McDonough, R. N., and Huggins, W. H. (1968), *IEEE Trans. Auto. Control AC-13*, 408.
Myhill, J. (1965), *Biophys. J. 5*, 89.
Perl, W. (1960), *Int. J. Appl. Radiat. Isotopes 8*, 211.
Richards, E. G., Teller, D., Hoagland, V. D., Jr., Haschemeyer, R., and Schachman, H. K. (1971a), *Anal. Biochem. 41*, 215.
Richards, E. G., Teller, D., and Schachman, H. K. (1971b), *Anal. Biochem. 41*, 189.
Robertson, J. S. (1957), *Physiol. Rev. 37*, 133.
Schuyler, R., and Isenberg, I. (1971), *Rev. Sci. Instrum.* (in press).
Smith, R. E., and Morales, M. F. (1944), *Bull. Math. Biophys. 6*, 133.
Sober, H. A., Ed. (1970), Handbook of Biochemistry, Cleveland, Ohio, The Chemical Rubber Co.
Svedberg, T., and Pederson, K. O. (1940), The Ultracentrifuge, New York, N. Y., Oxford University Press, Johnson Reprint Corp.
Tao, T. (1969), *in* Molecular Luminescence, Lim, E. C., Ed., New York, N. Y., W. A. Benjamin, p 851.
Van Liew, H. D. (1967), *J. Theor. Biol. 16*, 43.
Wahl, P., and Lami, H. (1967), *Biochim. Biophys. Acta 133*, 233.
Worsley, B. H. (1964), *Commun. Ass. Comput. Mach. 7*, 39.
Worsley, B. H., and Lax, L. C. (1962), *Biochim. Biophys. Acta 59*, 1.
Yphantis, D. (1964), *Biochemistry 3*, 297.

Equilibrium Centrifugation of Nonideal Systems. The Donnan Effect in Self-Associating Systems*

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ABSTRACT: The sedimentation-equilibrium concentration distribution of charged macromolecules is coupled to the equilibrium distribution of other ions, causing the macromolecular concentration to be a more gradual function of radius than if the macromolecules possessed no charge. This Donnan effect decreases the apparent molecular weight averages from their ideal values. A rigorous thermodynamic treatment of this effect is presented for systems containing one or more macromolecular species, such as self-associating systems, and in which the supporting electrolyte may sediment. A general restriction of this theory is that the charge-to-mass ratio of all macromolecular species be the same. An extension of the thermodynamic component definitions of Scatchard leads to simple expressions for the reciprocal apparent weight-average molecular weight. Except when the supporting electrolyte of a highly nonideal solute sediments appreciably, this reciprocal molecular weight average is separable into the sum of an ideal and a nonideal contribution, the latter in the form of a nonideal virial expansion. The nonideal virial coefficients depend on the stoichiometry of the supporting electrolyte: for a uni-univalent salt, the third nonideal virial coefficient is zero; while for a uni-divalent salt, the fourth nonideal virial coefficient is zero. Expressions are given for estimating the limiting macromolecular concentration, below which a particular nonideal virial coefficient has a negligible influence on the experimentally measured molecular weight average.

The equilibrium distribution in a centrifugation field of an ideal monodisperse neutral species sedimenting in an incompressible solvent is a simple exponential function

$$c_i = A_i e^{\sigma_i \xi} \tag{1a}$$

where

$$\sigma_i = \frac{M_i(1 - \bar{v}_i\rho)\omega^2}{RT} \tag{1b}$$

and

$$\xi = r^2/2$$

Here, c_i is the concentration on a weight basis of species i; σ_i has been termed the "effective reduced molecular weight" (Yphantis and Waugh, 1956; Yphantis, 1964); M_i and $\bar{v}_i$ are the molecular weight and partial specific volume of the species, respectively; ρ is the solution density; ω the angular velocity; and r the distance from the center of rotation. This equation can be derived by various mechanistic or, more properly, thermodynamic approaches (see, for instance, Svedberg and Pedersen, 1940; Goldberg, 1953; Schachman, 1959; Williams *et al.*, 1958). The resultant distribution is a consequence of the opposing tendencies of diffusion and sedimentation: the centrifugal field induces solute transport in the radial direction, while diffusion tends to equalize any concentration inbalance (and thus leads to a transport in the centripetal direction). Equation 1 reflects the balance of these two flows and depends

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on the molecular weight of the species in such a way that, if $\bar{v}_i$ and ρ are known, the molecular weight can be determined from the equilibrium distribution.

If several species are present, each species will sediment separately and in accordance with eq 1, so long as no interaction occurs between them or with the solvent. However, if the species interact the distributions become "coupled," and may no longer be described by eq 1. One simple type of interaction would be the reversible association of uncharged and otherwise ideal species. If the time for establishment of association equilibrium is small compared to the time required for essential attainment of sedimentation equilibrium, if the "law of mass action" is obeyed, and if there is no volume change upon association, then the individual species involved in the association, including the polymers, will continue to obey eq 1.

Excluded volume effects and Donnan effects are interactions that lead to the breakdown of eq 1. The former effect is usually (but by no means always, Godfrey and Harrington, 1970; W. F. Stafford and D. A. Yphantis, unpublished data) negligible at the low macromolecular concentrations required in sedimentation equilibrium studies performed with the Rayleigh interference optical system of the analytical ultracentrifuge. However, most biological macromolecules studied are polyelectrolytes. There is, then, an interaction between them and other charged ions: the macromolecular ions cannot redistribute independently in accord with eq 1, but their distributions must be coupled with those of the other electrolytes in such a way that electroneutrality is everywhere maintained. This coupled redistribution is the Donnan effect and is analogous to the familiar situation in an osmotic pressure experiment where the equilibrium distribution of the membrane-diffuseable salt ions depends on the distribution of the charged macromolecular species across the semipermeable membrane.

The interdependence of the distributions of the low molecular weight salt ions and the charged macromolecular species causes, as one would expect, the concentration distribution of the macromolecular species to be a more slowly increasing function of radius and the concentration distribution of the salt ions to be a somewhat more rapidly increasing function than would be the case if they were uncharged and could distribute independently: a "compromise" is reached. If one did not account, then, for the Donnan effect, the molecular weights determined from such nonideal sedimentation equilibrium experiments would be erroneously small. As in other situations (*e.g.*, osmotic pressure experiments) where the Donnan effect occurs, the error introduced becomes larger as the ratio of the macromolecular concentration to the concentration of low molecular weight electrolyte is increased, or as the charge-to-mass ratio for the macromolecular species is increased. These properties usually have led investigators to attempt, where possible, to design their experiments in such a way that the macromolecules possessed a minimum of charge and so that the solution with which they were equilibrated contained sufficient "supporting electrolyte" to "swamp" any remaining Donnan effect. Frequently these criteria cannot be met (*e.g.*, these conditions for minimizing the Donnan effect may cause unwanted aggregation of the macromolecules), and nonideal effects then can significantly influence the distribution. In such instances investigators have usually removed the effect of the nonideality by extrapolating the molecular weight averages to zero macromolecular concentration. Such a procedure, of course, severely limits investigation of associating systems or even of systems containing several species.

