

On Light-Scattering Studies of Isoionic Proteins

The calculations described here show that, in light-scattering studies on isoionic proteins, the attractive force due to charge fluctuations results in a considerable contribution to the derivative of the excess chemical potential of the protein with respect to its concentration in the presence of salt as well as in the salt-free case. This attractive force is sometimes sufficient to contribute significantly to the negative values of the slopes of light-scattering plots even in the case of proteins which carry a nonvanishing mean electric charge from ion-binding. Thus, it appears that the interaction of fluctuating charges provides at least a partial explanation for the negative slopes often observed in light-scattering experiments on isoionic proteins in the presence of salt.

THEORY AND CALCULATIONS

In recent years the method of light scattering has become a standard technique for studies of the sizes, shapes, and degrees of aggregation of natural and synthetic polymers (1). In the case of a two-component system, the relationship between the excess turbidity, τ , and the chemical potential, μ_2 , of the polymer is (2, 3):

$$H \frac{C_2}{\tau} = \frac{1}{M_2} \left[1 + \frac{C_2}{RT} \frac{\partial \mu_2^{(e)}}{\partial C_2} \right] \quad (1)$$

$$H = \frac{32\pi^3 n^2 (\partial n / \partial C_2)^2}{3\lambda^4 N}$$

$$\mu_2 = RT \log C_2 + \mu_2^{(e)} + \mu_2^0(T, p)$$

where C_2 is the concentration of the macromolecular species in grams/ml., T is the thermodynamic temperature, M_2 is the molecular weight of the macromolecular species, n is the refractive index of the solution, N is Avogadro's number, R is the gas constant, λ is the wavelength of the light, and $\mu_2^{(e)}$ is

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the excess chemical potential of the macromolecular component. The intercept of a plot of HC_2/τ vs. C_2 gives the molecular weight, while from the slope the derivative of the excess chemical potential of the protein with respect to its concentration may be calculated. This derivative is a measure of the departure of the solution from ideal behavior. Effective attractive forces between the macromolecules lead to negative values of the derivative, while repulsive forces may give rise to positive values. In the case of a three-component system (such as water = component 0, salt = component 1, protein = component 2), the light-scattering expression is more complicated. The multicomponent theory (3-5) shows that in such a case the light-scattering equation assumes the form:

$$\frac{HC_2}{\tau} = \frac{(\partial \mu_2 / \partial C_2) + (M_2/M_1) \frac{(\partial \mu_1 / \partial C_2)^2}{(\partial \mu_1 / \partial C_1)}}{RT \{1 - [\alpha (\partial \mu_1 / \partial C_2) / (\partial \mu_1 / \partial C_1)]^2\}} \quad (2)$$

$$\alpha = \left(\frac{\partial n}{\partial C_1} \right)_{c_2} / \left(\frac{\partial n}{\partial C_2} \right)_{c_1}$$

In the case when the protein carries an average net charge \bar{Z}_2 , it is convenient to define components according to Scatchard (6), i.e., in such a manner that addition of one mole of protein results in the net addition of only one mole of ions, with the preservation of electroneutrality. Thus, addition of one mole of protein involves the addition of $-\bar{Z}_2 Z_i m_i^0/\Gamma^0$ moles of each type of ions of the supporting electrolyte. The molar concentration m_i^0 of the i 'th ion of charge Z_i and the ionic strength, $\Gamma^0/2$, are those obtained as m_2 approaches zero.

Using this definition of components, and the multicomponent theory of light scattering, it can be shown that when component 1 is a 1-1 electrolyte the three-component equation becomes:

$$H \frac{C_2}{\tau} = \frac{1}{1+D}$$

$$\left\{ \frac{1}{M_2} + C_2 \left[\frac{M_1 \bar{Z}_2^2}{2M_2^2 C_1 \epsilon} + \frac{A_{22}}{M_2} - \frac{10^6}{M_1 M_2^2} \right. \right. \quad (3)$$

$$\left. \left. \frac{\left(\frac{M_2}{10^3} A_{12} - \frac{\bar{Z}_2^2 M_1^2 C_2}{2 \times 10^3 M_2 C_1^2 \epsilon} \right)}{\frac{2}{C_1 \epsilon} + A_{11}} \right] \right\}$$

