Alternative Equations for Mass and Molar Equilibrium Constants Derived from the Multinomial Theory†

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ABSTRACT: A method is described for the calculation of equilibrium constants of self-associating systems using the successive $d^{(r)}M_{w,app}/dc^r$. Using this method it is suggested that lysozyme solutions at pH 7.2, $I = 0.2$, and 4° contain monomers, dimers, and trimers. A small positive virial coefficient was computed for this system.

In previous papers (Derechin, 1968, 1969a,b, 1971, 1972) the multinomial theory for the analysis of self-associating systems was presented. The equations derived for the calculation of the (mass) equilibrium constants make use of the successive $(d^{(r)}Rk/dc^r)_{c=0}$, with $r = 1, 2 \ldots$ where Rk represents respectively the various ratios involving the monomer molecular weight as follows: $R1 = M_1/M_{n,app}(c)$, $R2 = M_1/M_{w,app}(c)$, and $R3 = M_{z,app}/M_1$, at $c = 0$. In this paper, equations that permit the calculation of mass equilibrium constants using the successive $(d^{(r)}M_{w,app}/dc^r)_{c=0}$ are presented, together with alternative equations for the calculation of molar equilibrium constants. Finally, methods described before (Derechin, 1971) for the analysis of experimental cases using the multinomial theory are applied to the new set of equations.

Theory

The equations for the calculation of the mass equilibrium constants of self-associating systems using $M_{z,app}(c)$ (Derechin, 1972) can be written

$$K_2 = X + \frac{1}{2M_1}\left(\frac{dM_{z,app}}{dc}\right)_{c=0} \tag{1}$$

$$K_3 = \frac{3}{2}X^2 + \frac{3}{2M_1}\left(\frac{dM_{z,app}}{dc}\right)_{c=0}X + \frac{1}{4M_1^2}\left(\frac{dM_{z,app}}{dc}\right)^2_{c=0} + \frac{1}{12M_1}\left(\frac{d^{(2)}M_{z,app}}{dc^2}\right)_{c=0} \tag{2}$$

$$K_4 = \frac{8}{3}X^3 + \frac{4}{M_1}\left(\frac{dM_{z,app}}{dc}\right)_{c=0}X^2 + \left[\frac{3}{2M_1^2}\left(\frac{dM_{z,app}}{dc}\right)^2_{c=0} + \frac{1}{3M_1}\left(\frac{d^2M_{z,app}}{dc^2}\right)_{c=0}\right]X + \frac{1}{8M_1^3}\left(\frac{dM_{z,app}}{dc}\right)^3 + \frac{1}{9M_1^2}\left(\frac{dM_{z,app}}{dc}\right)_{c=0} \times \left(\frac{d^{(2)}M_{z,app}}{dc^2}\right)_{c=0} + \frac{1}{72M_1}\left(\frac{d^3M_{z,app}}{dc^3}\right)_{c=0} \tag{3}$$

It has been shown (Adams, 1962; Adams and Filmer, 1966) that

$$M_{z,app}(c) = \frac{d}{dc}cM_{w,app}(c) \tag{4a}$$

that is,

$$M_{z,app}(c) = M_{w,app}(c) + c\frac{d}{dc}M_{w,app}(c) \tag{4b}$$

It follows that

$$\left(\frac{d^{(r)}M_{z,app}}{dc^r}\right)_{c=0} = (r+1)\left(\frac{d^{(r)}M_{w,app}}{dc^r}\right)_{c=0} \tag{5}$$

Combining eq 5 with eq 1–3, we obtain expressions for the equilibrium constants of self-associating systems using the successive derivatives of $M_{w,app}(c)$ with respect to c. These equations can be written as

$$K_2 = X + ma \tag{6}$$

$$K_3 = \frac{3}{2}X^2 + 3maX + m^2a^2 + \frac{1}{4}mb \tag{7}$$

$$K_4 = \frac{8}{3}X^3 + 8maX^2 + [6m^2a^2 + mb]X + m^3a^3 + \frac{2}{3}m^2ab + \frac{1}{18}mc \tag{8}$$

where $m = 1/M_1$, $X = BM_1$, and a, b, and c, respectively are the first, second, and third derivatives of $M_{w,app}(c)$ with respect to c at $c = 0$.

