = \ln \frac{m_r'}{m_r''} = \frac{z_r F}{RT}(\Phi'' - \Phi') \tag{5}$$

where K_r is the partition coefficient of the reference ion. Therefore, for an aqueous two-phase system at equilibrium, experimental measurements of m_r' and m_r'' give a clearly defined value for $\Delta\Phi$, the interfacial-electrostatic-potential difference.

A simple relation such as Eq. 5 does not exist if Eq. 1 is applied directly to each ionic species in the two-phase system at equilibrium. Substitution of Eq. 1 into Eq. 4 yields after some simplification,

$$\ln K_i = \ln \frac{m_i'}{m_i''} = \ln \frac{\Gamma_i''}{\Gamma_i'} + \frac{z_i F}{RT}(\Phi'' - \Phi') \tag{6}$$

where i represents any ionic species present in the system. In this case, knowledge of the partition coefficient of the electrolyte is not enough to determine $\Delta\Phi$; we also require knowledge of Γ_i''/Γ_i', a poorly defined quantity that cannot be measured or calculated. In fact, $\Delta\Phi$ defined in Eq. 6 is not unambiguously related to any measurable equilibrium property of the two-phase system. This severely restricts the usefulness of Eq. 6 for calculating interfacial-electrostatic-potential differences and suggests that such calculations be performed using quasi-electrostatic-potential theory. However, as shown by the pioneering work of Albertsson, Eq. 6 can be used to explain the nature of the interfacial-electrostatic-potential difference in aqueous two-phase systems.

Interpretation and Calculation of $\Delta\Phi$ for a Single Strong Electrolyte

We are concerned with the $\Delta\Phi$ that arises as a result of the addition of a strong electrolyte or salt(s) that fully dissociates into ν_+ cations of charge z_+ and ν_- anions of charge z_- to an aqueous two-phase system previously containing no charged species (e.g., a PEG/Dextran two-phase system). We take each phase to be electrically neutral; thus, for phase $'$ we require:

$$z_+ m_+' = -z_- m_-' \tag{7a}$$

and for phase $''$ we require:

$$z_+ m_+'' = -z_- m_-''. \tag{7b}$$

Dividing Eq. 7a by Eq. 7b gives,

$$K^+ = \frac{m_+'}{m_+''} = \frac{m_-'}{m_-''} = K^-. \tag{8}$$

Therefore, the phase electroneutrality condition forces the partition coefficients of the anion and the cation to be equal.

Interpretation of $\Delta\Phi$: the Albertsson model

Starting from Eq. 6 and assuming each phase to be electrically neutral, Albertsson derived the following relationship between the interfacial-electrostatic potential difference and the Γ_i's for the ions of the added electrolyte:

$$\Delta\Phi = (\Phi'' - \Phi') = \frac{RT}{(z_+ - z_-)F} ln\left[\frac{(\Gamma_-''/\Gamma_-')}{(\Gamma_+''/\Gamma_+')}\right]. \tag{9}$$

Equation 9 suggests that interfacial-electrostatic-potential differences between equilibrium phases develop as a result of unsymmetric "chemical" affinities of the ionic species for the two equilibrium phases (that is, in general, $\Gamma_-''/\Gamma_-' \neq \Gamma_+''/\Gamma_+'$). The Albertsson model, therefore, provides valuable insight into the origin of the microscopic charge separation in the interfacial region.

We can take the Albertsson model one step further by determining the relationship between the partition coefficient and the electrolyte and the Γ_i's of the ions making up the electrolyte. Substitution of Eq. 9 into Eq. 6 applied to the anion of the salt yields after simplification:

$$K_- = (\Gamma_-''/\Gamma_-')^{\frac{z_+}{z_+ - z_-}} (\Gamma_+''/\Gamma_+')^{\frac{-z_-}{z_+ - z_-}}. \tag{10}$$

Since $K_- = K_+ = K_S$, Eq. 10 shows how the partition coefficient of the electrolyte is related to the "chemical" activity coefficients of the ions of the salt at equilibrium.

Calculation of $\Delta\Phi$: quasi-electrostatic potential theory

A direct relation between interfacial-electrostatic-potential differences and measurable equilibrium properties of two-phase systems can be established through application of quasi-electrostatic potential theory. Applying quasi-electrostatic potential theory to the same system, we select the cation (+) as reference ion r. The partition coefficient of r is given by Eq. 5. The partition coefficient of the anion (−) is then given by,

$$\ln K_- = \frac{z_- F}{RT}(\Phi'' - \Phi') + \ln \frac{\gamma_-''}{\gamma_-'} - \frac{z_-}{z_r} \ln \frac{\gamma_r''}{\gamma_r'}. \tag{11}$$

We again take each phase to be electrically neutral; we can, therefore, equate the righthand sides of Eq. 5 and Eq. 11 to give:

$$\Delta\Phi = (\Phi'' - \Phi') = \frac{RT}{(z_r - z_-)F} \ln\left[\frac{(\gamma_-''/\gamma_-')}{('\gamma_r''/\gamma_r')^{z_-/z_r}}\right]. \tag{12}$$

