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Self-Associating Systems. II. Multinomial Theory for Nonideal Systems*

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ABSTRACT: A general expression for the calculation of equilibrium constants and virial coefficients of self-associating nonideal systems has been established. The derivations are an extension of the multinomial theory for ideal systems (Derechin, M. (1968), *Biochemistry 7*, 3253).

This general expression is applicable to association reactions of any degree of polymerization and is valid irrespective of whether the apparent weight-average, M_{app}, or number-average, $M_{\text{n app}}$, molecular weight is used in the calculations. Using this expression, equations to establish the equilibrium constants K_1, K_2, K_3, and K_4 have been derived. Directions for the calculation of virial coefficients are given.

In a previous paper (Derechin, 1969) self-association reactions of the type

$$nP_1 \leftrightarrows a_2P_2 + a_3P_3 + \cdots + a_mP_m$$

with

$$\sum_{i=2}^{m} ia_i = n \qquad (1)$$

for ideal systems have been examined. Many systems encountered in practice depart of course from ideality. It is, therefore, desirable to extend this theory to cover nonideal behavior.

Theoretical

It is assumed here that all species participating in the self-associating reaction have the same partial specific volume ($\bar{v}_1 = \bar{v}_2 = \cdots = \bar{v}$) and the same refractive index increment $(dn/dc_1)_{T,P} = (dn/dc_2)_{T,P} = \cdots = (dn/dc)_{T,P}$, and that for dilute systems the activity coefficient of each associating species can be represented by a series expansion as $\ln y_i = iB_iM_1c +$ higher terms in c, where B_i is the second virial coefficient of the species i, M_1 is the molecular weight of the monomer, and c is the total solute concentration in g/100 ml. Since we shall deal only with systems in dilute solution, higher powers in c are neglected. Also it is assumed that $B_1 = B_2 = \cdots = B$. In the particular case $i = 1$

$$\ln y_1 = BM_1c \qquad (2)$$

Then

$$\ln y_i = iBM_1c = \ln y_1{}^i \qquad (3a)$$

or

$$y_i = y_1{}^i \qquad (3)$$

The condition for chemical equilibrium can be stated in terms of the equilibrium constants, K_i, as

$$y_ic_i = K_iy_1{}^ic_1{}^i \qquad i = 1, 2, \ldots \qquad (4a)$$

or using eq 3,

$$c_i = K_ic_1{}^i \qquad i = 1, 2, \ldots \qquad (4)$$

Equation 4 is valid for ideal and nonideal systems. In the particular case $i = 1$, $K_i = 1$. Also since

$$c = \sum_{i=1}^{m} c_i \qquad i = 1, 2, \ldots, m \qquad (5)$$

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we can write, as shown by Adams and Fujita (1963)

$$c = \sum_{i=1}^{m} K_i c_1{}^i \qquad i = 1, 2, \ldots, m \tag{6}$$

Using eq 6 and the definitions of the weight-average, M_{wc}, and number-average, M_{nc}, molecular weight, it has been shown (Adams, 1965a,b; Derechin, 1968) that

$$\frac{cM_1}{M_{\text{nc}}} = \int_0^c \frac{M_1 \text{d}c}{M_{\text{wc}}} = \sum_{i=1}^{m} \frac{K_i}{i} c_1{}^i \tag{7}$$

Data obtained from experiment (*e.g.*, sedimentation equilibrium, osmotic pressure, etc.) permit the evaluation of apparent molecular weights ($M_{\text{w app}} = M_{\text{app}}$ or $M_{\text{n app}}$). The value of M_{app} (or $M_{\text{n app}}$) obtained by these procedures differs from the values of M_{wc} (or M_{nc}) by at least one term involving the virial coefficient(s). It has been shown (Adams and Fujita, 1963) that

$$\frac{1}{M_{\text{app}}} = \frac{1}{M_{\text{wc}}} + Bc \tag{8}$$

Multiplying eq 8 throughout by $M_1\text{d}c$, integrating, and combining with eq 7, we can write

$$\frac{M_1 c}{M_{\text{nc}}} = \int_0^c \frac{M_1 \text{d}c}{M_{\text{wc}}} = H(c) - \tfrac{1}{2}BM_1c^2 = \sum_{i=1}^{m} \frac{K_i}{i} c_1{}^i \tag{9b}$$

where

$$H(c) = \frac{M_1 c}{M_{\text{napp}}} = \int_0^c \frac{M_1 \text{d}c}{M_{\text{app}}}$$