It has been shown (Steiner, 1952) that the various equilibrium constants of a system containing various species are expressible in several interrelated forms, since the various descriptions are (Roark and Yphantis, 1969) thermodynamically equivalent. Using the symbols K_i and k_i, respectively, to represent the various mass and molar equilibrium constants with $i = 1, 2, \ldots, n$ for self-associating systems containing all the successive species from monomer to n-mer, K_i^* and k_i^* for the parallel equilibrium constansts for the various monomer–m-mer equilibria, with $m = 1, 2, \ldots, n$ and

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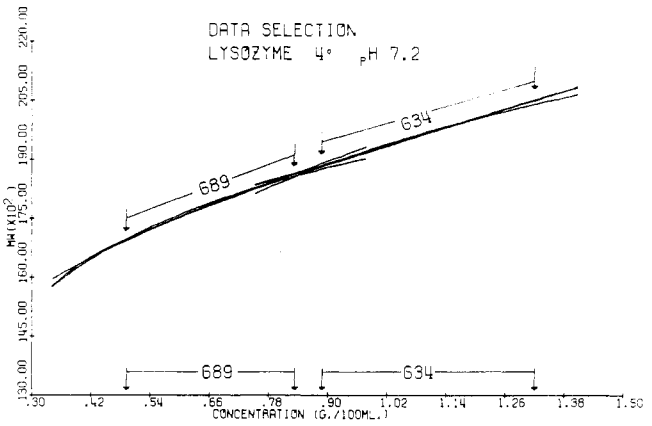

FIGURE 1: Results of computations with polynomials degree 2–4 for two experiments (689 and 634) showing the "cut-off" points for selection of data. Sections between vertical arrows were used, as shown.

P_i for the various associating species present, we can write

$$P_1 + P_1 \rightleftharpoons P_2$$

$$K_2 = K_2^* = \frac{c_2}{{c_1}^2};\ c_2 = K_2^* {c_1}^2$$

$$3P_1 \rightleftharpoons P_3$$

$$K_3^* = \frac{c_3}{{c_1}^3};\ c_3 = K_3^* {c_1}^3$$

$$P_2 + P_1 \rightleftharpoons P_3$$

$$K_3 = \frac{c_3}{c_2 c_1} = \frac{K_3^*}{K_2^*} \tag{9}$$

and so on. It can be seen that the various equilibrium constants for the sequential associations can be written

$$K_i = \frac{K_i^*}{K_{i-1}^*} \tag{10}$$

and, further, that the concentration of each associating species can be expressed in terms of the parallel, single-step, association constants, and the monomer concentration as

$$c_i = K_i^* {c_1}^i \tag{11}$$

Although eq 11 has been used by Adams *et al.* (1963, 1966) and Derechin (1968, 1969a,b, 1972) to derive their equations for the equilibrium constants, no explicit distinction between K_i and K_i^* was made in their derivations. Yet, this distinction is relevant since the equilibrium constants computed directly from their equations are the various K_i^* and not the K_i values. The latter can be obtained from the former, using eq 10.

The molar equilibrium constants can be expressed in terms of their mass counterparts. Using eq 11 and 12

$$A_i = \frac{10 c_i}{i M_1} \tag{12}$$

where A_i = molar concentration of species i and c is the concentration in g/100 ml

$$k_i = \frac{i-1}{i}\frac{M_1}{10}\frac{K_i^*}{K_{i-1}^*} = \frac{i-1}{i}\frac{M_1}{10}K_i \tag{13a}$$

and

$$k_i^* = \frac{{M_1}^{i-1}}{10^{i-1}}\frac{K_i^*}{i} \tag{13b}$$

Comparing eq 13 it can be seen that irrespective of the presence of associating species higher than the dimer $k_2 = k_2^*$.

Occasionally it is found convenient to assume ideality for an associating system. This assumption greatly simplifies derivations and computations since all terms in X in eq 1–3 and 6–8 vanish. In addition the mass equilibrium constants, K_i^*, for simple ideal monomer–n-mer equlibria can be easily calculated from the equations for the various K_i (*e.g.*, eq 1–3) by making $M_{z,\mathrm{app}}(c) = M_z(c)$, $M_{w,\mathrm{app}}(c) = M_w(c)$, and

$$\left(\frac{d^{(r)}M_z}{dc^r}\right)_{c=0} = \left(\frac{d^{(r)}M_w}{dc^r}\right)_{c=0} = X = 0 \tag{14}$$