The importance of Eq. 12 can be seen more clearly by applying it to the description of a two-phase system at equilibrium

containing a 1:1 electrolyte. For a 1:1 electrolyte, $z_-/z_r = -1$ and $z_r - z_- = 2$. In this case, Eq. 12 reduces to:

$$(\Phi'' - \Phi') = \frac{RT}{2F}\ln\left(\frac{\gamma_-''\gamma_r''}{\gamma_-'\gamma_r'}\right) = \frac{RT}{F}\ln\left(\frac{\gamma_\pm''}{\gamma_\pm'}\right) = \frac{RT}{F}\ln(K_s) \quad (13)$$

where, as usual,

$$\gamma_\pm^\nu = \gamma_+^{\nu_+}\gamma_-^{\nu_-}.$$

Here, $\gamma_\pm$ is the mean-ionic activity coefficient for the neutral salt (s), and $\nu = \nu_+ + \nu_-$. Mean-ionic activity coefficients are tabulated for most strong electrolytes in water at 25°C; for those salts or temperatures where such data are unavailable, $\gamma_\pm$ can be determined using one of many mean-ionic activity coefficient models for strong electrolytes, including Debye-Hückel theory (Newman, 1973), Pitzer's model (Pitzer, 1973), and the mean-spherical approximation of Blum (1980). The last equality in Eq. 13 holds because, at equilibrium,

$$m_s'\gamma_\pm' = m_s''\gamma_\pm''. \quad (14)$$

Equation 13, therefore, provides a means of directly calculating the interfacial electrostatic-potential difference from the equilibrium properties of a two-phase system; alternatively, Eq. 13 can be used to calculate equilibrium electrolyte partition coefficients from measured electrostatic potential differences.

Measurement of $\Delta\Phi$: Ag/AgCl capillary-electrode cell

Figure 1 shows a schematic of the Ag/AgCl capillary-electrode cell used to measure interfacial-electrostatic-potential differences between the phases of aqueous two-phase extraction systems. The cell consists of two identical Ag/AgCl capillary electrodes in contact with the same phase or the opposite phases of an aqueous two-phase system. Each electrode is composed of a Ag/AgCl wire electrode immersed in an aqueous 3-M KCl, saturated AgCl solution, all contained in a glass capillary pipette with an average internal tip diameter of 30 μm. The entire measurement assembly is enclosed in a Faraday cage.

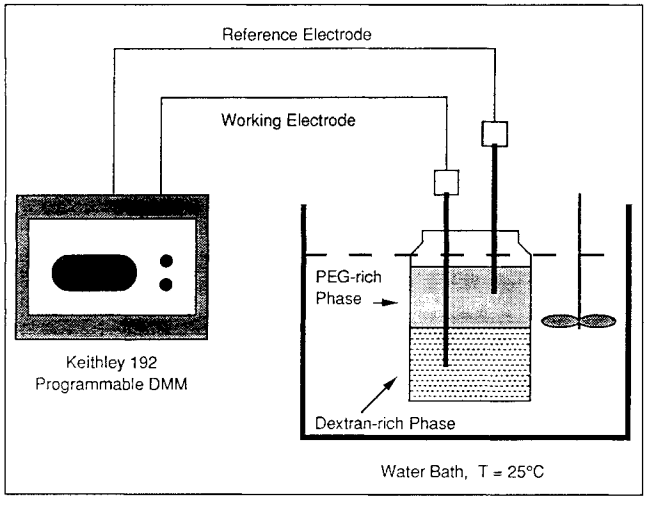

Figure 1. Ag/AgCl capillary-electrode cell used to measure interfacial-electrostatic-potential differences between the phases of aqueous two-phase extraction systems.

The concentration (solubility) of AgCl in the aqueous capillary-tube solutions is low enough that it can be ignored in our electro-mechanical analysis with no loss in accuracy. Thus, we can consider the electrolytic cell shown in Figure 2.

In general, the concentration of KI in phase ϵ is different from that in phase ϵ'. The transition regions (I, II, and III) are regions (interfaces) in the cell in which concentration gradients exist. The measured electrostatic-potential drop is the sum of the potential drops across these three liquid junctions.

Equilibrium among phases in contact with one another is described by Eq. 4: for example,

$$\mu_{K^+}^{\epsilon} = \mu_{K^+}^{\epsilon'}. \quad (15)$$

Similarly, chloride ions are equilibrated between the solution δ' and the electrode β', and so forth. However, not all of the contacting phases in the system are in equilibrium. The capillary-electrode experiment is designed such that phases δ and ϵ are not in equilibrium and phases δ' and ϵ' are not in equilibrium. Thus, Eq. 4 does not apply to the components found in transition regions I and III.

As shown by Newman (1973), the cell potential U is related to the electrostatic-potential difference between phases α and α', and to the difference in the electrochemical potentials of the electrons (e^-) in these leads by:

$$FU = z_e^- F(\Phi^\alpha - \Phi^{\alpha'}) = \mu_{e^-}^{\alpha} - \mu_{e^-}^{\alpha'}. \quad (16)$$

Applying Eq. 4 where appropriate, we can rewrite Eq. 16 as:

$$z_e^- F(\Phi^\alpha - \Phi^{\alpha'}) = (\mu_{Ag}^{\alpha} - \mu_{AgCl}^{\beta} + \mu_{KCl}^{\delta} - \mu_{K^+}^{\delta}) - (\mu_{Ag}^{\alpha'} - \mu_{AgCl}^{\beta'} + \mu_{KCl}^{\delta'} - \mu_{K^+}^{\delta'}). \quad (17)$$

Since the two electrodes in Figure 2 are identical, the chemical potentials of the nonionic components cancel and Eq. 17 reduces to:

$$z_e^- F(\Phi^\alpha - \Phi^{\alpha'}) = \mu_{K^+}^{\delta} - \mu_{K^+}^{\delta'} = \underbrace{(\mu_{K^+}^{\delta} - \mu_{K^+}^{\epsilon})}_{\text{I}} + \underbrace{(\mu_{K^+}^{\epsilon} - \mu_{K^+}^{\epsilon'})}_{\text{II}} + \underbrace{(\mu_{K^+}^{\epsilon'} - \mu_{K^+}^{\delta'})}_{\text{III}} \quad (18)$$

where I represents the contribution that transition region I makes to the measured electrostatic-potential difference. Similar statements hold for transition regions II and III.