$$1 + D = \left[1 - \frac{\alpha \left(A_{12} - \frac{10^3 \bar{Z}_2^2 M_1 C_2}{2 M_2^2 C_1} \right)^2}{\frac{2}{C_1 \epsilon} + A_{11}} \right]^2$$

$$\epsilon = 1 - \left(\frac{\bar{Z}_2 M_1 C_2}{2 M_2 C_1} \right)^2; \quad A_{ij} = \frac{1}{RT} \frac{\partial \mu_i^{(e)}}{\partial C_j}$$

The intercept of the HC_2/τ vs. concentration plot is the reciprocal of the sum of the molecular weight and a term describing the thermodynamic interaction of the protein with component 1. It is discussed in detail elsewhere (7-10).

In order to understand the significance of the coefficient of C_2 , it is necessary to know the various factors contributing to the thermodynamic interactions. The first term of this coefficient is a consequence of the component definition used and is always positive. The third term depends on the interaction

between the protein and component 1. The A_{22} term, representing protein-protein interaction, is composed of contributions from several attractive and repulsive forces. Among these are (a) the positive contribution of the excluded volume, (b) the positive contribution of the electrostatic repulsion due to the net charge of the protein, (c) the negative contribution of the dipole and multipole interactions, (d) the effect of various non-electrostatic forces, and (e) an additional attractive force which has been shown by Kirkwood and Shumaker (11) to be acting in the system as a result of the fluctuations in charge and charge configuration on a protein molecule.

It is the purpose of this paper to show that the attractive force resulting from charge fluctuations (11) can be invoked in certain cases to explain the negative sign of the coefficient of C_2 observed in light-scattering studies on isoionic proteins in the presence of neutral salts.

In the case of a salt-free isoionic protein of zero average net charge, the light-scattering equation assumes the form of a power series in $C_2^{1/2}$ (12-14):

$$H \frac{C_2}{\tau} = \frac{1}{M_2} (1 - a_1 C_2^{1/2} + a_2 C_2 + \dots) \quad (4)$$

where a_1 is a function of the fluctuating charge (12-14), and a_2 involves the effects of various electrostatic and non-electrostatic forces. When the protein has a nonzero net charge, a correction has to be made for its ionization (15).

It has been found that in the presence of salt the slopes of light-scattering curves for isoionic proteins can vary from positive values at high ionic strengths to negative values at low ionic strengths (13, 14, 16). The positive slopes are attributable to an electrostatic repulsive force between protein molecules. This repulsive force is caused by the binding of ions to the protein which would, of course, result in the presence of a nonzero mean charge on the protein molecules. The negative slopes which are observed in the presence of low salt concentrations and which are indicative of attractive forces between protein molecules have not received specific quantitative interpretation up to the pres-

ent. The contributions made to the slope by the protein-ion and ion-ion interaction terms A_{12} and A_{11} of Eq. (2) can be calculated from ion-binding data and thermodynamic measurements on protein-free salt solutions, respectively.

In order to evaluate A_{22} , it is necessary to have information on the contribution of the various electrostatic and non-electrostatic forces operative in the system. We have calculated the contribution of the fluctuating charge interactions to A_{22} , both in salt-free solutions and in the presence of low concentrations of salt, using expressions and methods described by Kirkwood and Shumaker (11). According to these authors the potential of mean force, $W(R)$, between two protein molecules, the centers of mass of which are separated by a distance R , is related to the electrostatic potential, V , by (11, 17):

$$W(R) = \langle V \rangle_{Av} - \frac{1}{2kT} [\langle V^2 \rangle_{Av} - \langle V_{Av} \rangle^2] \quad (5)$$

$$V = \sum_{i=1}^{\nu_1} \sum_{k=1}^{\nu_2} \frac{q_i^{(1)} q_k^{(2)}}{DR_{ik}^{(12)}}$$

where $R_{ik}^{(12)}$ is the distance between the i 'th ionogenic site on one molecule and the k 'th site on the other, D is the dielectric constant of the medium, $q_i^{(1)}$ and $q_k^{(2)}$ are the electric charges of the ionogenic sites on the molecules and ν_1 and ν_2 are the numbers of basic groups on each of the two protein molecules.