Equation 9b shows that M_{napp} can also be obtained by graphical integration of a plot of M_1/M_{app} *vs.* c. Further, a general analytical expression, from which the values of K_i ($i = 1, 2, \ldots, m$) and the virial coefficient can be obtained, can be derived. Expanding $H(c)$ in powers of c (Maclaurin's theorem) eq 9b can be written

$$\begin{aligned} \frac{M_1 c}{M_{\text{nc}}} = \int_0^c \frac{M_1 \text{d}c}{M_{\text{wc}}} &= \sum_{r=0}^{1} \frac{1}{r!}\left(\frac{\text{d}^{(r)}H}{\text{d}c^r}\right)_{c=0} c^r \\ &+ \frac{1}{2}\left[\left(\frac{\text{d}^{(2)}H}{\text{d}c^2}\right)_{c=0} - BM_1\right]c^2 \\ &+ \sum_{r=3}^{\infty} \frac{1}{r!}\left(\frac{\text{d}^{(r)}H}{\text{d}c^r}\right)_{c=0} c^r = \sum_{i=1}^{m} \frac{K_i}{i} c_1{}^i \end{aligned} \tag{9a}$$

Equation 9a can be expressed in a more general manner as

$$\frac{M_1 c}{M_{\text{nc}}} = \int_0^c \frac{M_1 \text{d}c}{M_{\text{wc}}} = \sum_{r=0}^{\infty} \frac{1}{r!} D_r c^r = \sum_{i=1}^{m} \frac{K_i}{i} c_1{}^i \tag{9}$$

where

$$D_r = \begin{cases} \left(\dfrac{\text{d}^{(r)}H}{\text{d}c^r}\right)_{c=0} & r = 0, 1, 3, 4, \ldots \\ \left(\dfrac{\text{d}^{(2)}H}{\text{d}c^2}\right)_{c=0} - BM_1 & r = 2 \end{cases}$$

Using c as defined in eq 6, we can write

$$\sum_{r=0}^{\infty} \frac{1}{r!} D_r\left[\sum_{i=1}^{m} K_i c_1{}^i\right]^r = \sum_{i=1}^{m} \frac{K_i}{i} c_1{}^i \tag{10}$$

The power term in brackets in eq 10 can be evaluated by making use of the multinomial theorem as shown before (Derechin, 1969), and eq 10 becomes

$$\sum_{\xi=1}^{m} \sum_{r=1}^{\xi} \frac{1}{r!} D_r G(\xi, r, K_i) c_1{}^{\xi} = \sum_{\xi=1}^{m} \frac{K_\xi}{\xi} c_1{}^{\xi} \tag{11}$$

where D_r is defined as in eq 9

$$\begin{aligned} G(\xi, r, K_i) = &\sum_{\alpha_2=0}^{r} \cdots \sum_{\alpha_m=0}^{r} \\ &\times \binom{r}{\left(\xi - \sum_{i=2}^{m} i\alpha_i\right), \alpha_2, \ldots \alpha_m} \prod_{i=2}^{m} K_i{}^{\alpha_i} \end{aligned}$$

$$\xi = \sum_{i=1}^{m} i\alpha_i = \alpha_1 + \sum_{i=2}^{m} i\alpha_i$$

$\alpha_1, \alpha_2, \ldots, \alpha_m$ take all positive integral values for which

$$\xi - r = \sum_{i=2}^{m} (i-1)\alpha_i \qquad \text{condition 1}$$

$$\xi - \sum_{i=2}^{m} i\alpha_i \geq 0 \qquad \text{condition 2}$$

and

$$\sum_{i=1}^{m} \alpha_i = r \qquad \text{condition 3}$$

Comparing the coefficients of the same power of c on both sides of eq 11 we have

$$\sum_{r=1}^{\xi} \frac{1}{r!} D_r G(\xi, r, K_i) = \frac{K_\xi}{\xi} \qquad \xi = 1, 2, \ldots, m \tag{12a}$$

