Sedimentation Equilibrium in Reacting Systems. II. Extensions of the Theory to Several Types of Association Phenomena[1,2]

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Prior theoretical treatments for the study of protein and detergent associations in systems at equilibrium, such as osmotic pressure, light scattering, and sedimentation equilibrium, and in systems during transport, to include chromatography, electrophoresis, and velocity sedimentation, are to be found in the literature. For the most part it is idealized behaviors which are described. Here are considered extensions of the sedimentation equilibrium approach, to both a description of the centrifugal behavior in the presence of such reactions, and an indication as to how the effects of solution nonideality may be included in the essential working equations without limiting the discussion to the simplest monomer–dimer case.

I. Introduction

The measurement and interpretation of the amount of scattering at the several angles as a beam of light passes through a solution of macromolecules may provide useful information about the size, shape, and configuration of the solutes. Studies of this kind, made possible largely by the pioneering investigations of Debye,[4] have been used by him and later by others with certain detergent solutions in order to determine the size of the micelles, or more precisely, the number of primary units of which they are composed. In addition Debye has provided a theory descriptive of the formation of the micelle, with its structure representing an equilibrium between the repulsive long-range Coulomb forces due to the charges concentrated in the "heads" of the detergent molecules and the short-range attractive van der Waals forces which are operative when the hydrocarbon portions of the molecules are forced from the surrounding water into the core of the structure.[5]

According to this exposition one would expect a substantially homogeneous population of micelles. Here sedimentation equilibrium experiments can give valuable information, including an estimate of the number of monomers associating to form the given micelle, and an indication at least of any appreciable polydispersity. Descriptions of experiments of this kind are now just beginning to make their appearance[6] and methods of interpretation of the data are being developed.

Association phenomena are also quite common in protein systems; representative references are here given.[7–16] In certain cases they appear to be quite involved, for they are believed to undergo monomer–dimer–n-mer associations instead of the formally simpler monomer–n-mer reactions. In others, the association number may be two, three, four, eight, etc., to correspond to a number of basic building blocks to form the particular protein. Presumably such numbers may be derived either from X-ray analysis of crystals or by such experiments as osmotic pressure, light scattering, and sedimentation, either equilibrium or transport, with solutions.

In a previous report from this laboratory,[7] there was presented a mathematical analysis to describe the combined sedimentation and chemical equilibrium in an associating solute system of the monomer–dimer type. Here, the attempt is to be made to extend the treatment to systems in which the solute is made up of monomer–n-mer, or monomer–dimer–n-mer in chemical equilibria with each other. As examples of these types of interaction detailed consideration will be given to monomer–dimer and monomer–n-mer equilibria, including micelle formation. Some additional remarks will be directed to those cases which contain several complexes in equilibrium with monomer. A short preliminary account of the work has already appeared.[2] In it, the final sentence should read: For monomer–dimer–trimer equilibria it appears one can only evaluate $K_2 M_1 - B_1 M_1{}^2$ and $2K_3 - 3K_2{}^2$.

II. Basic Equations

In the simplest of the associating systems, a monomer and its dimer form the solute species in a single solvent component. Such species are designated by the subscripts 1 and 2, or in general i. Although we propose detailed considerations of the monomer–dimer and the monomer–n-mer cases, a more complicated association, that of monomer–dimer–trimer, will be used as representative system in order to set down a few basic equations. It is represented as

$$n\mathrm{P}_1 \rightleftharpoons m\mathrm{P}_2 + q\mathrm{P}_3 \tag{1}$$
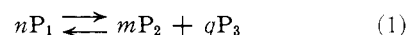
where P_1 represents the macromolecular species undergoing the indicated reaction. In sedimentation equilibrium experiments with such a solute system, especially with protein associations, two assumptions are usually made: one is that the partial specific volumes of the associating species are equal, and the other

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(2) For more detailed discussions of certain of these problems reference is made to the Ph.D. dissertation of E. T. A., Jr., University of Wisconsin, 1962. *Cf.* also, E. T. Adams, Jr., *Federation Proc.*, **23**, 215 (1964).

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requires that the refractive index increments are equal. The equilibrium constants K_2 and K_3 are defined by the equations

$$K_2 = \left(\frac{c_2}{c_1{}^2}\right)\left(\frac{y_2}{y_1{}^2}\right) = \frac{a_2}{a_1{}^2}; \quad K_3 = \left(\frac{c_3}{c_1{}^3}\right)\left(\frac{y_3}{y_1{}^3}\right) = \frac{a_3}{a_1{}^3} \quad (2)$$

with the concentrations, c, being expressed in grams per unit volume of solution, and the activity coefficients, y, measure nonideality on this concentration scale.

On a molar basis, the conditions for chemical equilibrium are

$$2\mu_1 = \mu_2$$
$$3\mu_1 = \mu_3 \quad\quad (3)$$
$$n\mu_1 = \mu_n$$

The chemical potentials, μ_i, are given in the units cal./mole. The condition for sedimentation equilibrium for the ith reactant is

$$d\mu_i - M_i\omega^2 r dr = 0 \quad\quad (4)$$

Thus for the constituent P_1 (constant temperature)

$$\left(\frac{\partial \mu_1}{\partial c_1}\right)_P \frac{dc_1}{dr} + \bar{v}M_1 \frac{dP}{dr} - M_1\omega^2 r = 0 \quad (4a)$$

In this expression μ_1 = chemical potential per mole of monomer, M_1 = monomer molecular weight, ω = angular velocity of the rotor, r = radial distance from the center of rotation, $\bar{v}_1$ = partial specific volume of the monomer, c_1 = concentration of monomer at any radial position (g. per dl.), and $dP/dr = \omega^2 r\rho$, where ρ is the density of the solution.