In the presence of neutral salt, for protein molecules with a mean net charge, $e\bar{Z}_2 = \langle q \rangle_{Av}$, and mean square charge, $e\langle Z_2^2 \rangle_{Av}^{1/2} = \langle \Delta q^2 \rangle_{Av}^{1/2}$, Eq. (5) becomes, upon introduction of proper Debye-Hückel screening:

$$W_{22}(R) = \frac{\bar{Z}_2^2 e^2}{DR} S - \frac{\langle Z_2^2 \rangle_{Av} e^4}{2kTD^2R^2} \quad (6)$$

$$W_{2i}(R) = \frac{\bar{Z}_2 Z_i e^2}{DR} S - \frac{\langle Z_2^2 \rangle_{Av} Z_i^2 e^4}{2kTD^2R^2} S^2$$

$$f_{ik} = \left\langle \frac{R^2}{(R_{ik}^{(12)})^2} \right\rangle_{Av}$$

$$S = \frac{e^{-\kappa(R-a)}}{1 + \kappa a}$$

where κ and a are the usual Debye-Hückel parameters, and e is the protonic charge.

Setting f_{ik} equal to unity, i.e., neglecting all terms except that in charge-charge interaction, substituting Eq. (6) in the expressions for the radial distribution function (18) used by Kirkwood and Shumaker (11), and performing the integration indicated by them,

$$g_{2i}(R) = e^{-W_{2i}(R)/kT} \quad (7)$$

$$G_{2i} = 4\pi \int_0^\infty R^2 [g_{2i}(R) - 1] dR,$$

we obtain for the fluctuating charge contribution to the electrostatic part of A_{22} , A_{22}^e :

$$A_{22}^e = \frac{1}{RT} \left(\frac{\partial \mu_2^{(e)}}{\partial C_2} \right) = \frac{N}{M_2} \sum_{i=2}^4 \nu_i G_{2i} \quad (8)$$

$$G_{2i} = -\frac{4\pi e^2 \bar{Z}_2 Z_i}{DkT\kappa^2} + \frac{\pi e^4 \langle Z_2^2 \rangle_{Av} Z_i}{D^2 k^2 T^2 \kappa (1 + \kappa a)^2}$$

$$\nu_3 = \left| \frac{\bar{Z}_2}{2} \right|; \quad \nu_4 = - \left| \frac{\bar{Z}_2}{2} \right|; \quad \nu_2 = 1$$

$$A_{22}^e = 2B_0 - \frac{\pi N e^4 \langle Z_2^2 \rangle_{Av}}{M_2 (DkT)^2 \kappa (1 + \kappa a)^2}$$

$$2B_0 = \frac{7\pi N b^3}{6M_2}$$

$$\kappa^2 = \frac{4\pi N e^2}{DkT} \left(\frac{\sum_j m_j Z_j^2}{1000} + \frac{\langle Z_2^2 \rangle_{Av} + \bar{Z}_2^2}{M_2} C_2 \right)$$

where m_j is the molar concentration of added electrolyte, b is the radius of the protein molecule, and ν_i is the number of moles of the i 'th ionic species associated with each mole of protein in Scatchard's (6) method of defining components. This expression is found to be identical with that obtained for the salt-free case. In the integration, the first term of Eq. (6) disappears as a result of electroneutrality while, as a result of definition of components, the fluctuating charge-charge interaction yields two identical terms of opposite sign, which cancel out.