It is the purpose of this paper to present a theory predicting (a) the resultant equilibrium concentration distributions, due to the Donnan effect, and (b) the most commonly determined local average of the molecular weight distribution, the apparent weight-average molecular weight. A fundamental restriction of the theory will be to a mixture of macromolecular species, all having the same charge-to-mass ratio. (Other restrictions will be detailed below.) The theory should allow estimation of the importance of the Donnan effect for various experimental conditions. Use will be made in a subsequent paper of the form of the equations developed here to suggest a new way of analyzing such nonideal experiments.[1] To this end, the approach here makes use of virial expansions for the contribution of the Donnan effect to the overall behavior of the macromolecular system.

Scatchard (1946) first suggested a certain "natural" definition of thermodynamic components for investigating the Donnan effect of a single macromolecular species and a salt in osmotic pressure experiments. He later applied his excellent treatment to sedimentation equilibrium (Johnson *et al.*, 1954), again restricting the theory to a single macromolecular species and a nonsedimenting uni-univalent salt. (The redistribution of the salt was assumed to be due strictly to the Donnan effect.) Extensions of these important treatments have been made (Johnson *et al.*, 1956, 1959) which accounted for sedimentation of the salt and derived expressions for the apparent weight-average and apparent Z-average molecular weights. These treatments apply to monodisperse systems.

Here we use the Scatchard treatment, extending it to a polydisperse system of macromolecules with each species having an identical charge-to-mass ratio, and including supporting electrolytes that may sediment and that may be assymmetric in valence type. Emphasis is placed on determination of virial expansions for the apparent weight-average molecular weight.

The mixture of macromolecules of primary interest here is a self-associating system. Under favorable conditions, the association may not substantially alter the effective charge-to-mass ratio, thus permitting application of this theory. The self-associating system may either be one in rapid chemical equilibrium, or it may be essentially incapable of reequilibration within the time course of the sedimentation equilibrium experiment.

Derivation of the Basic Equations Describing the Donnan Effect. We consider a system composed of one solvent component, one salt, and various macromolecular species, all having the same charge-to-mass ratio. In particular, we assume that the molecular weights of the species are integral multiples of the monomer molecular weight. Thus our analysis applies immediately to a self-associating system (provided the charge-to-mass ratio does not change upon association). However, the condition of rapid chemical equilibrium (*i.e.*, with rates of equilibration much faster than the rate of approach to sedimentation equilibrium) between the various species is not required: the analysis applies as well to permanently aggregated polymers of the monomer (*i.e.*, to chemical equilibration times much longer than those of the sedimentation equilibrium experiment).

In this section we derive the basic equations for the Donnan effect, relating the experimentally measured or "apparent" weight-average molecular weight to its ideal value. (The Donnan effect causes the experimentally measured value of the weight-average molecular weight to be less than its true value.) The only restrictions we make, at present, are that the partial specific volumes of all macromolecular species be the same,

[1] See also Roark and Yphantis (1969).

that the activity coefficients of all components be neglected (*i.e.*, the only nonideality we are considering is the Donnan effect), and that the solution be incompressible.

We first define the components of the system to be simple extensions of the component definitions of Scatchard, first presented in his classical study of the Donnan effect in osmotic pressure (Scatchard, 1946). Scatchard's definitions have the useful property that as the concentration of the macromolecular species goes to zero, the experimentally obtained value of an apparent molecular weight average approaches the molecular weight of the macromolecular component of his definition.

The definitions are as follows: component 1, solvent; component 2, macroion P_i, $[iZ/(\nu_+ + \nu_-)]$ ions $X^{-\nu_-}$, and $[-iZ/(\nu_+ + \nu_-)]$ ions B^{ν_+}. The monomer of component 2 has mass M_{21} and charge Z. The ith polymer has mass $M_{2i} = iM_{21}$ and charge iZ. Component 3: salt $B_{\nu_-}^{\nu_+} X_{\nu_+}^{-\nu_-}$, where ν_+ and ν_- are the absolute values of the valences of the positive ion B^{ν_+} and the negative ion X^{ν_-}, respectively.

We write the molal concentrations of the various species in terms of the component defined above as

$$m_{P_i} = m_{2i} \tag{2a}$$

$$m_B = \nu_- m_3 - \sum_i \frac{iZ}{\nu_+ + \nu_-} m_{2i} \tag{2b}$$

$$m_x = \nu_+ m_3 + \sum_i \frac{iZ}{\nu_+ + \nu_-} m_{2i} \tag{2c}$$

For convenience, we now define the following parameters of the salt stoichiometry as

$$\nu = \nu_+ + \nu_- \tag{3a}$$

$$\bar{\nu} = \nu_+ \nu_- \tag{3b}$$

$$\Delta\nu = \nu_- - \nu_+ \tag{3c}$$

The sums of eq 2 may be simplified as

$$\sum_i \frac{iZ}{\nu} m_{2i} = \frac{Z}{\nu M_{21}} c_2 \tag{4}$$

where c_2 is the total concentration of component 2 on the following weight basis:[2] grams of component 2 per kilogram of component 1. It is convenient to define here a parameter β to characterize the extent of the nonideality

$$\beta = \frac{Z}{\nu M_{21}} \frac{c_2}{m_3} \tag{5}$$

This quantity β is an extension of Scatchard's parameter η. Increasing values of β are associated with greater nonideality. With this definition and eq 4, we rewrite eq 2b,c as

$$m_B = \nu_- m_3 (1 - \beta/\nu_-) \tag{6a}$$

$$m_x = \nu_+ m_3 (1 + \beta/\nu_+) \tag{6b}$$

[2] Note that although useful, this is not a usual definition of c_2.