The total solute concentration, in the monomer–dimer–trimer system which has been selected is

$$c = c_1 + K_2c_1{}^2\left(\frac{y_1{}^2}{y_2}\right) + K_3c_1{}^3\left(\frac{y_1{}^3}{y_3}\right) \quad (5)$$

The weight-average molecular weight for this associating system (as in all others) varies with the total solute concentration; we adopt the symbol $M_{w(c)}$ to indicate this fact. Here

$$M_{w(c)}(= M_{wr}) = \frac{\sum\limits_{i=1}^{3} c_{ir}M_i}{\sum\limits_{i=1}^{3} c_{ir}}$$

$$= \frac{M_1}{c_r}\left((c_r + 2K_2c_{1r}{}^2\left(\frac{y_1{}^2}{y_2}\right)_r + 3K_3c_{1r}{}^3\left(\frac{y_1{}^3}{y_3}\right)_r\right) \quad (6)$$

The symbol c_{1r} represents the monomer concentration at position r in the cell.

III.   The Ideal Case

A.   Monomer–Dimer–Trimer Equilibria.—Ideal associating systems are defined as those in which excess chemical and electrical potentials are assumed to be absent. Thus, the theoretical working equations may be said to provide data to allow one to test for the ideality of solution behavior as well as for the association mechanism at appreciable solute concentrations, instead of being required to make evaluations in the

vicinity of infinite dilution. With the aid of the basic equations we write for the combined sedimentation and chemical equilibrium

$$\frac{1}{2A} \frac{1}{r} \frac{dc}{dr} = c_r M_{w(c)} = \Sigma c_{ir}M_i \quad (7)$$

where $A = (1 - \bar{v}\rho)\omega^2/RT$.

For the monomer, the sedimentation equilibrium is described by the formula

$$\frac{dc_1}{d(r_2)} = Ac_1M_1 \quad\quad (8)$$

In solution

$$c_{1r} = c_{1a}e^{A M_1(r^2 - a^2)} = c_{1a}e^{\phi_1} \quad (9)$$

The quantity a represents the radial distance to the meniscus in the cell, and $\phi_1 = A M_1(r^2 - a^2)$. Here the concentrations at any radial position, c_r, and the concentration at the meniscus, c_a, along with the radial distances r and a themselves, have been used as limits of integration. The equation could be generalized by using any chosen radial position, r_*, with the corresponding concentration, c_{r_*}, instead of the values at the meniscus, provided that $a \leq r_* \leq b$. The radial position b is that at the bottom of the cell.

For the dimer, trimer, etc., and in the same way we have

$$c_{2r} = K_2c_{1a}{}^2e^{2\phi_1} = c_{2a}e^{2\phi_1}, \text{ etc.} \quad (10)$$

Thus, for the system in discussion

$$\frac{1}{2A M_1} \frac{1}{r} \frac{dc}{dr} = c_{1r} + 2K_2c_{1r}{}^2 + 3K_3c_{1r}{}^3$$

$$(11)$$

$$= c_{1a}e^{\phi_1} + 2K_2c_{1a}{}^2e^{2\phi_1} + 3K_3c_{1a}{}^3e^{3\phi_1}$$

Dividing both sides of this equation by c_a, the total concentration of macromolecular component at the meniscus, we have

$$\frac{1}{2A M_1 c_a} \frac{1}{r} \frac{dc}{dr} = f_{1a}e^{\phi_1} + 2f_{2a}e^{2\phi_1} + 3f_{3a}e^{3\phi_1} \quad (12)$$

The quantities, f_{1a}, etc., represent the weight fractions of the several associating species, monomer, etc., at the meniscus. Dividing through by e^{ϕ_1}, we define a quantity

$$\psi \equiv \left\{\frac{1}{2A M_1 c_a} \frac{1}{r} \frac{dc}{dr}\right\} / e^{\phi_1}$$

$$(13)$$

$$= f_{1a} + 2f_{2a}e^{\phi_1} + 3f_{3a}e^{2\phi_1}$$

Since the sum of the weight fractions is one

$$f_{1a} = 1 - f_{2a} - f_{3a}$$

and

$$\psi - 1 = f_{2a}(2e^{\phi_1} - 1) + f_{3a}(3e^{2\phi_1} - 1) \quad (14)$$

Thus, a plot of $(\psi - 1)/(2e^{\phi_1} - 1)$ vs. $(3e^{2\phi_1} - 1)/(2e^{\phi_1} - 1)$ may be used to distinguish between a monomer–dimer association, and either a monomer–trimer or a monomer–dimer–trimer system. This is
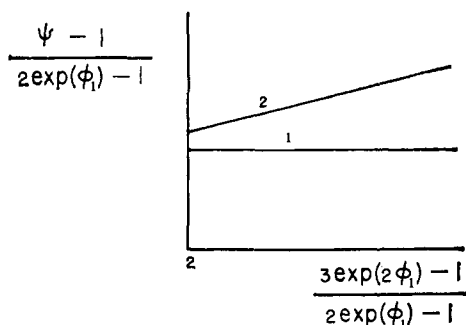
Fig. 1.—Plot based on eq. 14: Curve 1 would be given by a monomer–dimer association. The ordinate of this line has the value f_{2a}. Curve 2 could be given by a monomer–dimer–trimer association or a monomer–trimer association. The value of the abscissa at the meniscus is 2.

demonstrated in Fig. 1, in which it will be seen that for the monomer–dimer case the plot is a horizontal straight line, but in either of the other situations the line is inclined.