for all $r < i - 1$. For a monomer–trimer association this leads to

$$K_{3,\mathrm{ID}}^* = \frac{1}{12M_1}\left(\frac{d^{(2)}M_z}{dc^2}\right)_{c=0} = \frac{1}{4M_1}\left(\frac{d^{(2)}M_w}{dc^2}\right)_{c=0} \tag{15a}$$

and using eq 11

$$k_{3,\mathrm{ID}}^* = \frac{M_1}{3600}\left(\frac{d^{(2)}M_z}{dc^2}\right)_{c=0} = \frac{M_1}{1200}\left(\frac{d^{(2)}M_w}{dc^2}\right)_{c=0} \tag{15b}$$

$$k_{3,\mathrm{ID}} = \frac{1}{30}\left(\frac{dM_z}{dc}\right)_{c=0} + \frac{M_1}{90}\left(\frac{d^{(2)}M_z}{dc^2}\right)_{c=0}\Bigg/\left(\frac{dM_z}{dc}\right)_{c=0} \tag{15c}$$

$$= \frac{1}{15}\left(\frac{dM_w}{dc}\right)_{c=0} + \frac{M_1}{60}\left(\frac{d^{(2)}M_w}{dc^2}\right)_{c=0}\Bigg/\left(\frac{dM_w}{dc}\right)_{c=0}$$

Experimental Section

Data from low-speed sedimentation equilibrium experiments using lysozyme at pH 7.2, I = 0.2, and 4° examined before (Derechin, 1971) were analyzed here again. Experimental details described before (Derechin, 1971) are applicable here and will not be described again.

Preparation of the Data and Computations. For the selection of data, plots of $M_{w,\mathrm{app}}$ *vs.* c obtained with various polynomials were compared and sections of these data showing obvious discrepancies of respresentation (Figure 1) were removed. In addition the polynomials and data for $M_{w,\mathrm{app}}(c)$ from various experiments were examined so that the final selection resulted in the most continuous and smooth curve for experiments in successive concentration ranges (Figure 2). This condition is based on the assumption that the associating species being analyzed belong to one and only one thermodynamic system.

The computations were made on a CDC 6400 computer by methods described before (Derechin, 1971), except that

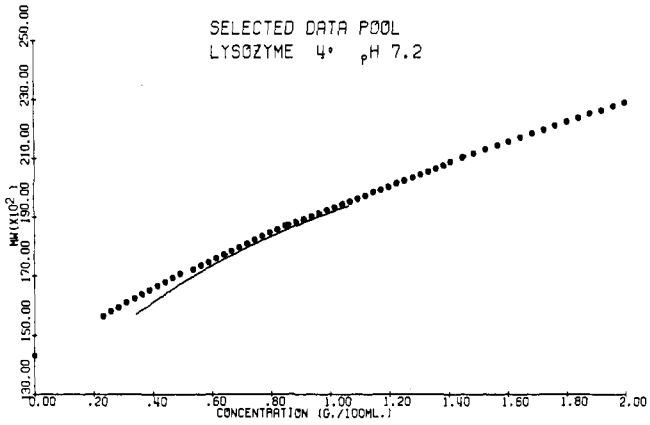

FIGURE 2: Final data pool, $M_{w,app}$ *vs.* concentration of lysozyme at pH 7.2, 4°, used in computations. Experiment shown by continuous line was not used.

the various derivatives, $d^{(r)}M_w/dc^r$, were calculated using the analytical derivative of the polynomial selected to represent their respective primitive function. Thus, $(dM_{w,app})/dc = (d/dc)P(c) = P'(c)$, where $P(c)$ is the polynomial selected to represent $M_{w,app}(c)$. In all cases, the coefficient of the linear term of the ploynomial selected for primitive function was taken as $(d^{(r)}M_{w,app}/dc^r)_{c=0}$ Although results obtained with this procedure were essentially the same as those calculated from $\Delta y/\Delta x$ at small increments of the independent variable, the computations were simplified.