For each component $2i$, the chemical potential, μ_{2i}, is given by

$$\frac{\mu_{2i} - \mu_{2i}^0}{RT} = \ln m_{P_i} + \frac{iZ}{\nu} \ln m_x - \frac{iZ}{\nu} \ln m_B \tag{7}$$

The chemical potential of component 3, μ_3, is given by

$$\frac{\mu_3 - \mu_3}{RT} = \nu_- \ln m_B + \nu_+ \ln m_x \tag{8}$$

We define σ_{2i} and σ_3 to be

$$\sigma_{2i} = \frac{M_{2i}(1 - \bar{v}_2\rho)\omega^2}{RT} \tag{9a}$$

$$\sigma_3 = \frac{M_3(1 - \bar{v}_3\rho)\omega^2}{RT} \tag{9b}$$

where $\bar{v}_2$ and $\bar{v}_3$ are the partial specific volumes of the macromolecular species[3] and of the salt, respectively, ρ is the density of the solution, and ω is the angular velocity of the rotor in radians per second. Then the conditions for equilibrium in the centrifugal field are (Goldberg, 1953)

$$\sigma_{2i} = \frac{1}{RT} \frac{d(\mu_{2i} - \mu_{2i}^0)}{d\xi} \tag{10}$$

$$\sigma_3 = \frac{1}{RT} \frac{d(\mu_3 - \mu_3^0)}{d\xi} \tag{11}$$

where $\xi = r^2/2$ (r is the radius). The equilibrium condition of eq 10, in conjunction with the expression for the chemical potential as given in eq 7, leads to

$$\sigma_{2i} = \frac{d \ln m_{P_i}}{d\xi} + \frac{iZ}{\nu}\left[\frac{d \ln m_x}{d\xi} - \frac{d \ln m_B}{d\xi}\right] \tag{12}$$

In this expression we replace the concentrations of the various species by the concentrations of the previously defined components through eq 2a and 6a,b. After some simplification, this replacement leads to

$$\sigma_{2i} = \frac{d \ln m_{2i}}{d\xi} + \frac{(iZ/\bar{\nu})\beta}{(1 + \beta/\nu_+)(1 - \beta/\nu_-)} \frac{d \ln \beta}{d\xi} \tag{13}$$

The second term on the right-hand side of the above equation arises from the Donnan nonideality and vanishes for an ideal system.

Equation 13 indicates how any species $2i$ is distributed at equilibrium. If the various species form a self-associating system whose monomer molecular weight is M_{21}, we require that the concentrations of the various macromolecular species be

[3] Note that all macromolecular species, $2i$, have the same partial specific volume, given, according to the Scatchard type of component definition, by

$$\bar{v}_2 = \frac{M_{P_1X_{Z/\nu_-}} \bar{v}_{P_1X_{Z/\nu_-}} - (Z/\nu)M_3\bar{v}_3}{M_{P_1X_{Z/\nu_-}} - (Z/\nu)M_3}$$

where $M_{P_1X_{Z/\nu_-}}$ and $\bar{v}_{P_1X_{Z/\nu_-}}$ are the molecular weight and partial specific volume of the macromolecular monomer species as usually defined, *i.e.*, as the macroion P_1^Z plus Z/ν_- counterions X^{ν_-}.

consistent with mass action equilibria. That is, we require that all species $2i$ be related by the set of equations

$$m_{2i} = K_i m_{21}^{\ i} \tag{14}$$

where the K_i are molal association constants. If we integrate eq 13, we have

$$m_{2i} = A_{2i} e^{\sigma_{2i}\xi}\left[\frac{1 - \beta/\nu_-}{1 + \beta/\nu_+}\right]^{iZ/\nu} \tag{15}$$

where the A_{2i} are constants of integration. Combination of eq 14 with eq 15 shows that

$$K_i = \frac{A_{2i}}{A_{21}^{\ i}} \tag{16}$$

and that eq 13 is consistent with the mass action equilibria of eq 14.

To proceed further, we temporarily turn our attention to component 3. Combination of eq 8 and the equilibrium condition, eq 11, yields

$$\sigma_3 = \nu_+\frac{\mathrm{d}\ln m_X}{\mathrm{d}\xi} + \nu_-\frac{\mathrm{d}\ln m_B}{\mathrm{d}\xi} \tag{17}$$

If the molalities of the salt ion species are replaced by molalities in terms of our components, then using eq 6 and carrying out the implied differentiation, we rewrite eq 17 as

$$\frac{\beta^2/\bar{\nu}}{(1 + \beta/\nu_+)(1 - \beta/\nu_-)}\frac{\mathrm{d}\ln\beta}{\mathrm{d}\xi} = \frac{\mathrm{d}\ln m_3}{\mathrm{d}\xi} - \frac{\sigma_3}{\nu} \tag{18}$$

Equations 13 and 18 can be seen to be very similar. The former relates the variation of $\beta(r)$ to the variation of $m_{2i}(r)$; while the latter relates the variation of $\beta(r)$ to $m_3(r)$. From eq 5 which defines β, we obtain

$$\frac{\mathrm{d}\ln m_3}{\mathrm{d}\xi} = \frac{\mathrm{d}\ln c_2}{\mathrm{d}\xi} - \frac{\mathrm{d}\ln\beta}{\mathrm{d}\xi} \tag{19}$$

Finally, we combine eq 13, 18, and 19 to eliminate derivatives of β and m_3

$$\sigma_{2i} = \frac{\mathrm{d}\ln m_{2i}}{\mathrm{d}\xi} + i\left[\frac{Z\beta}{\bar{\nu} + \Delta\nu\beta}\right]\left[\frac{\mathrm{d}\ln c_2}{\mathrm{d}\xi} - \frac{\sigma_3}{\nu}\right] \tag{20}$$

Let us, for convenience, call the terms in the first set of brackets δ

$$\delta \equiv \frac{Z\beta}{\bar{\nu} + \Delta\nu\beta} \tag{21}$$

Now, the ideal weight-average σ is

$$\sigma_{w,I} = \frac{\sum_i c_{2i}\sigma_{2i}}{c_2} \tag{22a}$$

and the apparent weight-average σ is

$$\sigma_{w,a} = \frac{\mathrm{d}\ln c_2}{\mathrm{d}\xi} \tag{22b}$$

(If Rayleigh interference optics are used, this quantity is not quite identical with the experimentally measured quantity. However, in the next section, we show that, for most cases, the apparent weight-average σ defined here differs negligibly from the experimentally measured quantity.) For an incompressible solution, therefore

$$\frac{1}{c_2}\sum_i c_{2i}\frac{\mathrm{d}\ln m_{2i}}{\mathrm{d}\xi} = \frac{\mathrm{d}\ln c_2}{\mathrm{d}\xi} = \sigma_{w,a} \tag{23}$$

Thus, if eq 20 is multiplied by c_{2i}, summed over i, and then divided by c_2; and if eq 22 and 23 are used, we will have

$$\sigma_{w,I} = \sigma_{w,a}\left[1 + \frac{\delta}{c_2}\sum_i ic_{2i}\right] - \frac{\delta\sigma_3}{\nu c_2}\sum_i ic_{2i} \tag{24}$$

But

$$\frac{1}{c_2}\sum_i ic_{2i} = \frac{1}{M_{21}c_2}\sum_i c_{2i}M_{2i} = \frac{M_{w,I}}{M_{21}} \tag{25}$$

where $M_{w,I}$ is the ideal weight-average molecular weight. (The ideal weight-average molecular weight and the ideal weight-average σ, and, indeed, any molecular weight and its corresponding σ, are related by the well-known relation of eq 1.) Combining eq 24 and 25, and noting that since there is no volume change upon association $M_{w,I}/M_{21} = \sigma_{w,I}/\sigma_{21}$, we get

$$\frac{1}{M_{w,a}} = \frac{\dfrac{1}{M_{w,I}} + \dfrac{\delta}{M_{21}}}{1 + \dfrac{\delta\sigma_3}{\nu\sigma_{21}}} \tag{26}$$