Now, in order to distinguish between a monomer–dimer–trimer and a monomer–trimer association, we rewrite eq. 14 as

$$\frac{\psi - 1}{3e^{2\phi_1} - 1} = f_{3a} + f_{2a}\left(\frac{2e^{\phi_1} - 1}{3e^{2\phi_1} - 1}\right) \quad (14a)$$

In Fig. 2 is shown a plot of $(\psi - 1)/(3e^{2\phi_1} - 1)$ vs. $(2e^{\phi_1} - 1)/(3e^{2\phi_1} - 1)$. For the monomer–trimer association a horizontal straight line results, but for the more complicated situation with monomer–dimer–trimer in equilibrium an inclined straight line would be found.

Having tested for the mechanism of the association by the methods given above for the more common sedimentation equilibrium experiment which makes use of schlieren optics to record the total concentration gradient of the redistributed components over the cell, the Rayleigh optical system now can be employed to evaluate the quantities c_{1a}, $K_2c_{1a}^2$, etc. Again, referring to this system, there are obtained from a generalized form of eq. 4 the set of equations

$$c_{r_1} = c_{1a}e^{AM_1(r_1^2 - a^2)} + K_2c_{1a}^2e^{2AM_1(r_1^2 - a^2)} +$$
$$K_3c_{1a}^3e^{3AM_1(r_1^2 - a^2)}$$
$$(15)$$
$$c_{r_2} = c_{1a}e^{AM_1(r_2^2 - a^2)} + K_2c_{1a}^2e^{2AM_1(r_2^2 - a^2)} +$$
$$K_3c_{1a}^3e^{3AM_1(r_2^2 - a^2)}$$

$$c_a = c_{1a} + K_2c_{1a}^2 + K_3c_{1a}^3$$

For ideal solutions the apparent and thermodynamic equilibrium constants are equal, thus a plot of $K_{2(app)}$ or $K_{3(app)}$ vs. c should give a horizontal straight line, Fig. 3. For the nonideal case, soon to be considered, concentration dependence is involved.

B. **Monomer-n-Mer Equilibria.**—With associations of the monomer-n-mer type such as occur in detergent solutions, the weight fraction, f, of monomer and therefore the concentration of monomer, c_1, can be obtained by the application of an equation of Steiner,[17] namely

$$\ln f = \int_0^c \left(\frac{M_1}{M_{w(c)}} - 1\right) d \ln c \quad (16)$$

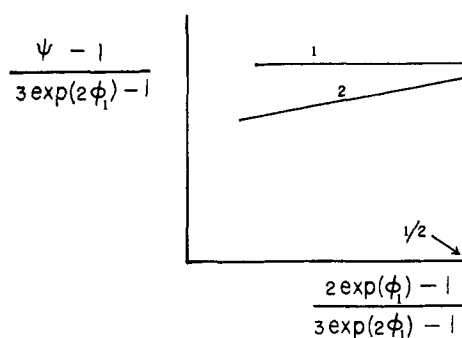(17) R. F. Steiner, Arch. Biochem. Biophys., **39**, 333 (1952); **49**, 400 (1954).

Fig. 2.—Plot based on eq. 14a: Curve 1 would be given by a monomer–trimer system only. The ordinate in this horizontal line has the value f_{3a}. Curve 2 would be representative only of a monomer–dimer–trimer association. The value of the abscissa at the meniscus is $1/2$.
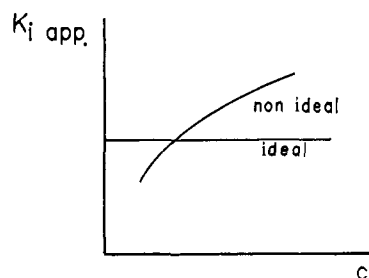

Fig. 3.—Plot of $K_{i(app)}$ vs. c: The quantity $K_{i(app)}$ is calculated from eq. 15. For ideal solutions it is independent of c, the total concentration of the macromolecular component. For nonideal systems concentration dependence will be exhibited, a possible form of which is indicated.

Then, remembering that

$$\frac{c - c_1}{c_1^n} = K_n$$

or

$$\log (c - c_1) = \log K_n + n \log c_1 \quad (17)$$

the value of n, the degree of aggregation, is available from a simple plot. For this case, the equation corresponding to (14) would read

$$\psi - 1 = f_{na}(ne^{\phi_1} - 1)$$

Thus, a plot of $\psi - 1$ vs. $(ne^{\phi_1} - 1)$ will provide an inclined line if only one species is present. If the n-mer is polydisperse upward curvature of the line will be found. This plot is more sensitive to polydispersity than is the plot based on eq. 17; however, the use of this equation is required for the estimate of the value of n.