Results

Using sets of equilibrium constants and virial coefficients in the range close to $K_2 = 0.43$, $K_3 = 0.20$, and $B \simeq 0$, a range suggested before (Derechin, 1971) for lysozyme at pH 7.2, $I = 0.2$, and 4°, the first and second $(d^{(r)}M_{w,app}/dc^r)$ (c) values obtained with model systems were compared with their experimental counterparts (D test). Figure 3 shows the best correspondence for the system $K_2 = 0.424$, $K_3 = 0.215$, and $BM_1 = 0.017$. This value of BM_1 was estimated using the D test (Derechin, 1971) as shown in Figure 4. It can be seen

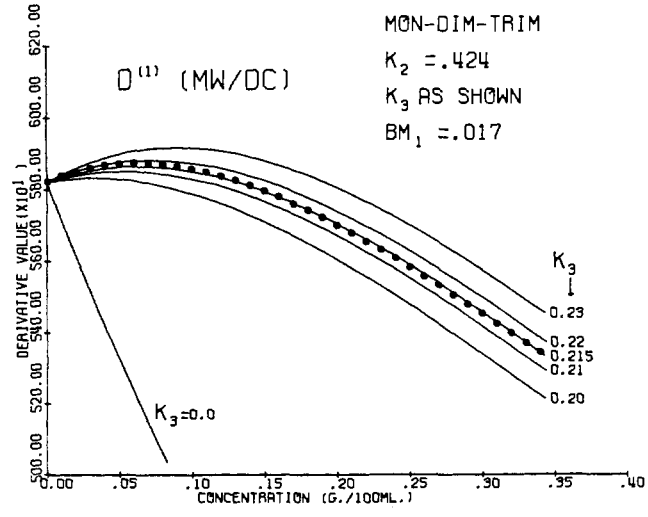

FIGURE 3: D test for a monomer–dimer–trimer system $D^{(1)}$ from experiment comparing symbols *vs.* $D^{(1)}$ from various systems (lines) as shown.

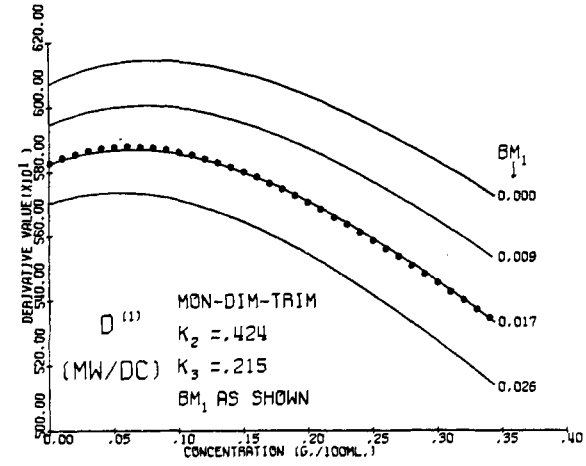

FIGURE 4: $D^{(1)}$ for a monomer–dimer–trimer system with $K_2 = 0.424$, $K_3 = 0.215$ to approximate the value of the virial coefficient.

(Figure 3) that the monomer–dimer model system ($K_3 = 0.0$) greatly departs from the behavior of the experimental case. On the contrary, models assuming a monomer–dimer–trimer system approximated very closely the behavior of the experimental data when model *vs.* experimental $D^{(1)}$ (Figure 3) were compared. As expected from the results shown in Figure 4, the comparison of monomer–dimer–trimer–tetramer model systems (Figure 5) showed that the case $K_4 = 0.0$ gave the best correspondence with experiment. When the $M_{w,app}(c)$ experimental was compared (M_w test) with the corresponding model function calculated using $K_2 = 0.424$, $K_3 = 0.215$, $K_4 = 0.0$, and $BM_1 = 0.017$, the correspondence obtained was excellent throughout, with the model system showing slightly lower (less than 1%) values of $M_{w,app}(c)$ at concentrations higher than 2 g/100 ml only. Table I summarizes the computation of mass and molar equilibrium constants and of the standard free energy, $\Delta F°$, for this study.