If eq 21 and 9 are used for δ, σ_{21}, and σ_3, respectively, we have what may be regarded as the fundamental equation describing the effect of Donnan equilibria in the equilibrium ultracentrifugation of self-associating systems

$$\frac{1}{M_{w,a}} = \frac{\dfrac{1}{M_{w,I}} + 2B_1(c_2)c_2}{1 + \left[\dfrac{2B_1(c_2)}{\nu}\dfrac{M_3(1 - \bar{v}_3\rho)}{(1 - \bar{v}_2\rho)}\right]c_2} \tag{27}$$

where $B_1(c_2)$ is given by

$$B_1(c_2) = \frac{Z^2}{2\nu\bar{\nu}M_{21}^{\ 2}m_3(c_2,\xi) + (2M_{21}Z\Delta\nu)c_2} \tag{28}$$

and where ν, $\bar{\nu}$, and $\Delta\nu$ are given by eq 3.

Examination of eq 27 shows that, at the limit $c_2 = 0$, the apparent weight-average molecular weight of component 2 becomes equal to its ideal value. As mentioned above, this "intuitively satisfying" limiting relation results from the definition chosen for the components. In the case of the usual definition (component 2 being macroion P_i and iZ/ν_- ions $X^{\nu-}$, that is, the polyelectrolyte plus its counterions), the limiting value of the apparent weight-average molecular weight, as c_2 approaches 0, is less than the defined ideal value.

Negligible Sedimentation of Component 3. If $\sigma_3 \ll \sigma_{21}$, that is, if

$$M_3(1 - \bar{v}_3\rho) \ll M_{21}(1 - \bar{v}_2\rho) \tag{29}$$

Equation 27 becomes

$$\frac{1}{M_{w,a}} = \frac{1}{M_{w,I}} + 2B_1(c_2)c_2 \tag{30}$$

To find how $B_1(c_2)$ varies with c_2, we first examine the variation of $m_3(c_2)$ with c_2. Although in this section we assume that there is negligible sedimentation of the salt, we do not assume negligible redistribution of the salt. Such redistribution may arise in two ways: through direct sedimentation of the salt and through movement to satisfy the condition of local electroneutrality when the charged macromolecule sediments. It is only the first that we neglect here.

To find how $m_3(c_2)$ varies with c_2, we integrate eq 18.

$$\int d \ln m_3 = \frac{1}{\bar{\nu}}\int \frac{\beta^2 \, d \ln \beta}{(1 + \beta/\nu_+)(1 - \beta/\nu_-)} + \frac{\sigma_3}{\nu}\int d\xi \tag{31}$$

In the above equation for the variation of $m_3(c_2)$, the first term on the right-hand side is the contribution to the variation from salt movement associated with satisfying local electroneutrality, as the charged macromolecules redistribute. The second term on the right is the contribution from direct sedimentation of component 3 (*e.g.*, the second term on the right expresses the entire variation of m_3 in the "solvent" or "reference" side of the centrifuge cell, where no macromolecules are present). In view of our approximation of neglecting sedimentation of component 3, we shall neglect this second term on the right. Thus, eq 31 may be integrated to give

$$m_3(c_2) = \frac{m_{3,0}}{(1 + \beta/\nu_+)^{\nu_+/\nu}(1 - \beta/\nu_-)^{\nu_-/\nu}} \tag{32}$$

where $m_{3,0}$ is an integration constant. As c_2 approaches zero, β will approach 0 and m_3 will approach $m_{3,0}$. Thus, we may interpret $m_{3,0}$ as the value of m_3 in the region of negligible macromolecular concentration, if such a region exists. If the solution has been properly equilibrated by dialysis before performing the centrifugation, then the salt concentration outside the dialysis membrane will be very nearly $m_{3,0}$. (Because of the coupling between species 2 and species 3, the proper[4] equilibration of species 3 by dialysis requires a specific concentration of species 2 within the dialysis membrane. This specific optimal concentration depends on the detailed conditions of the sedimentation equilibrium experiment, including the rotational speed. However, in most cases, use of a nonoptimal solute concentration for equilibration by dialysis will lead to a negligible difference between $m_{3,0}$ and the concentration of m_3 in the reference compartment.)

Equation 32 may be expanded in a Taylor series about $\beta = 0$. The leading terms of the expansion are

$$\frac{1}{m_3(c_2)} = \frac{1}{m_{3,0}}\left\{1 - \frac{\beta^2}{2\bar{\nu}} + \ldots\right\} \tag{33}$$

If, in the above equation, eq 5 is used for β, we have

$$\frac{1}{m_3(c_2)} = \frac{1}{m_{3,0}}\left\{1 - \left(\frac{Z^2}{2\nu^2\bar{\nu}M_{21}{}^2 m_{3,0}{}^2}\right)c_2{}^2 + \ldots\right\} \tag{34}$$

[4] "Proper" in the sense that $m_{3,0}$ be exactly equal to the dialysate concentration.

Returning to eq 28, we first expand $B_1(c_2)$ in a Taylor series about $c_2/m_3(c_2) = 0$

$$B_1(c_2) = \frac{Z^2}{2\nu\bar{\nu}M_{21}{}^2 m_3(c_2)}\left[1 - \frac{Z\Delta\nu}{\nu\bar{\nu}M_{21}}\frac{c_2}{m_3(c_2)} + \frac{Z^2(\Delta\nu)^2}{\nu^2\bar{\nu}^2M_{21}{}^2}\frac{c_2{}^2}{m_3{}^2(c_2)} + \ldots\right] \tag{35}$$

Then, eq 34 is substituted into eq 35 everywhere $m_3(c_2)$ occurs. The resulting final expression for $B_1(c_2)$ may then be used in eq 30 to yield a virial, or series, expansion for the reciprocal weight-average molecular weight.

$$\frac{1}{M_{w,a}} = \frac{1}{M_{w,I}} + \left(\frac{Z^2}{\nu\bar{\nu}M_{21}{}^2 m_{3,0}}\right)c_2 - \left(\frac{Z^3\Delta\nu}{\nu^2\bar{\nu}^2M_{21}{}^2 m_{3,0}{}^2}\right)c_2{}^2 - \left(\frac{Z^4}{2\nu^3\bar{\nu}^2M_{21}{}^4 m_{3,0}{}^3}\right)\left[1 - \frac{2(\Delta\nu)^2}{\bar{\nu}}\right]c_2{}^3 + \ldots \tag{36}$$

One of the most obvious, and most important, characteristics of the above equation is that the reciprocal apparent weight-average molecular weight is separable into two additive quantities: the first being the ideal contribution ($1/M_{w,I}$), and the second being a virial series of terms representing the nonideality. This separation has useful consequences that will be explored in a future paper.