IV. The Nonideal Case

A. **Monomer–Dimer–Trimer Equilibria.**—When the equations are extended to include thermodynamic nonideality effects one restricts at once the concentration range over which observations can be interpreted. The expression for the chemical potential becomes

$$\mu_i = (\mu_i^0)_c + RT \ln y_ic_i$$

The quantity $\ln y_i$ can be expanded in powers of c to give

$$\ln y_i = BM_1c + 0(c^2)$$

$$\ln y_i = BM_2c + 0(c^2)$$

or in general

$$\ln y_i = iBM_1c + 0(c^2)$$

only for very dilute systems. From eq. 4 there can be obtained for the solution as a whole the result

$$(1 + BcM_{w(c)}) \frac{dc}{d(r^2)} = c_r M_{w(c)}(1 - \bar{v}\rho) \frac{\omega^2}{2RT} \quad (18)$$

since

$$\frac{dc}{d(r^2)} = (1 + 2K_2c_1 + 3K_3c_1^2) \frac{dc_1}{d(r^2)}$$

(The symbol c_1 is here used to represent c_{1r}, the concentration of monomer at any radial distance, r, in the cell.) With the definition of $M_{w(c)}$ and some rearrangement, we find

$$\frac{1}{M_a} = \frac{1}{M_{w(c)}} + Bc =$$
$$\frac{1 + K_2c_1 + K_3c_1^2}{M_1(1 + 2K_2c_1 + 3K_3c_1^2)} + Bc \quad (19)$$

where

$$M_a = M_{\text{app}} = \frac{M_{w(c)}}{1 + BcM_{w(c)}}$$

and

$$\frac{1}{r}\frac{dc}{dr} = cM_a(1 - \bar{v}\rho) \frac{\omega^2}{RT}$$

From eq. 19 it will be noted that

$$\frac{1}{M_1} - \frac{1}{M_a} = \frac{K_2c_1 + 2K_3c_1^2}{M_1(1 + 2K_2c_1 + 3K_3c_1^2)} - Bc \quad (20)$$

and

$$\lim_{c \to 0} \frac{\left\{\dfrac{1}{M_1} - \dfrac{1}{M_a}\right\}}{c} = \frac{K_2}{M_1} - B \quad (20a)$$

Furthermore

$$\lim_{c \to 0} \frac{dM_a}{dc} = K_2M_1 - BM_1^2 \quad (20b)$$

and

$$\lim_{c \to 0} \frac{dM_{w(c)}}{dc} = K_2M_1 \quad (20c)$$

Thus, by plotting $\{(1/M_1 - (1/M_a)\}/c$ against c or $1/M_a$ against c a relation between K_2 and BM_1 is obtained. Defining $L = K_2 - BM_1$, we have

$$K_2 = L + BM_1 \quad (21)$$

The quantity L or its analogs often appear in equations descriptive of the associating system. For example it has been shown[7] to be involved in the equations of Steiner.[17]

In the next paragraphs we develop additional equations which involve the quantities K_2, K_3, and BM_1. The first step in the process is to define the apparent weight fraction of monomer, f_a. Going back to Steiner's relation, eq. 16, and using the apparent molecular weight, M_a, which is experimentally available,

an equation is obtained which defines the apparent weight fraction of monomer; thus

$$\ln f_a = \int_0^c \left(\frac{M_1}{M_a} - 1\right) d \ln c$$
$$= \int_0^c \left(\frac{M_1}{M_{w(c)}} - 1\right) d \ln c + \int_0^c BM_1 dc$$

or

$$f_a = fe^{BM_1c} \quad (22)$$

where $f = c_1/c$.

It should be noted that the integrand in eq. 22 is not an indeterminate form at infinite dilution of the macromolecular component ($c = 0$); thus it is readily shown for a monomer–dimer–trimer (or monomer–dimer–n-mer) association that

$$\lim_{c \to 0} \frac{1}{c}\left(\frac{M_1}{M_{w(c)}} - 1\right) = -K_2 \quad (23)$$

Equation 23 also gives $-K_2$ for a monomer–dimer association; however, for a monomer–n-mer association ($n \geq 3$) eq. 23 has the value zero. Combining eq. 5 and 22 we have

$$\left(\frac{1}{f_a} - 1\right)\bigg/ c = (K_2 - BM_1)c_1/c + \left(\left[K_3 - 2K_2BM_1 + \frac{(BM_1)^2}{2}\right]c_1^2/c + \ldots\right) \quad (24)$$

$$\lim_{c \to 0} \frac{\left(\dfrac{1}{f_a} - 1\right)}{c} = K_2 - BM_1 = L \quad (24a)$$

Also

$$\frac{\left(\dfrac{1}{f_a} - 1\right)}{c_1 e^{BM_1c}} = \left\{(K_2 - BM_1) + \left(\left[K_3 - 2K_2BM_1 + \frac{(BM_1)^2}{2}\right]c_1 + \ldots\right)\right\}e^{-BM_1c} \quad (24b)$$

and

$$\lim_{c \to 0} \frac{\left(\dfrac{1}{f_a} - 1\right)}{c_1 e^{BM_1c}} = K_2 - BM_1 = L \quad (24c)$$

It follows as well that

$$\lim_{c \to 0} \frac{dM_a}{d(c_1 e^{BM_1c})} = K_2 - BM_1 = L \quad (24d)$$

Several other equations may be derived from eq. 23; thus

$$\ln f_a + (K_2 - BM_1)c =$$
$$\int_0^c \left(\frac{M_1}{M_{w(c)}} - 1\right) d \ln c + K_2c \quad (25)$$

or

$$f_a e^{(K_2 - BM_1)c} = fe^{K_2c} \quad (25a)$$

We can also obtain, for example, using eq. 19 and 23

$$f_a e^{-M_1/M_a} = fe^{-M_1/M_{w(c)}} \quad (25b)$$

There are a variety of additional relationships which can be derived in similar manner. Thus, making use of a definition

$$\Lambda \equiv \frac{\left(1 - \frac{M_1}{M_a}\right)}{c_1 e^{\lambda c}} = e^{\lambda c}\left\{\frac{K_2 + 2K_3 c_1}{(1 + 2K_2 c_1 + 3K_3 c_1^2)} - BM_1 - K_2 BM_1 c_1 - \ldots\right\} \quad (26)$$

it is found that

$$\lim_{c \to 0}\left(\frac{d\Lambda}{dc}\right) = -\lambda(K_2 - BM_1) + (2K_3 - 2K_2^2 - K_2 BM_1) \quad (27)$$