or

$$\begin{aligned} \sum_{r=1}^{\xi} \frac{1}{r!} D_r\Bigg[\sum_{\alpha_2=0}^{r} \cdots \sum_{\alpha_m=0}^{r} &\binom{r}{\left(\xi - \sum_{i=2}^{m} i\alpha_i\right), \alpha_2, \ldots \alpha_m} \\ &\times \sum_{i=2}^{m} K_i{}^{\alpha_i}\Bigg] = \frac{K_\xi}{\xi} \end{aligned}$$

$$\xi = 1, 2, \ldots, m \tag{12}$$

with conditions 1, 2, and 3 applicable as before (see eq 11), and with D_r defined as in eq 9. Equation 12 represents a set of equations from which the equilibrium constants, $K_1, K_2, K_3, \ldots, K_m$, and the virial coefficient, B, can be determined.

Application of the Theory to Calculation of Equilibrium Constants. The general expression (eq 12) will be used here for the calculation of K_1, K_2, K_3, and K_4, and B, for nonideal self-associating systems defined by eq 1. The calculation of these particular cases will be made in some detail, so that the derivation of explicit expressions for other equilibrium constant or constants will be more readily available. The final equations will be presented here exclusively in terms of the experimentally available quantities M_{app} (or $M_{\mathrm{n\,app}}$).

Examining eq 12 we can see that all the summation terms are simultaneously multiplied by $r!$ (since it is in the multinomial coefficient) and also divided by $r!$ ($1/r!$ precedes D_r). Thus, the number $r!$ can be deleted from eq 12. Also noting that

$$\left(\frac{\mathrm{d}^{(r)}H}{\mathrm{d}c^r}\right)_{c=0} = \left(\frac{\mathrm{d}^{(r-1)}H}{\mathrm{d}c^{r-1}}\right)_{c=0} = \left(\frac{\mathrm{d}^{(r-1)}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^{r-1}}\right)_{c=0} = \left(\frac{r\mathrm{d}^{(r-1)}\left(\dfrac{M_1}{M_{\mathrm{napp}}}\right)}{\mathrm{d}c^{r-1}}\right)_{c=0} \qquad (13)$$

we can define

$$D_{r-1} = \begin{cases} \left(\dfrac{\mathrm{d}^{(r-1)}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^{r-1}}\right)_{c=0} = \left(\dfrac{r\mathrm{d}^{(r-1)}\left(\dfrac{M_1}{M_{\mathrm{napp}}}\right)}{\mathrm{d}c^{r-1}}\right)_{c=0} & r = 1, 3, 4, \ldots, m \\ \left(\dfrac{\mathrm{d}^{(r-1)}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^{r-1}}\right) - BM_1 = \left(\dfrac{\mathrm{d}r^{(r-1)}\left(\dfrac{M_1}{M_{\mathrm{napp}}}\right)}{\mathrm{d}c^{r-1}}\right) - BM_1 & r = 2 \end{cases} \qquad (14)$$

Now, eq 12 can be rewritten

$$\sum_{r=1}^{\xi} D_{r-1}\left[\sum_{\alpha_2=0}^{r} \cdots \sum_{\alpha_m=0}^{r} \left(\left(\xi - \sum_{i=2}^{m} i\alpha_i\right)\alpha_2, \alpha_3, \overset{1}{\ldots}, \alpha_m\right)\prod_{i=2}^{m} K_i^{\alpha_i}\right] = \frac{K_\xi}{\xi}$$

$$\xi = 1, 2, \ldots, \qquad (15)$$

Then, eq 15 can be used immediately with the quantity (as supplied by the experimental method used) indicated in the definition of D_{r-1} (see eq 14). For this purpose, the values of $\alpha_1, \alpha_2, \alpha_3, \ldots, \alpha_m$ must be calculated such that conditions 1, 2, and 3 (see eq 11) are satisfied in all cases. Since these values are the same as those calculated (Derechin, 1969) for the ideal case (for $\xi = 1, 2, 3,$ and 4) those latter values will be used.