Applications of quasi-electrostatic potential theory to the

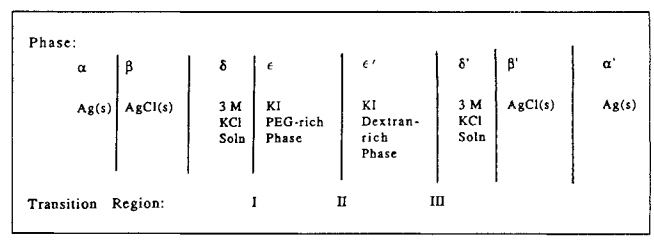

Figure 2. Ag/AgCl capillary electrode electrolytic cell.

electrolytic cell shown in Figure 2 (with K^+ taken as reference species r) yields:

$$\mu^{\delta}_{K^+} - \mu^{\epsilon}_{K^+} = RT \ln\left(\frac{m^{\delta}_{K^+}}{m^{\epsilon}_{K^+}}\right) + z_{K^+}F\Delta\Phi^{I} \tag{19a}$$

$$\mu^{\epsilon}_{K^+} - \mu^{\epsilon'}_{K^+} = 0 = RT \ln\left(\frac{m^{\epsilon}_{K^+}}{m^{\epsilon'}_{K^+}}\right) + z_{K^+}F\Delta\Phi^{II} \tag{19b}$$

and,

$$\mu^{\epsilon'}_{K^+} - \mu^{\delta'}_{K^+} = RT \ln\left(\frac{m^{\epsilon'}_{K^+}}{m^{\delta'}_{K^+}}\right) + z_{K^+}F\Delta\Phi^{III} \tag{19c}$$

where the electro-mechanical-potential difference associated with transition region II has been set equal to zero because phases ϵ and ϵ' are in equilibrium. Insertion of Eqs. 19a, 19b, and 19c into Eq. 18 gives:

$$z_{K^+}F(\Phi^{\delta} - \Phi^{\delta'}) = RT \ln\left(\frac{m^{\delta}_{K^+}}{m^{\epsilon}_{K^+}}\right) + z_{K^+}F\Delta\Phi^{I} + RT \ln\left(\frac{m^{\epsilon'}_{K^+}}{m^{\delta'}_{K^+}}\right)$$
$$+ z_{K^+}F\Delta\Phi^{III} = z_{K^+}F\Delta\Phi^{I} + z_{K^+}F\Delta\Phi^{II} + z_{K^+}F\Delta\Phi^{III} \tag{20}$$

where the second equality is derived using Eq. 19b and $m^{\delta}_{K^+} = m^{\delta'}_{K^+}$.

Equation 20 gives the relationship between the measured electrostatic-potential difference, $(\Phi^{\delta} - \Phi^{\delta'})$, and the interfacial-electrostatic-potential difference of interest, $\Delta\Phi^{II}$. In reporting interfacial-electrostatic-potential-difference data, Brooks et al. and King et al. have assumed that $(\Phi^{\delta} - \Phi^{\delta'})$ and $\Delta\Phi^{II}$ are the same; this is equivalent to assuming that:

$$z_{K^+}F\Delta\Phi^{I} + z_{K^+}F\Delta\Phi^{III} = 0. \tag{21}$$

King et al. indirectly validated this assumption by showing that a semitheoretical model, which assumes that all electrostatic effects in the system are described by a term of the form $z_pF(\Phi^{\delta} - \Phi^{\delta'})$ (where p is the net charge of the protein component), can predict the partition coefficient of a protein infinitely dilute in a PEG/Dextran aqueous two-phase system. We can establish the accuracy of assuming Eq. 21 to be valid more directly through application of the Henderson formula for the junction potential of a continuous-mixture junction (Newman, 1973). For example, application of the Henderson formula to transition region I yields:

$$\Delta\Phi^{I} = \Phi^{\delta} - \Phi^{\epsilon} = -\frac{RT}{F} A \frac{\ln\left(\frac{B^{\delta}}{B^{\epsilon}}\right)}{B^{\delta} - B^{\epsilon}} \tag{22}$$

where

$$A = \sum_i z_i u_i (c_i^{\delta} - c_i^{\epsilon}),\ B^{\delta} = \sum_i z_i^2 u_i c_i^{\delta},\ B^{\epsilon} = \sum_i z_i^2 u_i c_i^{\epsilon}$$

where i represents any ionic species, u_i is the mobility of species i, and c_i is the molar concentration of species i. The Henderson formula was derived for solutions so dilute that activity coefficients can be ignored, and it has been used to predict quantitatively liquid-junction potentials in such systems. For our system, where activity coefficients cannot be ignored and solute mobilities are not known well, the Henderson formula will not provide accurate liquid-junction potential data but can be used to estimate the relative magnitudes of three junction potentials ($\Delta\Phi^{I}$, $\Delta\Phi^{II}$, and $\Delta\Phi^{III}$).