In eq. 26 and 27 the quantity λ can take on the values BM_1, K_2, $-M_1/cM_{w(c)}$, and so on. So, when $\lambda = K_2$, eq. 27 becomes

$$\lim_{c \to 0}\left(\frac{d\Lambda}{dc}\right)_{\lambda = K_2} = 2K_3 - 3K_2^2 \equiv \Omega \quad (28)$$

It should also be noted that

$$\lim_{c \to 0}\frac{\left\{\frac{1}{M_a} - \frac{1}{M_1} + Lc\right\}}{c^2} = \frac{3K_2^2 - 2K_3}{M_1} \quad (29)$$

Using eq. 5, 19, 21, and 23, it can be shown that

$$\lim_{c \to 0}\frac{\left(\frac{M_1}{M_a} - f_a\right)}{c^2} =$$

$$K_2^2 + K_2 BM_1 - \frac{(BM_1)^2}{2} - K_3 \quad (30)$$

$$\lim_{c \to 0}\left\{\frac{f_a - 1}{Lc} + 1\right\}/c =$$

$$\frac{2K_2^2 - K_2 BM_1 - K_3 + (BM_1)^2/2}{K_2 - BM_1} \quad (31)$$

and

$$\lim_{c \to 0}\frac{\left(\frac{\left\{\frac{1 - f_a}{f_a}\right\}}{Lc_1 e^{BM_1 c}}\right) - 1}{c} =$$

$$K_3 - 3K_2 BM_1 - \frac{3(BM_1)^2}{2} \quad (32)$$

While it would appear that we have sufficient equations to evaluate K_2, K_3, and BM_1, we should examine what occurs when we substitute eq. 28 into eq. 30. We find

$$\lim_{c \to 0}\frac{\left(\frac{M_1}{M_a} - f_a\right)}{c^2} = K_2^2 + K_2 BM_1 - \frac{(BM_1)^2}{2} - K_3$$

$$= -\frac{\Omega}{2} - \frac{L^2}{2}$$

This provides no new information. Similar results are obtained with other combinations. The unfavorable result shows that these equations (30, 31, and 32, for example) are not mathematically independent. Thus, unless additional and independent relations which involve the quantities K_2, K_3, and BM_1 can be developed, all one can expect to evaluate from nonideal systems of the monomer–dimer–trimer (or n-mer) type are the quantities $L = K_2 - BM_1$ and $\Omega = 2K_3 - 3K_2^2$.

B. The Monomer–Dimer Equilibrium.—Where a simple monomer–dimer equilibrium exists, one may apply eq. 20a, 20b, 24a, 24c, and 24d, to obtain $L = K_2 - BM_1$. Having confidence that the monomer–dimer equilibrium is the only association reaction present, and representing the activity coefficient for each associating species in the conventional way, as has been indicated, the following equation can be applied[7]

$$\frac{2M_1}{M_a} - 1 = \frac{1}{(1 + 4K_2 c)^{1/2}} + 2BM_1 c \quad (34)$$

$$= \frac{1}{(1 + 4K_2 c)^{1/2}} + 2(K_2 - L)c \quad (34a)$$

At any value of c, eq. 34 is a cubic equation in K_2; it can be solved by standard algebraic methods. Alternatively, one can use the following equations to obtain K_2 and c_1.

$$cM_a - cM_1 = K_2 c_1^2 - BM_a M_{w(c)} c^2$$
$$= K_2 c_1^2 - BM_a M_1(c_1 + 2K_2 c_1^2)c \quad (35)$$

For the monomer–dimer association, eq. 5 reduces to

$$c = c_1 + K_2 c_1^2 \quad (36a)$$

or

$$K_2 = (c - c_1)/c_1^2 \quad (36b)$$

Then, with eq. 21, 36a, and 36b we obtain

$$c_1^2(cM_a - cM_1) = c_1^2(c - c_1) - \left\{(c_1 - c_1) - Lc_1^2\right\}(2c - c_1)cM_a \quad (36c)$$

At any given value of c, eq. 36c is cubic in the quantity c_1, and the quantity K_2 may be obtained from eq. 36b, once c_1 is known. As already suggested, Steiner's equations are valid.

Equation 28 or 29 will give K_2^2 for this system; thus there are several checks available. Of course, the previous results[7] could be applied, but this newer approach eliminates any necessity for calculating K_{app}.

C. The Monomer–n-Mer Equilibrium.—The monomer–n-mer equilibrium can be represented as

$$nP \rightleftharpoons P_n \quad (n \geq 3)$$
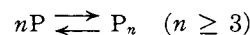
Assuming that the activity coefficients for the associating species can be represented in the usual fashion, we write

$$\frac{1}{M_a} = \frac{1}{M_{w(c)}} + Bc \quad (19)$$

However, for the monomer–n-mer equilibrium the quantity $M_{w(c)}$ becomes

$$M_{w(c)} = \frac{M_1(1 + nK_n c_1^{n-1})}{(1 + K_n c_1^{n-1})} \quad (37)$$

Thus

$$\lim_{c \to 0} \left\{ \frac{\left(\frac{1}{M_1} - \frac{1}{M_a} \right)}{c} \right\} = -B \qquad (38)$$

$$\lim_{c \to 0} \frac{dM_a}{dc} = -BM_1^2 \qquad (38a)$$

$$\lim_{c \to 0} \frac{dM_a}{d(c_1 e^{BM_1 c})} = -BM_1^2 \qquad (38b)$$

$$\lim_{c \to 0} \frac{\left(\frac{1}{f_a} - 1 \right)}{c} = -BM_1 \qquad (38c)$$

Again, the equations of Steiner are valid. Thus, eq. 16 applies and we have as before, $f_a = f e^{BM_1 c}$.