Equation 36 may be conveniently rewritten using the nonideal colligative viral coefficients defined by the relation

$$\frac{1}{M_{w,a}} = \frac{1}{M_{w,I}} + 2B_1c_2 + 3C_1c_2{}^2 + 4D_1c_2{}^3 + \ldots \tag{37}$$

Comparison of eq 36 and 37 identifies

$$B_1 = \frac{Z^2}{2\nu\bar{\nu}M_{21}{}^2 m_{3,0}} \tag{38a}$$

$$C_1 = \frac{-Z^3\Delta\nu}{3\nu^2\bar{\nu}^2M_{21}{}^3 m_{3,0}{}^2} \tag{38b}$$

$$D_1 = \left(\frac{Z^4}{8\nu^3\bar{\nu}^2M_{21}{}^4 m_{3,0}{}^3}\right)\left[1 - \frac{2(\Delta\nu)^2}{\bar{\nu}}\right] \tag{38c}$$

where B_1, C_1, and D_1 are the nonideal second, third, and fourth colligative virial coefficients, respectively. The virial expansion for the reciprocal apparent number-average molecular weight can be generated from eq 37 using a relationship between the weight-average and number-average molecular weights (this relation may be easily derived by expressing the molecular weight averages as ratios of radial derivatives of the concentration, as in Yphantis, 1964).

$$\frac{1}{M_w(c)} = \frac{d\left(\frac{c}{M_n(c)}\right)}{dc} \tag{39}$$

From this equation, and eq 37, we have

$$\frac{1}{M_{n,a}} = \frac{1}{M_{n,I}} + B_1c_2 + C_1c_2{}^2 + D_1c_2{}^3 + \ldots \tag{40}$$

We look next at the forms of eq 38 for particular types of

TABLE I: Significant Nonideal Virial Coefficients for a Molecule with $M = 2 \times 10^4$ and $Z = +10$ When $m_{3,0} = 10^{-2}$ m.

c_2 (g/l.)	Important Virial Coef
Uni-univalent Salt (BX)	
>5.06	2nd, 4th
$0.04 < c_2 < 5.06$	2nd
0.04	Ideal
Uni-divalent Salt (B_2X or BX_2)	
<3.79	2nd, 3rd
$0.12 < c_2 < 3.79$	2nd
<0.12	Ideal

salts. For salts. For a uni-univalent salt (B^+X^-), eq 38a–c are particularly simple

$$B_1 = \frac{Z^2}{4M_{21}^2 m_{3,0}} \tag{41a}$$

$$C_1 = 0 \tag{41b}$$

$$D_1 = -\frac{Z^4}{64M_{21}^4 m_{3,0}^3} = \frac{-B_1^2}{4m_{3,0}} \tag{41c}$$

We see that the third virial coefficient vanishes. (Whenever $\nu_+ = \nu_-$, the third virial coefficient will be 0.) The question arises at what concentration, c_2, does the fourth virial coefficient become important? If our criterion for neglecting the fourth virial coefficient is that it should influence $1/M_{w,a}$ to less than 1% of $1/M_{21}$, then the condition for neglecting D will be

$$c_2 < M_{21} m_{3,0}/1.84Z^{4/3} \tag{42}$$

where c_2 is in grams per liter. If this condition is met when component 3 is a uni-univalent salt, then it is reasonable to assume that the nonideality is limited to the second virial coefficient. We may pose the similar condition for neglecting the second virial coefficient; that is, that the contribution of the second virial coefficient to $1/M_{w,a}$ be less than 1% of $1/M_{21}$. This will be true if

$$c_2 < M_{21} m_{3,0}/50Z^2 \tag{43}$$

For a second example of a salt, we consider an asymmetric salt ($\Delta\nu \neq 0$). Here, the third virial coefficient will no longer be zero. For a monodivalent salt (B_2X or BX_2), eq 38 becomes

$$B_1 = \frac{Z^2}{12M_{21}^2 m_{3,0}} \tag{44a}$$

$$C_1 = \frac{\pm Z^3}{108M_{21}^3 m_{3,0}^2} \text{ (+ for } BX_2 \text{ and − for } B_2X) \tag{44b}$$

$$D_1 = 0 \tag{44c}$$

Note that the third virial coefficient will be positive if the charge on the macromolecule is of the same sign as that of the univalent ion of the salt; otherwise, the third virial coefficient will be negative. Unlike the case for symmetric salts (where $\Delta\nu = 0$), here the fourth virial coefficient is 0.

Again we consider conditions under which we may neglect various virial coefficients and use the same criterion as before: the virial coefficient may be neglected if it influences $1/M_{w,a}$ by less than 1% of $1/M_{21}$. The condition that will allow us to neglect the third virial coefficient, and consider only the second coefficient, is

$$c_2 < M_{21} m_{3,0}/1.67Z^{3/2} \tag{45}$$

If the concentration is further reduced, the system, for all practical purposes, may be considered ideal. This condition (that the second virial coefficient term be sufficiently small) is

$$c_2 < M_{21} m_{3,0}/16.7Z^2 \tag{46}$$

The results for these two types of salts can be summarized using as an example a protein of mol wt 20,000 with an effective charge of +10. We assume that $m_{3,0} = 0.01$ m. Table I presents a list, in decreasing order of macromolecular concentration, of the concentrations at which we may neglect the various terms of the virial expansion. Results for other salt stoichiometries may be obtained from eq 38.

In this analysis, we have assumed the salts to be fully ionized. If this is not the case, only the ionized fraction of the salt is reflected in $m_{3,0}$. Also, the charge on the macromolecule must be considered to be an "effective charge." In general, this will be less than the charge indicated by titration data, due to partial binding of ions to the macromolecule (Scatchard *et al.*, 1946; Tanford, 1961).

It is to be noted that a salt of the form B_2X or BX_2 is more effective in masking nonidealities than a monovalent salt of the form BX. A simple intuitive reason can be given for this effect. For a salt containing a greater charge per ion, fewer ions of the salt need redistribute to maintain local electroneutrality upon redistribution of the macromolecules. (The extent of the nonideality due to Donnan effect is dependent on the number of salt ions that need redistribute.) This analysis does not specifically apply to component 3 being a mixture of salts. An example of such a mixture would be a pH buffer containing both HPO_4^{2-} and PO_4^{3-}. By the above argument, the more highly charged PO_4^{3-} ions should more effectively mask the Donnan nonideality than do the HPO_4^{2-} ions. Thus, if we are interested in forming a conservative (minimal) estimate of the concentration above which there would be significant nonideality, we could assume that all the salt is present in the form HPO_4^{2-}.