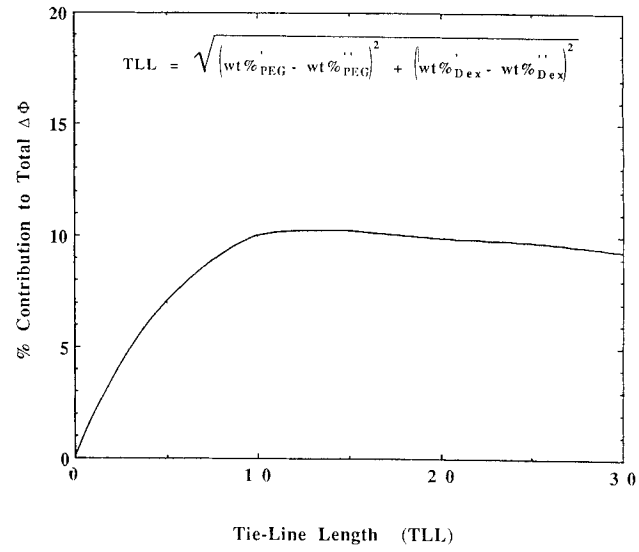

Figure 3. Application of the Henderson formula to the three liquid junctions in the electrolytic cell in Figure 2.

As shown in Figure 3, application of the Henderson formula to each liquid junction in the electrolytic cell shown in Figure 2 (with the phase-forming polymers PEG 3350 and Dextran T-70) suggests that the sum of the electrostatic-potential differences across transition regions I and III comprises no more than 10% of the total measured electrostatic-potential difference. In this analysis, all ion mobilities were determined from ion-diffusion-coefficient data via the Nernst-Einstein relation. As defined in Figure 3, the tie-line length is a useful parameter for correlating properties of aqueous two-phase systems; it is a representative measure of the composition difference of the two phases at equilibrium. Experimentally, an increase in the tie-line length causes a decrease in the partition coefficient of KI (as shown in Figure 5); the variation in the contribution that transition regions I and III make to the total $\Delta\Phi$ is due to this variation in the partition coefficient of KI.

In our interfacial-electrostatic-potential-difference experiments on PEG 3350/Dextran T-70 two-phase aqueous systems, experimental uncertainties range from 5 to 15% for each data point. Thus, for the PEG 3350/Dextran T-70 system, the added error associated with assuming Eq. 21 to be valid is relatively small. However, errors associated with application of Eq. 21 are unidirectional; the true $\Delta\Phi^{II}$ is always less than or equal to the measured $\Delta\Phi^{II}$.

A more general application of the Henderson formula to the analysis of measured $\Delta\Phi$'s for polymer/polymer aqueous two-phase systems containing a single strong electrolyte reveals that the sum of the contributions of transition regions I and III to the total measured $\Delta\Phi$ is small (i.e., $\leq 10\%$) if the mobilities of the ions do not differ by more than a factor of two. This analysis assumes that the Ag/AgCl capillary elec-

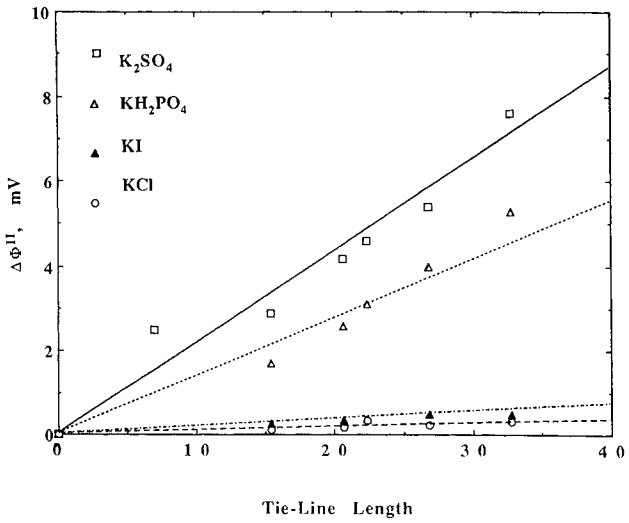

Figure 4. Interfacial-electrostatic-potential-difference data for the PEG 3350/Dextran T-70 aqueous two-phase system at 25°C as a function of the type of salt and the tie-line length.

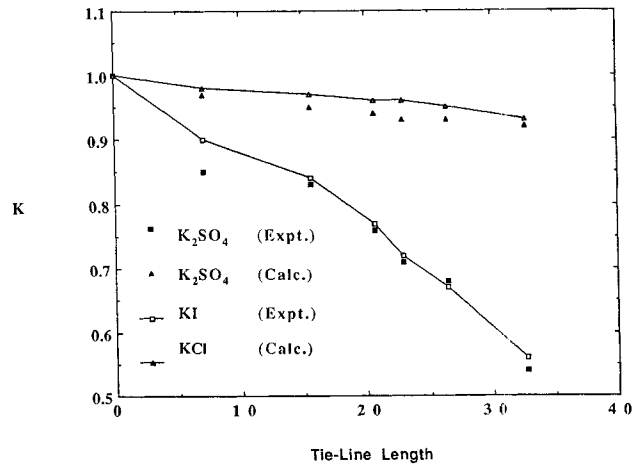

Figure 5. Measured and calculated chloride and sulfate partition coefficients in the PEG 3350/Dextran T-70 system at 25°C as a function of tie-line length.

trodes described in Figure 2 are used in the measurement; as before, all ion mobilities were determined from ion-diffusion-coefficient data.