Since we can evaluate the quantity BM_1 from eq. 38, we can readily estimate f and hence c_1. Knowing M_a and BM_1, we obtain $M_{w(c)}$ from (19) and the definition of M_a. It is apparent that if BM_1, c, and $M_{w(c)}$ are available, one can obtain n and K_n from eq. 17.

D. Charged Macromolecules and Further Remarks on Nonideal Systems.—Proteins and detergent micelles are usually charged macromolecules, and sedimentation equilibrium studies are generally carried out in buffers, often along with additional supporting electrolyte. By using the Scatchard–Bregman[18] or the Casassa–Eisenberg[19] definitions of components, one may reduce the sedimentation equilibrium equation to that of a two-component system.

These definitions of components have evolved from the Lamm[20] theoretical description of the sedimentation equilibrium of dissolved charged macromolecules in the presence of supporting electrolyte.

We consider a charged, associating macromolecule PX_z and a supporting electrolyte BX. The associating species are defined as $PX_z - \Gamma Z(BX)$ for the monomer, $[PX_z - \Gamma Z(BX)]_2$ for the dimer, and so on. The sedimentation equilibrium equation for the monomer becomes

$$A M_1 d(r^2) = d \ln c_1 + z f(z) dc + BM_1 dc \qquad (39)$$

Here

$$f(z) = \left\{ \frac{z \left[(1 - 2\Gamma)(1 - \Gamma) + \Gamma + \left(\frac{\Gamma^2 z c}{\theta M} \right) \right] dc}{M_1 \left(\theta + \frac{zc}{M_1} \right)} + \right.$$
$$\left. \frac{\left[1 - 2\Gamma - \left(\frac{\Gamma z c}{\theta M_1} \right) \right] dc_3}{M_3 \left(\theta + \frac{zc}{M_1} \right)} \right\}$$

where z = charge on the monomer, M_1 = molecular weight of monomer $[PX_z - \Gamma Z(BX)]$, M_3 = molecular weight of BX, c = total concentration (g./dl.) of macromolecular component at any radial position in the ultracentrifuge cell, $A = (1 - \bar{v}_{PX_z - \Gamma Z(BX)}\rho)\omega^2/2RT$, Γ = membrane distribution parameter of Casassa and

(18) G. Scatchard and J. Bregman, J. Am. Chem. Soc., 81, 6095 (1959).
(19) (a) E. F. Casassa and H. Eisenberg, J. Phys. Chem., 64, 753 (1960); (b) H. Eisenberg and E. F. Casassa, J. Polymer Sci., 47, 29 (1960).
(20) O. Lamm, Arkiv Kemi, Mineral. Geol., 17A, No. 25 (1943–1944).

Eisenberg[19b] $= i/2(1 - (iZcM_3/M_1 c_3) + \ldots)$, $\theta = (c_3/M_3) - (\Gamma Z c/M_1)$, and c_3 = concentration (g./dl.) of BX. The quantity i is a factor such that $0 \leqslant i \leqslant 1$. For a neutral macromolecule, $i = 0$; when a strong electrolyte is involved, $i = 1$. For macromolecules which behave as weak electrolytes the value of the quantity i lies between zero and one.

Remembering that the concentration for a monomer–dimer equilibrium can be expressed by eq. 36a and the concentration gradient by an equation of the same form as eq. 18, we obtain for the sedimentation equilibrium of a charged, associating monomer–dimer equilibrium

$$\frac{dc}{d(r^2)} \equiv \left[\frac{dc_1}{d(r^2)} \right] (1 + 2K_2 c_1) \qquad (40)$$

$$= A M_1 (c_1 + 2K_2 c_1^2) - (c_1 - 2K_2 c_1^2) \times$$
$$[f(z) + BM_1] \frac{dc}{d(r^2)}$$

or

$$\frac{1}{M_a} = \frac{1}{M_{w(c)}} + B^* c \qquad (40a)$$

Here, $B^* = BM_1 + f(z)/M_1$ and $cM_{w(c)}/M_1 = (c_1 + 2K_2 c_1^2)$. It should be noted that the second term in $f(z)$ is usually much, much smaller than the main term, so that the second term may be omitted, or one may make an approximation by assuming

$$\frac{dc_3}{dc} \simeq \frac{M_3(1 - \bar{v}_3 \rho)}{M_1(1 - \bar{v}_{PX_z - \Gamma Z(BX)}\rho)}$$

It is here taken for granted that the activity coefficients (i.e., the excess electrochemical potential) for each associating species can be expressed as

$$\ln y_i = i B^* M_1 c + 0(c^2) \qquad (40b)$$

It now follows from this convention that the data for charged, associating system can be analyzed in the manner already indicated in sections A, B, and C.