We remarked earlier that what we called the apparent weight-average σ, $\sigma_{w,a}$, defined by eq 22, differs from the actual experimentally obtainable quantity, which we shall denote by $\sigma_{w,a}^*$. The concentration is determined using measurements of fringe displacement when the Rayleigh interference optics are used. If component 2 is a charged macromolecule, the Donnan effect will lead to a difference in the salt gradient. Thus, the values of $\sigma_{w,a}$ are directly obtainable by experiment only if component 3 contributes negligibly to the fringe displacement. Here we examine the quantity $\sigma_{w,a}^*$ and compare its value to $\sigma_{w,a}$. The concentration as measured by fringe displacement will be given by

$$c_f = \frac{\Delta n_3 M_3 (m_3 - m_{3,0}) + \Delta n_2 c_2}{\Delta n_2} \tag{47}$$

where Δn_3 and Δn_2 are the refractive index increments, dn/dc, of components 3 and 2 on a weight–concentration basis (both are assumed to be constant), and $m_{3,0}$ is the (uniform) salt concentration in the reference side. The concentration has been normalized so that it becomes identical with c_2 whenever the concentration of component 3 in the reference sector is identical with that in the solution sector. The experimentally obtainable weight-average σ is then

$$\sigma_{w,a}^* = \frac{1}{c_f}\frac{dc_f}{d\xi} \tag{48}$$

If this is combined with eq 47, and if eq 22b is used for $\sigma_{w,a}$, one can show that

$$\frac{\sigma_{w,a}^*}{\sigma_{w,a}} = \frac{1 + \left(\frac{\Delta n_3 M_3}{\Delta n_2}\right)\frac{dm_3}{dc_2}}{1 + \left(\frac{\Delta n_3 M_3}{\Delta n_2}\right)\frac{m_3 - m_{3,0}}{c_2}} \tag{49}$$

From eq 34, we have that

$$m_3 - m_{3,0} = \left(\frac{Z^2}{2\nu^2\bar{\nu}M_{21}^2 m_{3,0}}\right)c_2^2 + \ldots \tag{50a}$$

and that

$$\frac{dm_3}{dc_2} = \left(\frac{Z^2}{\nu^2\bar{\nu}M_{21}^2 m_{3,0}}\right)c_2 + \ldots \tag{50b}$$

If eq 50 are substituted into eq 49, and the result is expanded in a Taylor series about $c_2 = 0$, the leading terms of the expansion are

$$\frac{\sigma_{w,a}^*}{\sigma_{w,a}} = 1 + (\Delta n_3/\Delta n_2)(M_3/\nu)B_1 c_2 + \ldots \tag{51}$$

Examination of eq 51 reveals that, for small differences, the fractional difference between $\sigma_{w,a}$ and the actual experimentally obtainable quantity, $\sigma_{w,a}^*$ is linearly proportional to the ratio between the refractive index increment of the salt and that of the macromolecule (typically, for proteins in dilute salt solutions this ratio ranges from 0.5 to 0.9), to the second virial coefficient, to the average mass/charge of the ions of the salt (M_3/ν), and to the concentration of component 2. The limiting value of c_2, below which $\sigma_{w,a}^*$ and $\sigma_{w,a}^*$ will have a fractional difference of less than 1% is

$$c_2 < \frac{\nu^2\bar{\nu}\Delta n_2 m_{3,0}M_{21}^2}{50\Delta n_3 M_3 Z^2} \tag{52}$$

As an example of the magnitude of this effect, let us consider a system in which component 3 is a 0.01 m solution of NaCl, and component 2 is a protein of 20,000 molecular weight with a charge of +10. Using eq 52, we find that there would be negligible differences between $\sigma_{w,a}$ and $\sigma_{w,a}^*$ up to protein concentrations of about 80 g/l. Thus, under most experimental conditions, $\sigma_{w,a}$ may be considered to be negligibly different from the experimentally obtainable quantity. However, under the conditions of very high nonideality and unusually large values of ($\Delta n_3 M_3$), the difference between $\sigma_{w,a}$ and $\sigma_{w,a}^*$ could become significant. It seems best to attempt experiments where the condition of eq 52 is met. If this is impossible, however, the scanning photoelectric system can often be used to obtain $\sigma_{w,a}$. However, for ordinary salts, such as NaCl, the difference between $\sigma_{w,a}$ and $\sigma_{w,a}^*$ does not become significant until the nonideality is so extreme as to have significant contributions from virial coefficients higher than the fourth.

Sedimentation of Component 3. Let us turn now to the case where the condition of eq 29 breaks down. This section deals with cases where sedimentation of salt cannot be neglected, and considers the presence of several macromolecular species (such as the several species making up a self-associating system), all having the same charge-to-mass ratio. These macromolecular species may or may not be in chemical equilibrium with each other.

The basic equation now becomes

$$\frac{1}{M_{w,a}} = \frac{\frac{1}{M_{w,I}} + 2B_1(c_2)c_2}{1 + 2B_1(c_2)M_{21}\alpha c_2} \tag{53}$$

where $B_1(c_2)$ is given by eq 28, and where

$$\alpha = \frac{M_3}{\nu M_{21}}\frac{(1 - \bar{\nu}_3\rho)}{(1 - \bar{\nu}_2\rho)} \tag{54}$$

α represents the extent to which sedimentation of the salt is important. (When no sedimentation of salt occurs, the value of α is 0.)

Consider the variable $\phi \equiv c_2 B_1(c_2)$ and expand eq 53 about $\phi = 0$.

$$\frac{1}{M_{w,a}} = \frac{1}{M_{w,I}} + \left(1 - \frac{M_{21}\alpha}{M_{w,I}}\right)\Big[2B_1(c_2)c_2 - 4B_1^2(c_2)M_{21}\alpha c_2^2 + 8B_1^3(c_2)M_{21}^3\alpha^2 c_2^3 + \ldots\Big] \tag{55}$$

This expansion for $1/M_{w,a}$ is distinctly more complicated than all so far examined. Here, the ideal contribution to $1/M_{w,a}$ and the nonideal contribution are not separable into two additive terms, but a factor, $(1 - M_{21}\alpha/M_{w,I})$, which is a function of c_2, multiplies the nonideal virial expansion.