Equations (8) may be used to calculate the contribution of the fluctuating charge to A_{22} for an isoionic protein in the presence of a neutral salt. $\langle Z_2^2 \rangle_{Av}$ is the sum of two terms: the variance of the bound proton fluctuation, and the variance of the fluctuation in bound salt ions:

$$\langle Z_2^2 \rangle_{AV} = \langle Z_{H^+}^2 \rangle_{AV} + \sum_j \langle Z_j^2 \rangle_{AV} \quad (9)$$

The first term can be obtained from light-scattering measurements on salt-free solutions (13, 14), and the second term may be calculated from the equation (11):

$$\langle Z_j^2 \rangle_{AV} = \sum_{r=1}^{\nu} \frac{1}{2 + m_j k_r + (m_j k_r)^{-1}} \quad (10)$$

where $\langle Z_j^2 \rangle_{AV}$ is the mean square charge due to fluctuations in bound ions of type j , m_j is the molar concentration of ions of type j in the added electrolyte, k_r is the intrinsic binding constant at the r 'th site, and ν is the number of sites at which ions j may be bound. Z_2 can be calculated from the dissociation of the protein at its isoionic point (15). In the calculation of A_{22} , the parameter a in the second equation of Eq. (8) varies with ionic strength. At salt concentrations where the ionic strength is due principally to protein, a is the distance of closest approach of two protein molecules. At high salt concentrations it is the distance of closest approach of the protein and small salt ion. At intermediate salt concentrations, a will take on values determined by both distances. As a crude approximation, a weighted average value of $(1 + \kappa a)^2$ can be used. Fortunately, at low ionic strengths this results in only a small error.

The quantity A_{12} in Eq. (3) may be calculated from salt-binding data by equating the activity coefficient of the protein to the fraction, f_2 , of protein molecules without bound ions j :

$$\frac{1}{RT} \frac{\partial \mu_2^{(e)}}{\partial m_1} = \frac{\partial \ln f_2}{\partial m_1} \quad (11)$$

$$f_2 = \prod_{r=1}^s \left(1 - \frac{k_r \gamma_1 m'_j e^{-2W(\bar{z}_2 + \bar{\nu} z_j) z_j}}{1 + k_r \gamma_1 m'_j e^{-2W(\bar{z}_2 + \bar{\nu} z_j) z_j}} \right)^{n_r}$$

$$\frac{1}{RT} \frac{\partial \mu_2^{(e)}}{\partial m_1} = - \sum_{r=1}^s$$

$$\left(\frac{n_r k_r e^{-2W(\bar{z}_2 + \bar{\nu} z_j) z_j}}{1 + k_r \gamma_1 m'_j e^{-2W(\bar{z}_2 + \bar{\nu} z_j) z_j}} \right) \frac{\partial (\gamma_1 m_j)}{\partial m_1}$$

$$m'_j = m_j - \bar{\nu} m_2$$

$$W = \frac{e^2}{2DkT} \left[\frac{1}{b} - \frac{\kappa}{1 + \kappa a} \right]$$

$$A_{12} = - \frac{10^8}{M_2} \sum \nu_i \frac{\bar{\nu}}{m_1} (\sum \nu_i + A_{11} C_1)$$

Here γ_1 is the activity coefficient of component 1, $\sum \nu_i$ is the total number of ions into which component 1 dissociates, n_r is the number of binding sites of type r , $\bar{\nu}$ is the average number of ions j bound per protein molecule when the j 'th ion concentration = m_j . The exponential terms in the second and fourth lines of Eq. (11) account for the electrostatic contribution to binding [cf. Scatchard (6)]. In this calculation long-range electrostatic interactions between small ions and the protein are neglected.

The quantity A_{11} can be obtained from independent thermodynamic measurements on pure component 1.

In this manner, the coefficient of C_2 in Eq. (3) was calculated for bovine serum albumin (BSA), bovine serum mercaptalbumin (BMA), and conalbumin in the presence of low concentrations of sodium chloride. In each case it was found that a small upward curvature is predicted in the light-scattering plot at low salt concentrations. This curvature is caused by the dependence of κ on C_2 . The curvature diminishes as the salt concentration increases. A_{12} was calculated according to Eq. (11) using the chloride-binding data of Scatchard *et al.* (19, 20). A_{11} values were taken from the literature (21). $\langle Z_2^2 \rangle_{AV}$ was obtained from published values of $\langle Z_{H^+}^2 \rangle_{AV}$ (13, 14) and Eq. (10). The calculated values of the interaction terms for BSA are given in Table I.