E. Other Activity Coefficient Treatments.—To this point use has been made of a single virial coefficient. Additional terms can be included but they make the equations (and calculations) much more complicated. Thus, if the activity coefficient for each associating species can be represented by the series

$$\ln y_i = i B_1 M_1 c + i B_2 M_1 c^2 +$$
$$0(c^3), \ i = 1, 2, 3, \ldots \ldots \qquad (41)$$

we still have

$$c = c_1 + K_2 c_1^2 + K_3 c_1^3$$

for a monomer–dimer–trimer association. It also follows that

$$\frac{1}{M_a} = \frac{1}{M_{w(c)}} + B_1 c + 2B_2 c^2 \qquad (42)$$

$$f_a = f e^{(B_1 M_1 c + B_2 M_1 c^2)} \qquad (43)$$

$$\lim_{c \to 0} \frac{dM_a}{dc} = K_2 M_1 - BM_1^2 \qquad (20b)$$

Equations 20a, 24a, 24c, and 24d are also valid in this instance.

There may be systems which are nonideal in more complicated ways than can be described by the conventions for the activity coefficient we have used. In this event, the activity coefficients may be described as

$$\ln y_1 = B_1 M_1 c + B_2 M_1 c^2 \tag{44}$$

for the monomer. Now for a monomer–dimer association

$$2P \underset{\leftarrow}{\overset{\rightarrow}{\rightleftharpoons}} P_2 \tag{45}$$

and

$$K_2 = \frac{a_2}{a_1{}^2} = \left(\frac{c_2}{c_1{}^2}\right)\left(\frac{y_2}{y_1{}^2}\right) = \frac{K'(c)y_2}{y_1{}^2} \tag{46}$$

Thus

$$y_2 = \frac{Ky_1{}^2}{K'(c)} \tag{47}$$

On expansion

$$K'(c) = K + \left(\frac{\mathrm{d}K'}{\mathrm{d}c}\right)_{c=0} c + \ldots \tag{48}$$

Then

$$y_2 = \frac{y_1{}^2}{\left[1 + \left(\dfrac{\partial \ln K'}{\mathrm{d}c}\right)_{c=0} c + \ldots\right]} = \frac{y_1{}^2}{(1 + \alpha c + \ldots)} \tag{49}$$

Hence

$$c = c_1 + K_2 \frac{c_1{}^2 y_1{}^2}{y_2} = c_1 +$$
$$K_2 c_1{}^2 (1 + \alpha c + \ldots) \tag{50}$$

It can then be shown that

$$\frac{\mathrm{d}c}{\mathrm{d}(r^2)} \{1 - \alpha K_2 c_1{}^2\} = \frac{\mathrm{d}c_1}{\mathrm{d}(r^2)} \times$$
$$\{1 + 2K_2 c_1 (1 + \alpha c)\} \tag{51}$$
$$= A c M_{w(c)} - B_1 c M_{w(c)} \frac{\mathrm{d}c}{\mathrm{d}(r^2)} -$$
$$2 B_2 c^2 M_{w(c)} \frac{\mathrm{d}c}{\mathrm{d}(r^2)} + \ldots$$

or

$$\frac{1}{M_a} = \frac{1}{M_{w(c)}} (1 + \alpha K_2 c_1{}^2 + \ldots) +$$
$$B_1 c + 2 B_2 c^2 \tag{51a}$$
$$= \frac{1}{M_{w(c)}} + B_1 c + 2 B_2 c^2 + \frac{\alpha K_2 c_1{}^2}{M_1} + \ldots$$

Equations 20b and 20c are still true for the monomer–dimer (and n-mer) association and for the monomer–

trimer (or n-mer) associations, respectively, but the Steiner equations now become much more complicated.

V. Discussion

An ever increasing number of dissociation–association reactions are being studied in the ultracentrifuge, along with other methods. Involved may be proteins, surfactants, and other molecules in solution. As of now the experimental results of such investigations, either restricted to the sedimentation methods or with the inclusion of the other approaches, are at considerable variance. Thus, as an immediate objective it has seemed worthwhile to attempt to put the theory of the combined chemical and sedimentation equilibrium into improved form for later experimental programs, eventually to eliminate some of these inconsistencies.

There is, of course, the more common sedimentation transport experiment for the purpose in discussion. Gilbert and associates[21] have been concerned with the modification of the form of the boundary gradient curve as the combined transport and reaction process proceeds. In order to simplify some of the mathematical problems which have arisen certain rather restrictive assumptions have been required, but the theoretical description does provide for a monomodal boundary for a monomer–dimer system and a bimodal one in the case of a monomer–n-mer (n > 2) reaction, as observed experimentally. Also the more classical combination of sedimentation velocity and diffusion experiments has been used to study associations both in protein and in surfactant systems, with the object being to evaluate the association number. For the paraffin-chain salt case the necessary theory has been recently set down and developed at considerable length by Mijnlieff[22]; it will be strictly correct only if the micellar system which forms is monodisperse.

We have elected to look into the possibilities of the sedimentation equilibrium experiment for several reasons. It has the more sound theoretical foundation, with it solution nonideality effects may be more readily taken into account, and presumably the experiments may be carried out at lower solute concentrations. Such experiments have not been popular in the past because of the relatively long time to attain equilibrium, but the situation in this respect is now changed because of the re-introduction of the use of short solution columns, already described in the Svedberg and Pedersen monograph,[23] by van Holde and Baldwin,[24] and by Yphantis.[25]

In the earlier report[7] there was presented an elaborate mathematical analysis for a monomer–dimer system at sedimentation equilibrium, one which included the effects of solution nonideality. Here, additional methods have been developed for the study of this system, and extensions have been provided whereby monomer–n-mer (n > 2) systems are included. Further, it appears that the theory in its present state

(21) (a) G. A. Gilbert, Discussions Faraday Soc., 20, 68 (1955); Proc. Roy. Soc. (London), A250, 377 (1959); A253, 420 (1959); (b) G. A. Gilbert and R. C. L1. Jenkins, "Ultracentrifugal Analysis in Theory and Experiment," J. W. Williams, Ed., Academic Press, Inc., New York, N. Y., 1963, pp. 59, 73.