However, a simple relation between the measured $\Delta\Phi$ and $\Delta\Phi^{II}$ does not exist for polymer/salt aqueous two-phase systems; here, the sum of $\Delta\Phi^{I}$ and $\Delta\Phi^{III}$ is no longer negligible. For these systems, each of the various liquid-junction potentials can be determined from a flux balance over the interfacial region (Newman, 1973):

$$F\nabla\Phi = -\frac{F}{\kappa}\mathbf{i} - \mathrm{RT}\sum_i \frac{t_i^o}{z_i}\nabla \ln c_i - \mathrm{RT}\sum_i \frac{t_i^o}{z_i}\nabla\left(\ln f_i - \frac{z_i}{z_r}\ln f_r\right) \quad (23)$$

where Φ is the quasi-electrostatic potential referred to species r, κ is the conductivity (mho/cm), $\mathbf{i}$ is the current density, t_i^o is the transference number of species i with respect to the velocity of the solvent, and f_i is the molar activity coefficient of ionic species i. Electrostatic-potential differences are calculated by integrating Eq. 23 across the junction region in the absence of current.

Experimental Studies

Materials

Polymers. Poly(ethylene glycol) fractions were purchased from Union Carbide Corporation, Chemical and Plastics Division, New York, NY, as Carbowax 3350 and Carbowax 8000. Dextran fractions were purchased from Pharmacia Inc., Piscataway, NJ, as Dextran T-70 (lot #00356) and Dextran T-500 (lot #38624). α-Cyclodextrins were purchased from Sigma Chemical Co., St. Louis, MO.

Salts. Potassium chloride, potassium iodide, and potassium phosphate were obtained from Fisher Scientific, Fair Lawn, NJ. Potassium sulfate was obtained from Allied Chemical, New York, NY.

Proteins. Bovine Serum Albumin (Σ# A-7030), α-Chymotrypsin (Σ# C-4129), and Lysozyme (Σ# L-6976) were purchased from Sigma Chemical Co., St. Louis, MO. Ag/AgCl microelectrodes were purchased from Microelectrodes Inc., Londonderry, NH.

Procedure

All interfacial-electrostatic-potential-difference measurements (with the exception of one set of experiments on a PEG/Dextran two-phase system containing KCl) were performed according to the protocol established by Brooks et al. (1984) and Sharp (1987) and used by King et al. (1988).

A single set of electrostatic-potential-difference experiments was performed on a PEG/Dextran two-phase aqueous system containing KCl by removing the Ag/AgCl wire electrodes from the capillaries and immersing them directly into the two-phase system.

Potassium-ion partition coefficients were measured by atomic absorption. Atomic absorption experiments were performed on a Perkin-Elmer model 2280 atomic-absorption spectrophotometer.

Results and Discussion

Figure 4 shows interfacial-electrostatic-potential-difference data for the PEG 3350/Dextran T-70 aqueous two-phase system at 25°C as a function of the type of salt and tie-line length. The electrostatic-potential differences measured are in the range 0 to 10 mV. Previous studies (Reitherman, 1973; Johansson, 1973; Brooks et al., 1984; King et al., 1988) have also reported values in this range, although for systems with different salt concentrations and polymer molecular weights. For each salt system studied, the interfacial-electrostatic-potential difference, $\Delta\Phi^{II}$, was zero at zero tie-line length and increased linearly with rising tie-line length. For a given tie-line length, the magnitude of $\Delta\Phi^{II}$ depends on the anion of the salt; sulfate gives the largest $\Delta\Phi^{II}$, then phosphate, then iodide, and finally chloride.

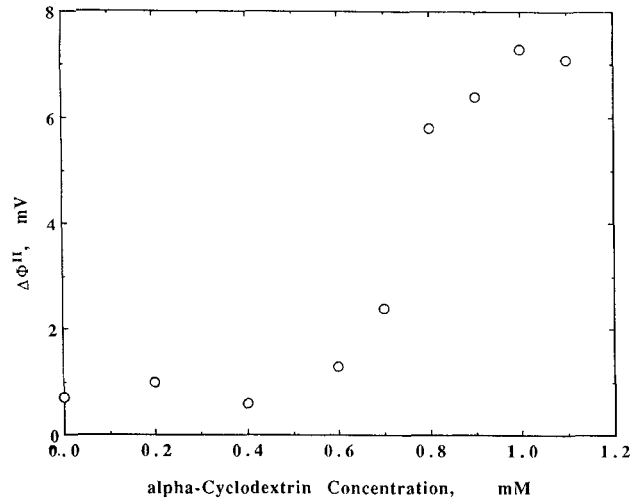

Figure 6. Interfacial-electrostatic-potential-difference data for an aqueous two-phase system containing 1.21-g Dextran T-70, 1.82-g PEG 3350, 16.97-g water, 1-mM KI, and varying amounts of α-cyclodextrin.

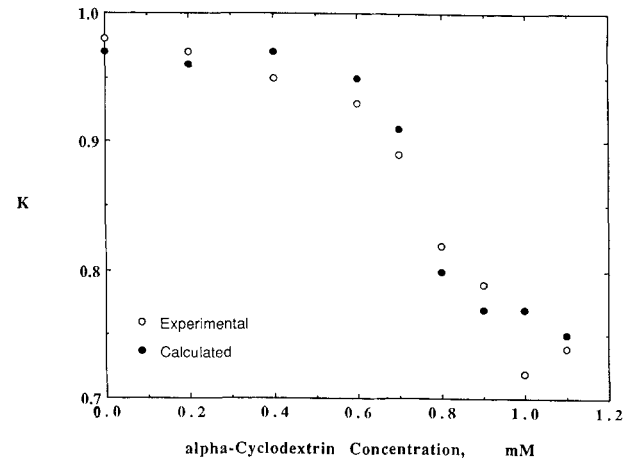

Figure 7. Measured and calculated iodide partition coefficients in an aqueous two-phase system containing 1.21-g Dextran T-70, 1.82-g PEG 3350, 16.97-g water, 1-mM KI, and varying amounts of α-cyclodextrin.