Series expansions for the apparent weight-average molecular weight in a system in which the salt sediments become significantly simpler if only one macromolecular species is present: $M_{w,I} = M_{21}$. For this case, eq 55 becomes

$$\frac{1}{M_{w,a}} = \frac{1}{M_{21}} + 2B_1(c_2)(1 - \alpha)c_2 - 4B_1^2(c_2)M_{21}\alpha(1 - \alpha)c_2^2 + 8B_1^3(c_2)M_{21}^2\alpha^2(1 - \alpha)c_2^3 + \ldots \tag{56}$$

Here, the ideal and nonideal contributions to $1/M_{w,a}$ are separable into two additive terms. At this point it is useful to expand $B_1(c_2)$ as a power series in c_2. As before, we first expand $1/m_3(c_2)$ using eq 32, but now retain the last term which has its origin in the sedimentation of the salt. Thus

$$m_3(c_2) = \frac{m_{3,0}\exp\{\sigma_3(\xi - \bar{\xi}/\nu\}}{(1 + \beta/\nu_+)^{\nu_+/\nu}(1 - \beta/\nu_-)^{\nu_-/\nu}} \tag{57}$$

where

$$\bar{\xi} = (r_m^2 + r_b^2)/4 \quad (58)$$

and where r_m and r_b are the radii of the meniscus and base, respectively. If the sedimentation of the salt is not too extreme, one can assign a useful physical meaning to $m_{3,0}$. Van Holde and Baldwin (1958) showed that if the parameter $H = \sigma_i(r_b^2 - r_m^2)/4$ is sufficiently small (*e.g.*, less than 0.25) then the concentration of species i at the point $\bar{\xi}$ is negligibly different from the initial loading concentration. (For a salt the effective σ_i will be σ_3/ν.) Thus, at the point ξ, in the absence of any macromolecule, $m_3(c_2)$ will have a value very nearly equal to the initial salt concentration. Therefore, $m_{3,0}$ will be approximately the final salt concentration against which the solution was dialyzed prior to equilibrium centrifugation.

Equation 57 may now be expanded to yield an equation analogous to eq 34

$$\frac{1}{m_3(c_2)} = \frac{\exp\{-\sigma_3(\xi - \bar{\xi})/\nu\}}{m_{3,0}}\left\{1 - \left(\frac{Z^2}{2\nu^2\bar{\nu}M_{21}^2m_{3,0}^2}\right)\exp\{-2\sigma_3(\xi - \bar{\xi})/\nu\}c_2^2 + \ldots\right\} \quad (59)$$

Substitution of this result into 35 gives the expansion of $B_1(c_2)$ in terms of c_2.

Finally, this expansion is substituted into eq 56, everywhere $B_1(c_2)$ occurs, to yield the desired expansion

$$\frac{1}{M_{w,a}} = \frac{1}{M_{21}} + \left[\frac{Z^2(1 - \alpha)}{\nu\bar{\nu}M_{21}^2m_{3,0}}\right]c_2' - \left\{\frac{Z^3(1 - \alpha)[\Delta\nu + Z\alpha]}{\nu^2\bar{\nu}^2M_{21}^3m_{3,0}^2}\right\}c_2'^2 - \left\{\frac{Z^4(1 - \alpha)\left\{1 - \frac{2}{\bar{\nu}}[\Delta\nu + Z\alpha]^2\right\}}{2\nu^3\bar{\nu}^2M_{21}^4m_{3,0}^3}\right\}c_2'^3 + \ldots \quad (60)$$

where

$$c_2' \equiv c_2 \exp\{-\sigma_3(\xi - \bar{\xi})/\nu\} \quad (61)$$

Notice that this modified virial expansion is no longer in terms of the variable c_2 but rather c_2': this new variable is a "transformed concentration" that reduces to the true macromolecular concentration in the limiting case when the salt does not sediment. No reasonably convergent series expansion about $c_2 = 0$ is possible when sedimentation of component 3 must be taken into account. However, the use of c_2' poses no serious problems, since the conversion of the experimental data from c_2 to c_2' can be easily performed through eq 61.[5]

Returning to the more general case when more than one macromolecular species is present, it is useful to consider when the expansion of eq 55 can be approximated by a virial expansion in which the ideal and nonideal terms occur separately and are additive. The expansion used is identical with that of eq 56, except that M_{21} has been replaced by $M_{w,I}$ in the leading term.

$$\frac{1}{M_{w,a}} \cong \frac{1}{M_{w,I}} + 2B_1(c_2)(1 - \alpha)c_2 - 4B_1^2(c_2)M_{21}\alpha(1 - \alpha)c_2^2 + 8B_1^3(c_2)M_{21}^2\alpha^2(1 - \alpha)c_2^3 + \ldots \quad (62)$$

To calculate the error entailed in using this expansion, we subtract this expansion, eq 62, from eq 53. If we employ our usual criterion for the validity of these approximations (that they shall introduce an error in $1/M_{w,a}$ no larger than 1% of $1/M_{21}$), then eq 62 will be useful provided that

$$c_2 \leqslant \left(\frac{\nu^2\bar{\nu}m_{3,0}M_{w,I}M_{21}(1 - \bar{v}_2\rho)}{100Z^2M_3(1 - \bar{v}_3\rho)}\right)\frac{M_{21}}{M_{w,I} - M_{21}} \quad (63)$$

Equation 63 is a reasonable condition that the virial expansion for $1/M_{w,a}$ be separable into an ideal and a nonideal contribution which are additive.

In the limit that the salt does not sediment, α vanishes and the virial coefficients of eq 60 become identical with those of eq 36, as, of course, they must. The question arises as to when it is satisfactory to neglect the sedimentation of the salt and to use the simpler eq 36, instead of the expansions given in this section. Subtracting eq 53 from eq 30, and requiring that this error be less than 1% of $1/M_{21}$, we have as the general condition for neglecting the sedimentation of the salt

$$c_2 \leqslant \frac{\nu^2\bar{\nu}m_{3,0}M_{w,I}M_{21}(1 - \bar{v}_2\rho)}{100Z^2M_3(1 - \bar{v}_3\rho)} \quad (64)$$

Notice that the limiting concentrations c_2, given by eq 63 and 64 depend not only on the ratio of σ_2 to σ_3, as we would expect, but also upon such factors as determine the extent of Donnan nonideality: the charge on the macromolecule and the salt molality. Indeed, for a given macromolecular concentration, the greater the nonideality from Donnan equilibrium, the greater will be the effect of salt sedimentation on the apparent weight-average molecular weight.

Except under extremely nonideal conditions, the expansion of eq 36, which neglects direct sedimentation of the salt, is valid. As an example of the limiting macromolecular concentration, below which sedimentation of the salt may be neglected, consider the nonideal system consisting of a protein with monomer mol wt 20,000 and charge +10 in a 0.01 *m*

[5] This same development can be applied to systems with more than one solute species and results in an equation analogous to eq 60 but with the term $1/M_{21}$ replaced by $1/M_{w,I}$ and all the factors $1 - \alpha$ replaced by $1 - M_{21}\alpha/M_{w,I}$. This gives then an implicit relation for $M_{w,I}$ in terms of $M_{w,a}$ and the other variables that can then be rearranged to yield $M_{w,I}$ explicitly but only as a rather "messy expression."