Derivation of Explicit Expressions for Equilibrium Constants and the Virial Coefficient Using Determination of M_{app}. *Derivation of* K_1. The only case is $\xi = 1$, $r = 1$. Conditions 1, 2, and 3 are satisfied for $\alpha_1 = 1$, $\alpha_i = 0$, $i > 1$, only. Substituting these values into eq 15, we have

$$\left(\frac{M_1}{M_{\mathrm{app}}}\right)_{c=0} \frac{1}{1!0!0!\ldots0!} K_2^{\,0}K_3^{\,0}\ldots = \left(\frac{M_1}{M_{\mathrm{app}}}\right)_{c=0} = \frac{K_1}{1} \qquad (16)$$

Derivation of K_2. For the first term (for $\xi = 2, r = 1$), conditions 1, 2, and 3 are satisfied for $\alpha_2 = 1, \alpha_1 = 0$, $i > 1$, only. With these values we can write the first term required by eq 15, as follows

$$\left(\frac{M_1}{M_{\mathrm{app}}}\right)_{c=0} \frac{1}{0!1!0!0!\ldots0!} K_2^{\,1}K_3^{\,0}K_4^{\,0}\ldots = K_2 \qquad (17c)$$

For the second summation term (for $\xi = 2$, $r = 2$) conditions 1, 2, and 3 are satisfied only if $\alpha_1 = 2$, $\alpha_i = 0$, and $i > 1$. We can therefore write a second summation term

$$\left[\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]\frac{1}{2!0!0!\ldots0!} = \frac{1}{2}\left[\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right] \qquad (17b)$$

Taking these two summation terms for $\xi = 2$, eq 15 is now complete and can be written

$$\frac{K_2}{2} = K_2 + \frac{1}{2}\left[\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right] \qquad (17a)$$

and solving for K_2, we have

$$K_2 = BM_1 - \left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} \qquad (17)$$

Derivation of K_3. For the first summation term (for $\xi = 3$, $r = 1$), conditions 1, 2, and 3 are obeyed only for $\alpha_3 = 1$, $\alpha_i = 0$, $i \neq 3$. Using these values and eq 16, the first term for $\xi = 3$ becomes

$$\left(\frac{M_1}{M_{\mathrm{app}}}\right)_{c=0} \frac{1}{0!0!1!0!\ldots0!} K_2^{\,0}K_3^{\,1}K_4^{\,0}\ldots = K_3 \qquad (18d)$$

For the second summation term (for $\xi = 3$, $r = 2$), conditions 1, 2, and 3 are satisfied only for $\alpha_1 = 1$, $\alpha_2 = 1$, $\alpha_i = 0$, $i > 2$. Then, the second summation term for $\xi = 3$ is

$$\left[\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]\frac{1}{1!1!0!0!\ldots0!} \times K_2^{\,1}K_3^{\,0}K_4^{\,0}\ldots = \left[\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]K_2 \qquad (18c)$$

For the third summation term (for $\xi = 3$, $r = 3$), conditions 1, 2, and 3 are satisfied only for $\alpha_1 = 3$, $\alpha_i = 0, i > 1$. Then, the third summation term becomes

$$\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0}\frac{1}{3!0!0!\ldots0!} = \frac{1}{6}\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0} \qquad (18\mathrm{b})$$

Adding all summation terms obtained for $\xi = 3$, eq 15 is now complete and can be written

$$\frac{K_3}{3} = K_3 + \left[\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]K_2 + \frac{1}{6}\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0} \qquad (18\mathrm{a})$$

Solving for K_3 and using eq 17, we have

$$K_3 = \frac{3}{2}(BM_1)^2 - 3\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0}(BM_1) + \left[\frac{3}{2}\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)^2_{c=0} - \frac{1}{4}\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0}\right] \qquad (18)$$

Derivation of K_4. For the first term (for $\xi = 4$, $r = 1$), conditions 1, 2, and 3 are satisfied only for $\alpha_4 = 1$, $\alpha_i = 0$, $i \neq 4$. Then, the first summation term in eq 15 becomes

$$\left(\frac{M_1}{M_{\mathrm{app}}}\right)_{c=0}\frac{1}{0!0!0!1!0!\ldots0!}K_2{}^0K_3{}^0K_4{}^1K_5{}^0\ldots = K_4 \qquad (19\mathrm{f})$$