Discussion

A new method for the calculation of the equilibrium constants of nonideal associating systems based on the multinomial theory is presented. Using this method, the analysis of data obtained with lysozyme at pH 7.2, $I = 0.2$, and 4°

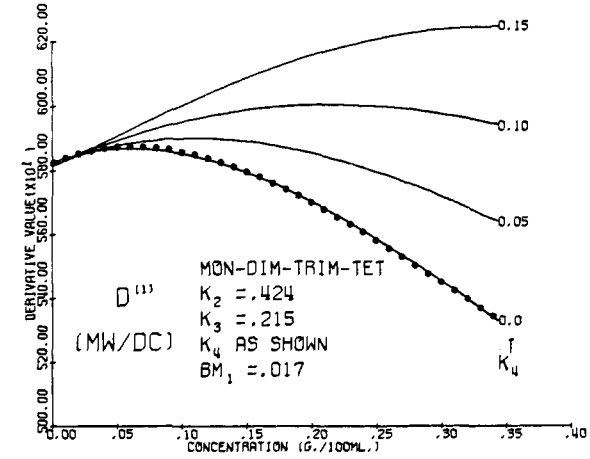

FIGURE 5: D test for a monomer–dimer–trimer–tetramer system, using $D^{(1)}$.

TABLE I: Constants Computed for Lysozyme at pH 7.2, 4°, $I = 0.2$.

Constants	Mass Equilibrium Constant	Molar Equilibrium Constant	$\Delta F°$
K_2 or k_2	0.430	300	−3150
K_3 or k_3	0.500	480	−3400
K_3* or k_3*	0.215	1.47×10^5	−6550

again showed that these protein solutions contain monomers, dimers, and trimers. In addition a small positive virial coefficient was computed for this system. Besides being an alternative procedure for the analysis of associating systems, this method is much more sensitive to variations in the magnitude of B than the procedures using the successive

$$\frac{d^{(r)}}{dc^r} R_{app} = \frac{d^{(r)}}{dc^r} (R + BM_1c) \quad (16)$$

with $R = R1$ or $R = R2$, since here the term in BM_1 vanishes after the first derivative. On the contrary, an increasing number of terms in BM_1 appear in the successive derivatives

$$\frac{d^{(r)}}{dc^r} M_{w,app} = \frac{d^{(r)}}{dc^r} \left(\frac{M_w(c)}{1 + BM_w(c)} \right) \quad (17a)$$

as used in the present method. The same applies to the method employing the successive

$$\frac{d^{(r)}}{dc^r} M_{z,app} = \frac{d^{(r)}}{dc^r} cM_{w,app} \quad (17b)$$

as in eq 1–3. The assumption of ideality as a simplifying factor in the computations involves the possible incorporation of an error which is larger the higher the associating species. Thus, in the case of a monomer–dimer–trimer association with $K_2 = K_3 = 1.0$ and $BM_1 = 0.1$, computations using equations for the ideal case would lead to results such as $K_2 = 0.90$, $K_3 = 0.71$, $K_4 = -0.5$. Besides leading to error in the calculated constants, these results frequently suggest the presence of species not existing in the system. For the case of a monomer–dimer equilibrium and the assumption of ideality the use of eq 1, 11, and 6 leads to

$$k_2 = \frac{1}{40} \left(\frac{dM_z}{dc} \right)_{c=0} = \frac{1}{20} \left(\frac{dM_w}{dc} \right)_{c=0} \quad (18)$$

This relation was suggested to this author by an anonymous referee of paper III of this series (Derechin, 1969b) with the correct comment that eq 18 permits the calculation of the molar dimerization constant without the knowledge of M_1. Unfortunately the use of eq 18 without the knowledge of M_1 appears infeasible, since this information is essential for the computation of the functions $M_w(c)$ and $M_z(c)$ down to $c = 0$. However, if the value of M_1 is available, this relationship may be of value.

The need for high-degree polynomials to represent the function $M_{w,app}(c)$ and to permit calculation of the equilibrium constants is greater in the present method than with procedures using the successive derivatives $d^{(r)}(M_1/M_{k,app})/dc^r$. This follows from the fact that the method described here calls for derivatives of functions of the type $y = x^n$, whereas the previous methods require derivatives of functions of the type $y = x^{-n}$, with $n = 2, 3, \ldots$.

Adams (1965) and Adams and Filmer (1966) described a method that permits the description of associating systems and is less sensitive to the usual experimental error than the methods of the multinomial theory. However, due to lower sensitivity at concentrations below 1 g/100 ml, that latter method may not be suitable for the analysis of proteins available in minute quantities. On the contrary, this is not an obstacle for the application of the multinomial theory. The limitation here is determined by the quality of the experimental data. Thus, in parallel with advances in the methodology, the multinomial theory should be useful for the analysis of self-associating systems, if not always alone, then combined with other methods (*e.g.*, Adams, 1965; Adams and Filmer, 1966; Roark and Yphantis, 1969).

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