The theoretical values of the slopes are compared with experimental data in Fig. 1 for BSA and BMA and in Fig. 2 for conalbumin. In the calculation of A_{22} , it was assumed that the near-balance between various short range nonionic attractive and repulsive forces, reflected in low values of the coefficient of C_2 in the absence of salt (12-14), is still applicable in the presence of salt. The contribution of such forces to A_{22} would then remain constant in the presence of low concentrations of salt, and the experimental value of a_2/M_2 in Eq. (4) can be added directly to the coefficient of C_2 calculated from the electrostatic considerations discussed above. In the case of BSA, a_2 is small an

negative (-1.4); for BMA it is small and positive (3.0). The agreement obtained between the calculated and the experimentally measured slopes (Fig. 1) is good in view of the assumptions made.

In the case of conalbumin, a_2 is negative and ten times larger than for BSA (-30.0). The agreement between theory and experiment shown in Fig. 2 is very good.

In these calculations the term ϵ of Eq. (3) was found to be significant at salt concentrations up to $1 \times 10^{-4} M$.

At high salt concentrations, the proteins acquire considerable net average charges due to chloride binding. This results in an additional positive contribution to A_{22} due to electrostatic repulsion. At the present time, this cannot be evaluated rigorously. At low salt concentrations (when binding is small) this contribution can be considered to be very small. At $3 \times 10^{-4} M$ NaCl, the positive contribution of this term to A_{22} is estimated to be equal to about half of the contribution of the A_{12} term.³

An attempt has been made also to calculate the value of a_2 , the coefficient of C_2 in the light-scattering equation of an isoionic protein in the absence of salt [Eq. (4)]. Such a protein has a mean charge $e\bar{Z}_2$ of zero, but nonzero mean square charge, $e\langle Z_2^2 \rangle_{AV}$.⁴ In order to do this it is necessary to have an explicit form for f_{ik} in Eq. (6), i.e., definite

³ This approximate calculation was carried out by using the methods of Eqs. (7) and (8) with a screened coulombic potential. This is adequate for the present approximate calculation. The integration was carried out numerically. Thus, for repulsion

$$G_{22} =$$

$$4\pi \int_a^\infty R^2 \left\{ \exp \left(-\frac{\bar{Z}_2^2 e^2}{DRkT} \frac{e^{-\kappa(R-a)}}{1 + \kappa a} \right) - 1 \right\} dR$$

The value of 0.60×10^{-4} ml./g. for a charge, \bar{Z}_2 , of -0.5 seems to be in full agreement with the experimental slope. Similar calculations for other systems seem also to give a reasonable estimate of the values observed experimentally (22).

⁴ If the isoionic point is not at pH 7.0, the protein will carry a nonzero net average charge (15). This factor can be accounted for as shown previously (15). In the present calculation it is assumed that the net charge effect has been properly accounted for.

TABLE I
INTERACTION CONSTANTS^a OF ISOIONIC BOVINE SERUM ALBUMIN IN NaCl SOLUTIONS

NaCl concn.	$\langle Z_{Cl}^2 \rangle_{AV}$ ^b	$(A_{22}^c + 2B_0 + a_2)^c$		A_{12}^d	Slope $\times 10^7$ (ml.-g. ⁻¹)	
		10 g./l.	5 g./l.		10 g./l.	5 g./l.
moles/l.						
3×10^{-5}	0.01	-7.7	-12.3	-44	-1.3	-1.9
1×10^{-4}	0.05	-6.7	-9.6	-33	-1.4	-1.8
3×10^{-4}	0.15	-5.1	-6.8	-28	-1.3	-1.5
1×10^{-3}	0.48	-2.4	-2.7	-19	—	—

^a Assumed mol. wt. 75,000.

^b Calculated with Eq. (10).

^c Calculated with Eq. (8).

^d Calculated with Eq. (11).

^e Contribution from a net average charge, \bar{Z}_2 , of -0.5 is estimated to be 0.60×10^{-4} .