A simpler and more useful expression for $M_{w,I}$ can be derived, however. Rearrange eq 53 to give

$$\frac{1}{M_{w,I}} = \frac{1}{M_{w,a}} - \left(1 - \frac{M_{21}\alpha}{M_{w,a}}\right)B_1(c_2)c_2$$

Then insert the expression derived for $B_1(c_2)$ and the definition of α and obtain an explicit form for $M_{w,I}$ as

$$\frac{1}{M_{w,I}} = \frac{1}{M_{w,a}} - \left(1 - \frac{M_3(1 - \bar{v}_3\rho)}{\nu M_{w,a}(1 - \bar{v}_2\rho)}\right)\left(\frac{Z^2c_2'}{\nu\bar{\nu}M_{21}^2m_{3,0}} - \frac{Z^3\Delta\nu(c_2')^2}{\nu^2\bar{\nu}^2M_{21}^3m_{3,0}^2} - \frac{Z^4\left(1 - \frac{2(\Delta\nu)^2}{\bar{\nu}}\right)(c_2')^3}{\nu^3\bar{\nu}^2M_{21}^4m_{3,0}^3} + \ldots\right)$$

where c_2' has been defined in eq 61. This expression enables direct estimates of $M_{w,I}$ from the observed $M_{w,a}$ for systems where the (uniform) charge density, Z/M_{21}, is known.

Na^+Cl^- aqueous solvent. If we assume no self-association, evaluation of eq 64 indicates a limit for c_2 of about 16 g/l. (If the protein self-associates ($M_{w,I} > M_{21}$), the limiting concentration as calculated by eq 64 would be even larger.) For systems having a lower monomer molecular weight, or a salt that sediments more, this limit for c_2 would be lower. The use of salts that have small values of $(1 - \bar{v}_3\rho)$, such as triethylammonium chloride (Yphantis, 1964) is useful for highly nonideal solutions.

Appendix

Concentration Distributions for Nonideal Systems. Here, we predict the equilibrium concentration distributions of the components 2 (the macromolecular species) and component 3 (the "supporting electrolyte") of a model self-associating system exhibiting nonideality from the Donnan effect. Again we assume that all macromolecular species have the same charge-to-mass ratio. The technique of predicting the equilibrium concentration distribution involves writing down an equation for $\beta(\xi)$ which may be solved by iteration at a series of positions $\{\xi_i\}$. The parameter, β, defined by eq 5, is a measure of the system's nonideality. The distribution of each species $2i$ is given in terms of β by eq 15. If this set of equations is multiplied by iM_{21}, to generate the concentrations on a weight basis, we have

$$c_{2i}(\xi) = iM_{21}A_{2i}e^{i\sigma_{21}\xi}\left[\frac{1 - \beta/\nu_-}{1 + \beta/\nu_+}\right]^{iZ/\nu} \quad \text{(A-1)}$$

Similarly, from eq 57 we have the distribution of component 3 in terms of β

$$m_3(\xi) = \frac{A_3e^{(\sigma_3/\nu)\xi}}{(1 + \beta/\nu_+)^{\nu_+/\nu}(1 - \beta/\nu_-)^{\nu_-/\nu}} \quad \text{(A-2)}$$

The total concentration of species $2i$ may be generated from eq A-1 by

$$c_2(\xi) = \sum_i c_{2i}(\xi) \quad \text{(A-3)}$$

Then, combining eq 5 and A-1, -2, and -3, we have an expression for β

$$\beta(\xi) = \left(\frac{Ze^{-(\sigma_3/\nu)\xi}}{\nu A_3}\right)\sum_i iA_{21}e^{i\sigma_{21}\xi}\left[\frac{(1 - \beta/\nu_-)^{(iZ+\nu_-)/\nu}}{(1 + \beta/\nu_+)^{(iZ-\nu_+)/\nu}}\right] \quad \text{(A-4)}$$

If component 3 is a uni-univalent salt, eq A-4 can be well approximated, for moderate monideality

$$\beta(\xi) = \left(\frac{Ze^{-(\sigma_3/2)\xi}}{2A_3}\right)\sum_i \{iA_{2i}e^{i(\sigma_{21}\xi - Z\beta)}\} \quad \text{(A-5)}$$

At a series of positions in the cell $\{\xi_j\}$ either eq A-4 or -5 may be solved for $\beta(\xi)$ by iteration. Then, the concentrations $c_{2i}(\xi)$ may be found at the series of positions $\{\xi_j\}$ by using eq A-1. Finally, the total concentration, $c_2(\xi)$, is determined for the series $\{\xi_j\}$ by eq A-3. One may also determine the concentration distribution for component 3 at the series $\{\xi_j\}$ by using eq A-2.

If component 3 is a uni-univalent salt, and if the nonideality is only moderate, eq A-1 and -2 may be well approximated by

$$c_{2i}(\xi) = iM_{21}A_{2i}e^{i(\sigma_{21}\xi - Z\beta)} \quad \text{(A-6)}$$

$$m_3(\xi) = A_3e^{[(\sigma_3/2)\xi + \beta^2/2]} \quad \text{(A-7)}$$

(Equation A-7 is a useful approximation even for relatively severe nonidealities.) On examining eq A-6 and -7, one notices that the effect of the nonideality is to cause $c_{2i}(\xi)$ to be a more slowly increasing function of ξ and $m_3(\xi)$ to be a slightly more rapidly increasing function of ξ than would be the case if the system were ideal.

Once the values of $c_{2i}(\xi)$ have been determined for the set $\{\xi_j\}$, by iteration of eq A-4 or -5 for $\beta(\xi)$, the values of $M_{w,I}(\xi)$, the ideal weight-average molecular weight, may be calculated from its defining relation, and then values of $M_{w,a}(\xi)$, the apparent weight-average molecular weight, may be calculated from eq 26.

References

Godfrey, J. F., and Harrington, W. F. (1970), *Biochemistry 9*, 886, 894.

Goldberg, R. J. (1953), *J. Phys. Chem. 57*, 194.

Johnson, J. S., Kraus, K. A., and Holmberg, R. W. (1956), *J. Amer. Chem. Soc. 78*, 26.

Johnson, J. S., Kraus, K. A., and Scatchard, G. (1954), *J. Phys. Chem. 58*, 1034.

Johnson, J. S., Scatchard, G., and Kraus, K. A. (1959), *J. Phys. Chem. 63*, 787.

Roark, D. E., and Yphantis, D. A. (1969), *Ann. N. Y. Acad. Sci. 164*, 245.

Scatchard, G. (1946), *J. Amer. Chem. Soc. 68*, 2315.

Scatchard, G., Batchelder, A. C., and Brown, A. (1946), *J. Amer. Chem. Soc. 68*, 2320.

Schachman, H. K. (1959), Ultracentrifugation in Biochemistry, New York, N. Y., Academic Press.

Svedberg, T., and Pedersen, K. O. (1940), The Ultracentrifuge, London, Oxford University Press.

Tanford, C. (1961), Physical Chemistry of Macromolecules, New York, N. Y., John Wiley.

Van Holde, K. E., and Baldwin, R. L. (1958), *J. Phys. Chem. 62*, 734.

Williams, J. W., Van Holde, K. E., Baldwin, R. L., and Fujita, H. (1958), *Chem. Rev. 58*, 715.

Yphantis, D. A. (1964), *Biochemistry 3*, 297.

Yphantis, D. A., and Waugh, D. (1956), *J. Phys. Chem. 60*, 623.