Figure 5 shows measured chloride and sulfate (King et al., 1988) partition coefficients in the PEG 3350/Dextran T-70 system at 25°C as a function of tie-line length. Both ion partition coefficients decrease with rising tie-line length, with the partition coefficient for sulfate decreasing much more sharply than that for the chloride. Also shown in Figure 5 are sulfate partition coefficients calculated using quasi-electrostatic potential theory (with the sulfate ion taken as species r) and the $\Delta\Phi^{II}$ data shown in Figure 4. Agreement of the calculations with experiment is striking, particularly when taking into account the errors associated with application of Eq. 21. A similar level of agreement is seen with the chloride ion, which indicates that quasi-electrostatic potential theory provides an unambiguous linkage between measured electrostatic-potential differences and thermodynamics. As demonstrated in an earlier publication (Haynes et al., 1989), the calculations shown in Figure 6 can be reversed; if the compositions of the two equilibrium phases are known either through experiment or through phase-equilibrium calculations, the interfacial-electrostatic-potential difference can be directly calculated using the equilibrium partition coefficient for the reference ion. In this case, knowledge (measurement) of $\Delta\Phi^{II}$ clearly is not necessary for the description of the equilibrium compositions of the two phases but can be used to test the accuracy of phase-diagram data or activity-coefficient models.

Although the thermodynamic analysis developed by Albertsson leads to expressions of limited applicability, Eqs. 9 and 10 provide valuable insight into the mechanism of electrostatic-potential-difference formation. Equation 9 indicates that electrostatic-potential gradients develop when the quantity $[(\Gamma_-''/\Gamma_-')/(\Gamma_+''/\Gamma_+')]$ diverges from unity. This implies that the interfacial-electrostatic-potential difference can be controlled (altered) by controlling (altering) the value of $[(\Gamma_-''/\Gamma_-')/(\Gamma_+''/\Gamma_+')]$. Figure 6 shows $\Delta\Phi^{II}$ data for an aqueous two-phase system at 25°C containing 1.21-g Dextran T-70, 1.82-g PEG 3350, 16.97-g water, 1-mM KI, and varying amounts of α-cyclodextrin. As the concentration of α-cyclodextrin rises from 0 to 1 mM, $\Delta\Phi^{II}$ increases from 0.7 mV to 7.3 mV. Over this range of α-cyclodextrin concentrations, the formation of iodide-cyclodextrin inclusion complexes changes the partition coefficient of the iodide ion from 0.97 to 0.72, as shown in Figure 4; however, no change was observed in the tie-line length or in the partition coefficient of α-cyclodextrin ($K=0.23$). These results clearly support the concepts embodied in Eqs. 9 and 10. The presence of a small amount of cyclodextrin has little effect on the relative "chemical" affinity of the potassium ion for the two equilibrium phases (i.e., $\Gamma_{K+}^{\delta\prime}/\Gamma_{K+}^{\delta}$) but has a large effect on $\Gamma_{I-}^{\delta\prime}/\Gamma_{I-}^{\delta}$ as a result of inclusion-complex formation. The difference in α-cylcodextrin's effect on the individual ions gives rise to an increase in $\Delta\Phi^{II}$.

Also shown in Figure 7 are iodide partition coefficients calculated using quasi-electrostatic potential theory and the $\Delta\Phi^{II}$ data shown in Figure 6. Once again, the calculated values agree well with experiment.

As reported previously by our group (Haynes et al., 1989; King et al., 1988) and others (see, for example, Walter et al., 1986), changes in salt partitioning, and therefore in the interfacial-electrostatic-potential difference, can significantly alter protein partition coefficients. For example, Figure 8 shows partition-coefficient data for three difference proteins in the aqueous two-phase system described in Figure 6 as a function of α-cyclodextrin concentration. In these experiments, the pH of the system was maintained at 6.9±0.2 by the presence of KI. Albumin, which is positively charged at pH 6.9, partitions strongly to the dextran-rich phase when no α-cyclodextrin is present. When α-cyclodextrin is added to the system, the partition coefficient for albumin rises as a result of the increase in the interfacial-electrostatic-potential difference. More dramatic partitioning effects are seen with α-chymotrypsin and lysozyme. As shown in Figure 8, both proteins partition preferentially to the PEG-rich phase when no α-cyclodextrin is present. Lysozyme and α-chymotrypsin are both negatively charged at pH 6.9. Therefore, we expect the partitioning trend for these proteins to be opposite to that for albumin; a decrease in the partition coefficients for lysozyme and α-chymotrypsin

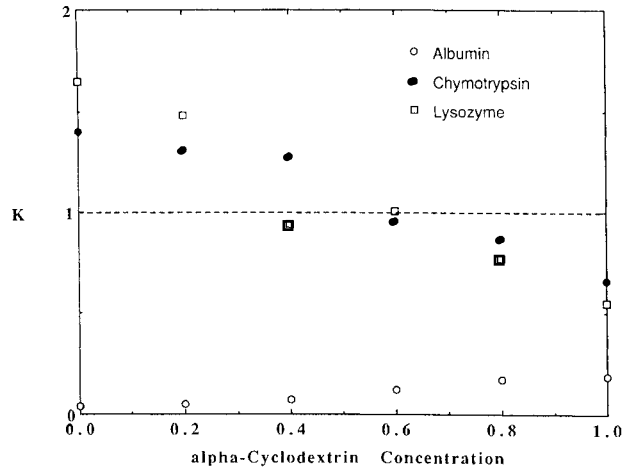

Figure 8. Albumin, α-chymotrypsin, and lysozyme partition-coefficient data as a function of α-cyclodextrin concentration for an aqueous two-phase system containing 1.21-g Dextran T-70, 1.82-g PEG 3350, 16.97-g water, 1-mM KI, and varying amounts of α-cyclodextrin.