For the second summation term (for $\xi = 4$, $r = 2$), conditions 1, 2, and 3 are satisfied in two cases: (a) $\alpha_1 = 1$, $\alpha_2 = 0$, $\alpha_3 = 1$, $\alpha_i = 0$, $i > 3$, and (b) $\alpha_1 = 0$, $\alpha_2 = 2$, $\alpha_i = 0$, $i > 2$. Thus, two summation terms are generated for $\xi = 4$, $r = 2$, as follows

(a)

$$\left[\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]\frac{1}{1!0!1!0!0!\ldots0!} \times K_2{}^0K_3{}^1K_4{}^0\ldots = \left[\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]K_3 \qquad (19\mathrm{e})$$

and

(b)

$$\left[\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]\frac{1}{0!2!0!\ldots0!}K_2{}^2K_3{}^0K_4{}^0\ldots = \frac{1}{2}\left[\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]K_2{}^2 \qquad (19\mathrm{d})$$

Summation term for $\xi = 4$, $r = 3$. Conditions 1, 2, and 3 are satisfied for $\alpha_1 = 2$, $\alpha_2 = 1$, $\alpha_i = 0$, $i > 2$, only. Then, another summation term can be written

$$\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0}\frac{1}{2!1!0!0!\ldots0!}K_2{}^1K_3{}^0K_4{}^0\ldots = \frac{1}{2}K_2\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0} \qquad (19\mathrm{c})$$

For the fifth summation term (for $\xi = 4$, $r = 4$), conditions 1, 2, and 3 are satisfied only for $\alpha_1 = 4$, $\alpha_i = 0$, $i \neq 4$. Thus, a final summation term is generated for $\xi = 4$

$$\left(\frac{\mathrm{d}^{(3)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^3}\right)_{c=0}\frac{1}{4!0!0!\ldots0!}K_2{}^0K_3{}^0K_4{}^0\ldots = \frac{1}{24}\left(\frac{\mathrm{d}^{(3)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^3}\right)_{c=0} \qquad (19\mathrm{b})$$

Adding all summation terms obtained for $\xi = 4$, eq 15 is complete and can be written

$$\frac{K_4}{4} = K_4 + \left[\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]K_3 + \frac{1}{2}\left[\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} - BM_1\right]K_2{}^2 + \frac{1}{2}K_2\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0} + \frac{1}{24}\left(\frac{\mathrm{d}^{(3)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^3}\right)_{c=0} \qquad (19\mathrm{a})$$

Solving for K_4, using eq 17 and 18 and defining for simplicity

$$\left(\frac{\mathrm{d}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c}\right)_{c=0} = a$$

$$\left(\frac{\mathrm{d}^{(2)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^2}\right)_{c=0} = b$$

$$\left(\frac{\mathrm{d}^{(3)}\left(\frac{M_1}{M_{\mathrm{app}}}\right)}{\mathrm{d}c^3}\right)_{c=0} = d$$

$$BM_1 = X$$

$$\left(\tfrac{8}{3}a^3 + ab - \tfrac{1}{18}d\right) = c = K_{4(\mathrm{ideal})}$$

we can write

$$K_4 = \tfrac{8}{3}X^3 - 8aX^2 + (8a^2 - b)X + c \qquad (19)$$

Using the same definitions of a, b, and X, eq 18 can be written as

$$K_3 = \tfrac{3}{2}X^2 - 3aX + e \qquad (18)$$

with $e = \tfrac{3}{2}a^2 - \tfrac{1}{4}b = K_{3(\text{ideal})}$.