(22) P. J. Mijnlieff, "Ultracentrifugal Analysis in Theory and Experiment," J. W. Williams, Ed., Academic Press, Inc., New York, N. Y., 1963, p. 81.

(23) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon Press, Oxford, 1940, pp. 57, 305.

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

(25) D. A. Yphantis, Ann. N. Y. Acad. Sci., 88, 586 (1960).

cannot be enlarged to describe the behavior of monomer–dimer–n-mer associations.

When applying the theory to the nonideal situations it has to be recognized that data in very dilute solutions are required. With the introduction of the Rayleigh methods and of 30-mm. double sector cells for the experiment, sufficiently greater precision in such dilute systems is promised and one is encouraged to essay such experiments. Incidentally, it is of interest that the theory developed by Steiner for *ideal* associating systems requires that the equilibrium constants be evaluated from limits taken at infinite dilution; this restriction is now relaxed.

A main objective is the determination of the quantity $L = K_2 - BM_1$. The equation of Steiner, subject to its restriction, provides it through his equation

$$\frac{\left(\frac{M_{w(c)}}{f_1 M_1} - 1\right)}{f_1 c} = \frac{4K_2}{M_1} + \frac{9K_3 f_1 c}{M_1{}^2} + \ldots$$

Here f_1 is the weight fraction of the monomer. It can be calculated by means of our eq. 16. Plotting the quotient against the quantity $f_1 c$, the intercept, $4K_2/M_1$, and the limiting slope at infinite dilution, $9K_3/M_1{}^2$, are available. For nonideal systems undergoing the monomer–dimer association Adams and Fujita[7] already have shown that this intercept is the desired quantity $K_2 - BM_1$.

It has been proposed, for quite different purposes to be sure, that the micelles formed in solutions of paraffin-chain salts may serve as convenient models for proteins. Problems of the kind we have discussed may lead one to be able to state just how far the approach used by Debye in his study of micelle formation

can be applied in protein physical chemistry. As a longer range objective it has been our purpose to indicate new means to converge on problems of this kind.

For ideal associating systems there exists another and simpler manner for testing the association mechanism instead of using eq. 14 and 14a. Using eq. 5, with all $y_i = 1$ for an ideal solution, and eq. 11 one obtains

$$(1/2A\,M_1 r)\frac{dc}{dr} - c = K_2 c_{1a}{}^2 e^{2\phi_1} + 2K_3 c_{1a}{}^3 e^{3\phi_1} \quad (52)$$

This may be rearranged to give

$$\frac{(1/2A\,M_1 r)\dfrac{dc}{dr} - c}{c_a e^{2\phi_1}} = f_{2a} + 2f_{3a} e^{\phi_1} \quad (53)$$

A plot of the quantity on the left-hand side of eq. 53 against e^{ϕ_1} gives a horizontal line for a monomer–dimer association and an inclined line for a monomer–dimer–trimer or monomer–trimer association. We can distinguish between a monomer–dimer–trimer and a monomer–trimer association with a second plot; we note that

$$\frac{3c - (1/2A\,M_1 r)dc/dr}{c_a e^{\phi_1}} = 2f_{1a} + f_{2a} e^{\phi_1} \quad (54)$$

Here a plot of the left-hand term of eq. 54 against e^{ϕ_1} gives a horizontal line for a monomer–trimer association and an inclined straight line for a monomer–dimer or monomer–dimer–trimer association.

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Morphology of Colloidal Gold—A Comparative Study

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A comparative study of the morphology of colloidal gold was made using light absorption and scattering, X-ray diffraction line broadening, and electron microscopy. A series of colloidal gold samples ranging in average particle "diameter" from 10 to 400 Å. and exhibiting a wide range of crystallite morphology was prepared and examined by each method. Extinction coefficients, absolute light scattering coefficients, and depolarization factors were measured for wave lengths between 4000 and 6500 Å. Using the theory of Mie for the interaction of light with conducting spheres and the extension of this theory by Gans to ellipsoids of various axial ratios, the approximate shape of the crystallites was calculated. The pure diffraction line broadening of the (200), (111), (220), and (311) reflections was measured, and by applying the Scherrer equation, the mean crystallite dimension along [100], [111], [110], and [311] was calculated. The morphology as calculated from light absorption and scattering and X-ray diffraction was found to be in fairly close agreement with the electron microscopic findings for most colloidal gold samples. However, each technique, including electron microscopy, was found to have limitations, in particular particle size ranges.

I. Introduction

Beginning with the slit ultramicroscope which was developed shortly after 1900, several indirect methods have been employed to study the morphology of colloidal particles. One method is that of light absorption and scattering. In 1908 Mie[2a] presented a theoretical treatment of the interaction of light with

small, conducting spheres. On the basis of Maxwell's electromagnetic theory, Mie calculated the true absorption and the intensity and polarization properties of light scattered by spherical particles of varying size in terms of the macroscopic optical properties of the metal. In 1912 Gans[2b] extended the calculations of Mie by generalizing the particle shape to prolate and oblate ellipsoids of revolution. Whereas Mie's cal

(2) (a) G. Mie, *Ann. Physik*, **25**, 377 (1908); (b) R. Gans, *ibid.*, **37**, 833 (1912).