should be observed with increasing $\Delta\Phi^{II}$. The results shown in Figure 8 verify our expectations. For both proteins, an increase in $\Delta\Phi^{II}$ from 0.7 mV to 7.3 mV lowers the partition coefficient from a value greater than unity to a value less than unity. Here, we clearly see the importance of $\Delta\Phi^{II}$. By changing $\Delta\Phi^{II}$, we can force proteins which initially partition preferentially into the PEG-rich phase to partition preferentially into the Dextran-rich phase and *vice versa*.

Cost and low solubility may make the addition of cyclodextrins to aqueous two-phase systems undesirable. However, the formation of inclusion complexes is not necessary for generating the partitioning trends seen in Figure 8. For example, Figure 9 shows partition-coefficient data for α-chymotrypsin in the PEG 3350/Dextran T-70 system as a function of tie-line length and type of salt. Once again, an

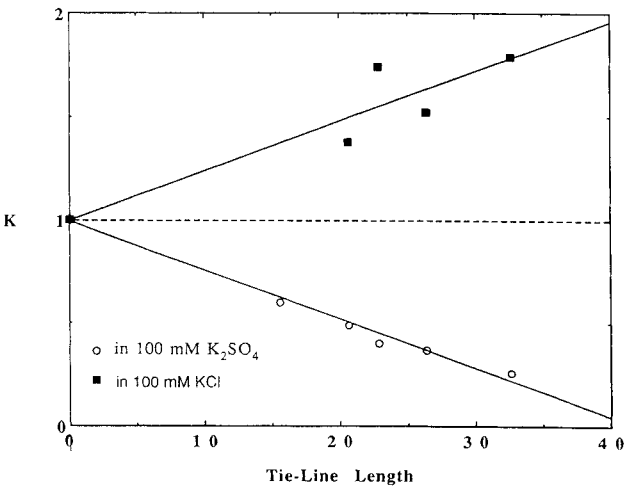

Figure 9. Partition-coefficient data for α-chymotrypsin in the PEG 3350/Dextran T-70 system at 25°C as a function of tie-line length and type of salt.

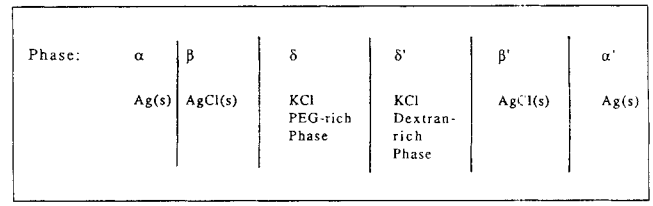

Figure 10. Electrolytic cell for zero potential-difference measurement.

increase in $\Delta\Phi^{II}$ causes protein to move from the PEG-rich phase to the Dextran-rich phase until equilibrium is achieved. Similar results have been reported by others.

One final concern should occupy our attention. Maurer (1989) conjectured that the immersion of capillary electrodes in a two-phase system and subsequent completion of the circuit significantly perturbs the compositions of the two phases from their equilibrium values. If this were true, our quasi-electrostatic potential theory analysis would be altered because system constraints of the form of Eq. 19b would no longer be valid. To refute this idea, we conducted a set of electrostatic-potential-difference experiments on a PEG/Dextran aqueous two-phase system containing 100-mM KCl, in which the Ag/AgCl wire electrodes were removed from their capillaries and immersed directly into the two-phase system. This leaves us with the electrolytic cell in Figure 10.

There are no capillary liquid junctions in this system; the cell potential is therefore given by,

$$FU = z_e^- F(\Phi^{\delta} - \Phi^{\delta'}) = \mu^{\delta}_{Cl^-} - \mu^{\sigma^-}_{Cl^-} \quad (=0 \text{ at equilibrium}). \qquad (24)$$

Thus, for the electrolytic cell shown in Figure 10, an interfacial-electrostatic-potential difference of zero will always be measured if the two phases remain in equilibrium. Figure 11 shows interfacial-electrostatic-potential-difference data as a function of tie-line length for the electrolytic cell shown in Figure 10. The data indicate that the aqueous two-phase system is not significantly perturbed from equilibrium during the measurement of electrostatic-potential differences.

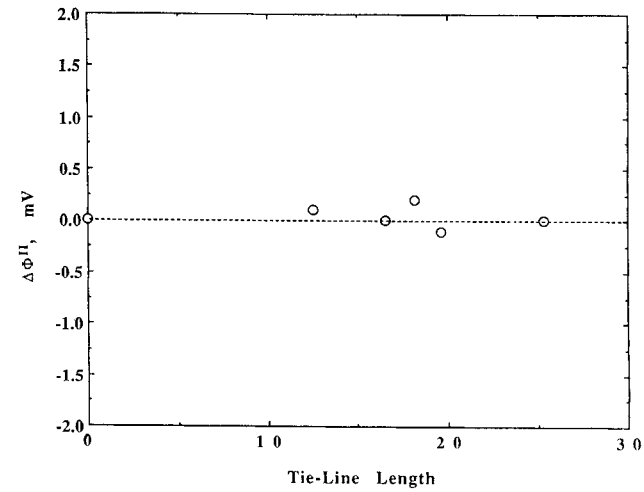

Figure 11. Interfacial-electrostatic-potential-difference data as a function of tie-line length for the electrolytic cell in Figure 10.