Derivation of Equations for the Virial Coefficient. Examination of eq 16–19 shows that any equilibrium constant for a nonideal system can be expressed as a polynomial in BM_1 whose coefficients can be obtained by the use of the experimentally determined quantity M_{app}. Thus, the solution of any of these polynomials requires that for that particular case, $K_i = 0$. Then, the value of BM_1 sought would be one of the roots of the given polynomial. A number of these roots (*e.g.*, imaginary roots) can be discarded at once. The remaining roots will have to be examined by inserting them in the equations for $K_i \neq 0$. Here, only roots leading to positive values of K_i would be acceptable. It is clear that this procedure will supply the value of BM_1 immediately in a number of cases, particularly when one or more associating species are missing at an early stage of the association reaction. However, cases such as indefinite states of association may be more difficult to deal with. In this type of case, an approximate value of BM_1 might be obtained by assuming a value of zero for the equilibrium constant of some species existing at a very low concentration.

Derivation of Equations to Determine Equilibrium Constants and Virial Coefficients Using $M_{\text{n app}}$. The approach and derivations necessary to obtain the value of equilibrium constants and virial coefficient from the apparent number-average molecular weight, $M_{\text{n app}}$, are essentially the same as with M_{app}. The only difference is the experimental information supplied, $M_{\text{n app}}$, obtained from osmotic pressure measurements or calculated by other means (*e.g.*, using eq 9). Only final equations need be given, therefore, as follows

$$K_1 = \left(\frac{M_1}{M_{\text{napp}}}\right)_{c=0} \qquad (20)$$

$$K_2 = -2\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\text{napp}}}\right)}{\mathrm{d}c}\right)_{c=0} + BM_1 \qquad (21)$$

$$K_3 = \frac{3}{2}(BM_1)^2 - 6\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\text{napp}}}\right)}{\mathrm{d}c}\right)_{c=0} BM_1 + \left[6\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\text{napp}}}\right)}{\mathrm{d}c}\right)^2 - \frac{3}{4}\left(\frac{\mathrm{d}^{(2)}\left(\dfrac{M_1}{M_{\text{napp}}}\right)}{\mathrm{d}c^2}\right)\right]_{c=0}$$

$$K_4 = \tfrac{8}{3}X^3 - 16aX^2 + (32a^2 - 3b)X + c \qquad (22)$$

with

$$a = \left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\text{napp}}}\right)}{\mathrm{d}c}\right)_{c=0}$$

$$b = \left(\frac{\mathrm{d}^{(2)}\left(\dfrac{M_1}{M_{\text{napp}}}\right)}{\mathrm{d}c^2}\right)_{c=0}$$

$$d = \left(\frac{\mathrm{d}^{(3)}\left(\dfrac{M_1}{M_{\text{napp}}}\right)}{\mathrm{d}c^3}\right)_{c=0}$$

$$c = -\tfrac{64}{3}a^3 + 6ab - \tfrac{2}{9}d = K_{4(\text{ideal})}$$

$$X = BM_1$$

It must be noted that in all cases the equilibrium constants obtained when $BM_1 = 0$ are identical with the equilibrium constants of the ideal case (Derechin, 1968).

Discussion

Steiner (1952, 1954) derived equations for the analysis of ideal associating systems. His derivations make use of the weight fraction of monomer and the definitions of either M_n or M_w, which are not presented as functionally interrelated. Thus, two independent methods were offered for computing the equilibrium constants from osmotic pressure and light scattering experiments, respectively. Adams and Fujita (1963) initiated the analysis of sedimentation and chemical equilibrium of nonideal systems, for which Adams (1965a,b) and Adams and Lewis (1968) derived expressions that permit the analysis of experimental results to yield the values of the equilibrium constants and virial coefficients. They also used the weight fraction of monomer. In addition, Adams (1965a,b) first established a functional relation between M_n and M_w and produced a unified theory that can be applied to ideal or nonideal systems. Derechin (1968) derived a theory for self-association reactions for ideal systems. This theory, like the present derivations, differs from those of the previous workers in several respects: (1) no use is made of the weight fraction of monomer; (2) a power expansion is used to represent the functional relation involving $M_{\text{w}c}$ and $M_{\text{n}c}$ (here $M_{\text{app}(c)}$ and $M_{\text{n app}(c)}$); (3) the concentration of the self-associating species, c, in this power expansion is expressed in terms of the equilibrium constants; (4) the resulting power expansion of the sum $\sum K_i c^i$ is solved by means of the multinomial theorem (see Parzen, 1960); (5) to obtain BM, no iterative process of successive approximations is needed here; (6) the theory presented here is equally valid for discrete (Adams, 1965a,b) and for indefinite (Adams and Lewis, 1968) states of association; (7) in contrast to treatments used by some other workers (Elias and Lys, 1966; Elias and Bareiss, 1967; Van Holde and Rossetti, 1967; Adams and Lewis, 1968) when dealing with indefinite state of associations no assumption is made here as to the identity or difference of the equilibrium constants.