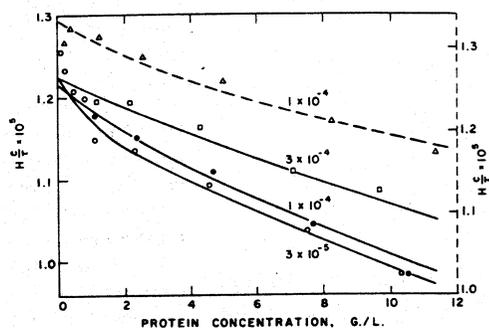


FIG. 1. Comparison of calculated and experimental light-scattering curves for isoionic BSA and BMA. Solid lines: calculated curves; symbols: experimental points (13). Left ordinate: BSA; right ordinate: BMA. Numbers on figure refer to molar concentration of NaCl in solvent.

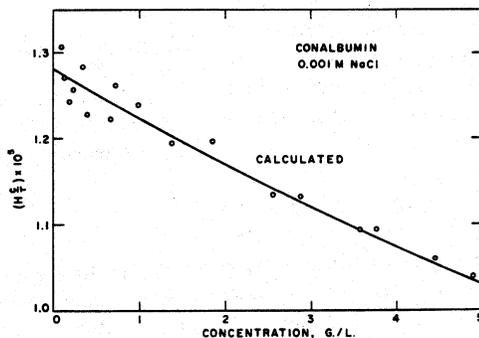


FIG. 2. Comparison of calculated and experimental light-scattering curves for isoionic conalbumin in $1 \times 10^{-3} M$ NaCl. Solid line: calculated curve; circles: experimental points (14).

assumptions must be made about the shape of the molecule and the geometrical configuration of the ionogenic sites. If it is assumed that all fluctuations occur uniformly and randomly on the surface of spheres of radius b , (b = radius of the protein molecules), then $f_{ik} = f$ is independent of both i and k and may be factored out of the summation in Eq. (6). Applying Eq. (10) of Ref. (11), we obtain

$$f = \frac{1}{z^2} \ln(1 - z^2) + \frac{1}{z} \ln \frac{1+z}{1-z} \quad (12)$$

$$z = \frac{2b}{R}$$

Using the identity $f = 1 - (1 - f)$ in Eq. (6), and performing the operations shown in Eq. (7), we obtain

$$a_2 = 2B_0 + 2B_1 + 2B_2 + 2B_3 \quad (13)$$

$$2B_0 = \frac{7\pi N b^3}{6M_2}$$

$$2B_1 = \frac{2\pi N e^4 \langle Z_2^2 \rangle_{Av}^2 a}{M_2 (DkT)^2}$$

$$2B_2 = -\frac{4\pi N e^4 \langle Z_2^2 \rangle_{Av}^2 a}{3M_2 (DkT)^2} (1 - \ln 2)$$

The first term, $2B_0$, reflects the contribution of the excluded volume effect to the second virial coefficient. The second term, $2B_1$, represents a positive contribution to the second virial coefficient due to the charge-charge interactions. It arises from the binomial expansion of the factor $(1 + \kappa a)^{-2}$ in the S^2 term of Eq. (6). The third term, $2B_2$, is the negative contribution caused by the attractive forces resulting from the higher fluctuating multipoles. The last term, $2B_3$, represents the effect of all other intermolecular forces (Van der Waals forces, fixed multipole moments, etc.).

Values of $2B_2$ have been calculated for BSA, BMA, human serum mercaptalbumin, and conalbumin from previously reported values of mean-square fluctuating charges (13, 14). These values of $2B_2$ are -8.88 , -9.04 , -12.56 , and -8.96 ml.-mole 2 -g. $^{-3}$, respectively. Although, at the present time, it is not possible to calculate theoretical values of a_2 , because of lack of knowledge of $2B_3$ and of the great uncertainty in the higher

terms of the Debye-Hückel equation [$2B_1$ in Eq. (13)], it seems of interest, nevertheless, to point out that interactions between fluctuating multipole moments can make a significant contribution to the excess chemical potential and may be partially responsible for the experimentally observed balancing out of the various short-range attractive and repulsive forces for the three serum proteins (12, 13) and for the negative value of the coefficient of C_2 for conalbumin (14).