Acknowledgment

This work was supported in part by NSF grants CBT-8705530, CBT-8715908, and ECE-85005848. The authors are especially grateful to John Newman for help in all areas of the project. The authors also wish to thank Heriberto Cabezas, Donald E. Brooks, Robert King, Eric Anderson, and Horacio Corti for helpful discussions.

Notation

c_i = molar concentration of species i, mol/L
F = Faraday's constant, 96,487 C/equiv
f_i = molar activity coefficient of species i
$\mathbf{i}$ = current density, A/cm^2
K_i = molal partition coefficient of species i
m_i = molality of species i, mol/kg
R = universal gas constant
r = reference ion
T = absolute temperature, K
t_i^o = transference number of species i with respect to the velocity of species 0
U = cell potential, mV
u_i = mobility of species i, cm^2•mol/J•s
z_i = charge number of species i

Greek letters

Φ = electric potential, mV
Γ_i = "chemical" molal activity coefficient of species i
γ_i = molal activity coefficient of species i
$\gamma_\pm$ = molal mean-ionic activity coefficient of species i
κ = conductivity, mho/cm
μ_i = chemical or electrochemical potential of species i, J/mol
ν = number of moles of ions which a mole of electrolyte dissociates

Literature Cited

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

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

Blum, L., "Primitive Electrolytes in the Mean-Spherical Approximation," *Theoretical Chemistry: Advances and Perspectives*, Vol. 5, p. 1, H. Eyring and D. Henderson, eds., Academic Press, New York (1980).

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

Gelb, R. I., L. M. Schwartz, M. Radeos, and D. A. Laufer, "Cycloamylose Complexation of Inorganic Anions," *J. Phys. Chem.*, **87**, 3349 (1983).

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

Haynes, C. A., H. W. Blanch, and J. M. Prausnitz, "Separation of Protein Mixtures By Extraction: Thermodynamic Properties of Aqueous Two-Phase Polymer Systems Containing Salts and Proteins," *Fluid Phase Equil.*, **53**, 463 (1989).

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

King, R. S., H. W. Blanch, and J. M. Prausnitz, "Molecular Thermodynamics of Aqueous Two-Phase Systems for Bioseparations," *AIChE J.*, **34**(10), 1585 (1988).

Kula, M.-R., K. H. Kroner, and H. Hustedt, "Purification of Enzymes by Liquid-Liquid Extraction," *Adv. Biochem. Eng.*, **24**, 73 (1982).

Maurer, G., question posed at *Int. Conf. on Partitioning in Aqueous Two-Phase Systems*, Assmanshausen, Germany (Aug. 27, 1989).

Newman, J. S., *Electrochemical Systems*, Prentice-Hall, Englewood Cliffs, NJ (1973).

Nichols, III, A. L., and L. R. Pratt, *J. Chem. Phys.*, **80**(12), 6225 (1984).

Pitzer, K. S., "Thermodynamics of Electrolytes: I. Theoretical Basis and General Equations," *J. Phys. Chem.*, **77**, 268 (1973).

Reitherman, R., S. D. Flanagan, and S. H. Barondes, "Electromotive Phenomena in Partition of Erythrocytes in Aqueous Polymer Two-Phase Systems," *Biochem. Biophys. Acta*, **297**, 193 (1973).

Sharp, K. A., "Protocol for Measurement of Electrostatic Potentials," personal communication (1987).

Smyrl, W. H., and J. S. Newman, "Potentials of Cells with Liquid Junctions," *J. Phys. Chem.*, **72**, 4660 (1968).

Walter, H., D. E. Brooks, and D. Fisher, *Partitioning in Aqueous Two-Phase Systems*, Academic Press, London (1985).

Appendix

According to Guggenheim, the electrochemical potential of an ion i in solution is given by:

$$\mu_i = \mu_i^\theta + \mathrm{RT}\,\ln(m_i\gamma_i) \tag{A1}$$

where γ_i is the activity coefficient of ion i, which includes all contributions from electrostatic and nonelectronic forces. For the arbitrary reference ion r, we solve Eq. 2 for the quasi-electrostatic potential, Φ:

$$\Phi = \frac{\mu_r^\theta}{z_r F} + \frac{\mathrm{RT}}{z_r F}\ln(\gamma_r). \tag{A2}$$

The electrochemical potential for any other ion in the system must now be determined. We define the electrochemical potential of any other ion i as:

$$\mu_i = \mu_i^\theta + \mathrm{RT}\,\ln(m_i\gamma_i) = \mathrm{RT}\,\ln(Q) + z_i F\Phi \tag{A3}$$

where Q is that contribution to the electrochemical potential of ion i which is independent of the electrical state of the system. Substituting Eq. A2 into Eq. A3 and solving for Q gives:

$$\mathrm{RT}\,\ln(Q) = \mu_i^\theta - \frac{z_i}{z_r}\mu_r^\theta + \mathrm{RT}\,\ln(m_i\gamma_i) - \mathrm{RT}\frac{z_i}{z_r}\ln(\gamma_r). \tag{A4}$$

Manuscript received Jan. 24, 1991, and revision received Aug. 7, 1991.