The evaluation of the equilibrium constants by the present method must be preceded by the evaluation of the term BM_1. For this purpose, the type of population present in the system (*e.g.*, the highest state of aggregation or the absence of a given molecular species) must be known or assumed. This will allow the expression for a given equilibrium constant to be equated to zero. Once this is done, BM_1 is obtained as a root of that equation, for which purpose standard methods of algebra are available. For example, the absence of dimer (*e.g.*, $k_2 = 0$) would offer the "ideal" case of a nonideal system since in this case, by virtue of eq 17 and 21, the value of BM_1 is

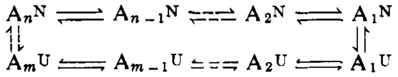
$$BM_1 = \left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\text{app}}}\right)}{\mathrm{d}c}\right)_{c=0} = 2\left(\frac{\mathrm{d}\left(\dfrac{M_1}{M_{\text{n app}}}\right)}{\mathrm{d}c}\right)_{c=0} \tag{23}$$

Similarly, the absence of trimer would offer uncomplicated access for determining the value of BM_1. Further, the simultaneous absence of dimer, trimer, etc., in the *initial* stages of the association reaction would offer a set of polynomials with at least one common root, namely, BM_1.

The evaluation of the successive derivatives of the function $M_1/M_{\text{app}(c)}$ (or $M_1/M_{\text{n app}(c)}$) in the vicinity of $c = 0$ for the associating solute may present some difficulties. However, where precise determinations of $c(r)$ are available in this concentration range, then, by a combination of analytical and graphical procedures these functions and their successive derivatives can be analyzed and hence a certain number of derivatives can be obtained. The solution to this problem is, therefore, largely dependent upon the availability of accurate measurements in the low concentration range. The use of the photoelectric scanner in the absorption optical system (Hanlon *et al.*, 1962; Schachman *et al.*, 1962) for ultracentrifuge work is one method that can provide increased sensitivity. A recent method using interference optics (Derechin, 1969) also offers increased sensitivity and precision in sedimentation equilibrium measurements.

An additional problem may arise in practice from the occurrence of secondary aggregation superimposed on the fundamental dissociation reaction. The present theory starts at eq 1, and with the assumption that the reaction goes from left to right. However, many biological materials (*e.g.*, subunit type enzymes) reach the experimental bench in one form of aggregation or another. The study of the chemical equilibrium of these systems must be preceded by the finding of experimental conditions that can lead to dissociation; hence, the expected reaction will proceed from right to left. This is important since the forces that keep the molecules in the associated form are usually of the same nature as the forces that keep each individual molecule in a given folded configuration. Then, any change in the system that can lead to dissociation may lead to an unfolding followed by secondary aggregation due to newly exposed groups (*e.g.*, hydrophobic or SH groups). As examples of such phenomena, our recent work on yeast hexokinases A and B (Lazarus *et al.*, 1968) shows in certain experimental conditions the concomitant occurrence of dissociation and aggregation; also, with prostatic acid phosphomonoesterase M. Derechin, W. Ostrowski, and E. A. Barnard, (to be published) show that the dissociation of the molecule in all conditions studied is always accompanied by aggregation, even under very mild conditions: extensive dissociation is combined with extensive aggregation, slight dissociation with slight aggregation, etc. Although the theory predicts the complete dissociation of all aggregates, without enquiry as to whether they are primary or secondary, into monomers at high dilution, this may happen at a concentration so low as to be beyond our capability of examination. Further, even if this dissociation does occur in a concentration range amenable to physical examination, the unfolded molecules may then enter into a new association distinct from that directly available to the folded form. Of course, if an essentially irreversible process can affect one component, as in the case of oxidation of SH groups in an unfolded monomer, the equilibrium is altered and apparent equilibrium constants would be misleading. It should be noted that, usually, the analysis of an associating macromolecular system is intended to describe the behavior of the biologically "native" molecules and the forms immediately derived therefrom (either subunits or aggregates). However, we are emphasizing here the probably frequent occurrence of secondary association reactions (*e.g.*, of unfolded subunits), so that a sequence of dissociations and associations involving both multimolecular and unimolecular components (Scheme I) should then be analyzed. If there is any risk of a behavior such as shown in Scheme I interfering, then it must be remembered that it will be necessary to distinguish and interpret the various constants obtained in these analyses in the light of the complexity of the real systems involved.