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APPENDIX

The expression for $A^{\epsilon_{22}}$ [Eq. (8)] in the case of an isoionic protein of zero mean charge is a direct consequence of the fact that the charge on protein molecules at any given conditions is distributed about \bar{Z}_2 .

At the isoionic point of a protein, there are many possible ionic configurations of the molecules of essentially equal free energies, and protons can migrate from one group to another resulting in fluctuations in charge and charge configuration on the protein molecules (11). Thus, if a group of protein molecules having an average charge \bar{Z}_2 is considered at any instant, it will be found to be composed of a number of ionized species with charges distributed about \bar{Z}_2 (23). The average charge of such a system, composed of species with charges from $-S$ to $+S$, is

$$\bar{Z}_2 = \frac{\sum_{i=-S}^{+S} n_i Z_i}{\sum n_i} \quad (14)$$

where n_i is the number of molecules with the charge Z_i .

In the case of a neutral isoionic protein in salt-free solution, $\bar{Z}_2 = 0$, the various ionized species act as gegenions for each other and

$$\sum_{i=-S}^0 n_i Z_i = - \sum_{i=0}^{+S} n_i Z_i.$$

Within the Debye-Hückel approximation, the excess chemical potential, $\mu_i^{(e)}$, of ionic species i with charge Z_i is

$$\mu_i^{(e)} = -\frac{e^2 \kappa Z_i^2}{2DkT(1 + \kappa a)} \quad (15)$$

where e is the protonic charge, D is the dielectric

constant of the medium, k is Boltzmann's constant, T is the thermodynamic temperature, a is twice the radius of the protein molecule and κ is the Debye-Hückel parameter. The excess chemical potential $\mu_2^{(e)}$ of the protein is given by

$$\mu_2^{(e)} = \frac{\sum_{i=-S}^{+S} \mu_i^{(e)} n_i}{\sum n_i} = -\frac{e^2 \kappa}{2DkT(1 + \kappa a)} \quad (16)$$

$$\frac{\sum_{i=-S}^{+S} n_i Z_i^2}{\sum n_i} = -\frac{e^2 \kappa \langle Z_2^2 \rangle_{AV}}{2DkT(1 + \kappa a)}$$

Thus, the electrostatic portion of the activity coefficient of a neutral protein is given by the Debye-Hückel law with the mean square charge of the protein, $\langle Z_2^2 \rangle_{AV}$.

Since the mean square charge of a neutral protein is given by the slope of the titration curve at the isoionic point (24), the excess chemical potential of such a protein may be readily calculated from its acid-base equilibria.

In the case of a neutral protein in salt-free medium, κ is given by

$$\kappa^2 = \frac{4\pi e^2 N}{DkT} \frac{\sum_{i=-S}^{+S} C_i}{M_2} Z_i^2 = \frac{4\pi e^2 N \langle Z_2^2 \rangle_{AV} C_2}{DkTM_2} \quad (17)$$

where C_i is the concentration in grams/ml. of ionic species i , C_2 is the total protein concentration, M_2 is its molecular weight, and N is Avogadro's number.

Using Eqs. (16) and (17), the derivative of the excess chemical potential of the protein, $\mu_2^{(e)}$, with respect to protein concentration is:

$$\frac{1}{RT} \frac{\partial \mu_2^{(e)}}{\partial C_2} = \frac{-\pi^{1/2} e^3 \langle Z_2^2 \rangle_{AV}^{3/2} N^{1/2}}{2M_2^{1/2} (DkT)^{3/2} (1 + \kappa a)^2 C_2^{1/2}} \quad (18)$$

$$\mu_2 = RT \log C_2 + \mu_2^{(e)} + \mu_2^0(T, p)$$

Equation (18) is seen to be identical with Eq. (8) and with the first term of the results of Kirkwood and Shumaker in their examination of the potential of mean force between protein molecules due to fluctuations in charge and charge configuration (11). It should be noted that while the method of Kirkwood and Shumaker yields also higher fluctuating multipole interaction terms, the present heuristic derivation is limited strictly to the fluctuating charge interaction.

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