SCHEME I[a]



[a] One of a family of patterns that may occur to complicate the self-association equilibria being analyzed. The superscripts N and U indicate, respectively, the native and unfolded forms of a protomer, A. Under various conditions (involving in some cases perhaps simply different concentration ranges) the association of the folded or unfolded forms may be preferred. The broken lines indicate that some of the equilibria may be inaccessible.

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Self-Associating Systems. III. Multinomial Theory for Ideal Systems Using the *z*-Average Molecular Weight*

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ABSTRACT: A general expression for the calculation of equilibrium constants of self-associating systems of ideal systems using the *z*-average molecular weight has been established. The derivations make use of the multinomial theorem but represent an independent method from that described (Derechin, M. (1968), *Biochemistry 7*, 3253) for use with the number, M_n, and weight-average, M_w, molecular weights.

In a previous paper (Derechin, 1968) self-association reactions of the type

$$nP_1 \rightleftarrows a_2P_2 + a_3P_3 + \cdots + a_mP_m$$

with

$$\sum_{i=2}^{m} ia_i = n \qquad (1)$$

for ideal systems have been examined. In that work, equations for the calculation of equilibrium constants from experimentally determined values of the number- and weight-average molecular weights were derived making use of multinomial theory. In this paper an independent theory that uses the same formal approach as before was derived for the analysis of self-association reactions using the *z*-average molecular weight.

Theoretical

It is assumed here that all species participating in the self-associating reaction have the same partial specific volume ($\bar{v}_1 = \bar{v}_2 = \cdots = \bar{v}$) and the same refractive index increment $(dn/dc_1)_{T,P} = (dn/dc_2)_{T,P} = \cdots = (dn/dc)_{T,P}$ and that the activity coefficient of each associating species can be represented by a series expansion as

$$\ln y_i = iBM_1c + \text{higher power of } c \qquad (2)$$

where B is the virial coefficient, M_1 is the molecular weight of the monomer, and c is the total solute concentration. At low solute concentration the higher powers in c are neglected. In the particular case, $i = 1$

$$\ln y_1 = BM_1c \qquad (2a)$$

Using eq 2 and 2a and the nomenclature of Adams and Fujita (1963), the concentration of each associating species and the total solute concentration, respectively, for a system at equilibrium can be stated as

$$c_i = K_i\frac{y_1^{\,i}}{y_i}c_1^{\,i} = K_ic_1^{\,i} \qquad i = 1, 2, \ldots \qquad (3a)$$

and

$$c = \sum_{i=1}^{m} K_ic_1^{\,i} \qquad i = 1, 2, \ldots \qquad (3)$$

By virtue of eq 3, $K_1 = 1$. Differentiating eq 3 and rearranging we have

$$\frac{dc}{dc_1} = \sum_{i=1}^{m} iK_ic_1^{\,i-1} \qquad (4)$$

The *z*-average molecular weight, M_{zc}, is defined here following suggested nomenclature of Adams and Williams, (1964); the subscript *c* is added to M_z to indicate a concentration dependent quantity. Thus

$$M_{zc} = \frac{\Sigma c_iM_i^{\,2}}{\Sigma c_iM_i} = \frac{M_1\Sigma i^2c_i}{\Sigma ic_i} \qquad (5)$$

Using eq 3a, 4, and 5, we can write

$$\frac{M_{zc}dc}{M_1} = \sum_{i=1}^{m} i^2K_ic_1^{\,i-1}dc_1 \qquad (6)$$

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