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ANALYSIS OF VARIOUS INDEFINITE SELF-ASSOCIATIONS OF THE AK TYPE

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Although we and others have developed equations to analyze for some indefinite self-associations that might be encountered, it is felt in some cases that these models, known as the sequential, equal equilibrium constant (SEK) models, might overestimate the size of aggregates encountered at higher solute concentrations. Thus, Garland and Christian proposed two attenuated equilibrium constant (AK) models that might overcome this problem. Their methods were restricted to ideal solutions and to osmometric procedures. We have removed these restrictions, and we have developed equations for analyzing four AK models that might be encountered. Various tests to aid in distinguishing these models are presented. These procedures have been tested with two simulated examples of a Type III AK indefinite self-association.

1. Introduction

Self-associations that appear to continue without limit are known as indefinite self-associations [1–4]. In order to analyze these self-associations some assumptions had to be made so that infinite series could be developed to describe the total concentration (c) and the number (M_{nc}) and weight (M_{wc}) average molecular weights or their apparent values (M_{na} and M_{wa}) under nonideal conditions. In order for these series to converge it was necessary to assume that the product of the intrinsic equilibrium constant (k) and the monomer concentration (c_1) in g/ℓ or other related products was restricted to a range between 0 and 1, i.e., $0 \leqslant kc_1 < 1$. The model has been described as the sequential equal equilibrium constant (SEK) model [3,5]. In a previous publication from this laboratory, some methods have been developed for analyzing four types of SEK model indefinite self-associations [1]. Here we present some methods for analyzing four analogous, indefinite self-associations, but in this model, known as the attenuated equilibrium constant (AK) model, one does not have to restrict values of kc_1 (c_1 in g/ℓ) or other related quantities to a range between zero and one, i.e., here $0 < kc_1 < \infty$.

Studies on the self-association of nucleic acid nitrogen bases, their derivatives, and on some nucleosides in aqueous solution have led to the conclusion that these solutes self-associate in stacks that are made up of the planar base moiety [5,6]. This interaction, known as base stacking, is attributed to hydrophobic interactions rather than hydrogen bonding. The SEK Model Type I indefinite self-association (also known as the random, open or isodesmic self-association) and other SEK models have been used to analyze these associations. For some of these materials that associate to a very high degree, the Type I SEK model may overestimate the degree of association at higher solute concentrations [5–8]. Attempts to correct for this have involved using a two equilibrium constant model (the Type III SEK Model) [5,9], placing an artificial limit on the size of the stack [7,8], and introducing activity coefficients (calculated from statistical thermodynamic models) for the aggregates, which leads to a sequence of decreasing association constants [10].

Garland and Christian [5] have suggested another approach to this problem, and they have tested their idea experimentally with kinetic and thermodynamic data. In the SEK model it has been assumed for any association step

$$P_1 + P_{i-1} \rightleftharpoons P_i \quad (i = 2, 3, ...) \tag{1}$$

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that a) the standard enthalpy-change is the same for each step ($\Delta H_2^0 = \Delta H_3^0 = ... = \Delta H^0$), and b) that the standard entropy change for all steps is constant ($\Delta S_2^0 = \Delta S_3^0 = ... = \Delta S^0$). Since a chemical equilibrium is present, the base molecules of the various stacks are able to exchange reversibility with one another, and Garland and Christian pointed out that it seemed more reasonable that there should be a varying entropy change for each step. Their arguments led to these relations [5]:

$$\Delta S_i^0 = -R \ln i + \text{constant} \quad (i = 2, 3, ...) \tag{2}$$

and

$$K_i = \exp(-\Delta G_i^0/RT) = \text{constant}$$

$$\times \exp(-\ln i)\exp(-\Delta H^0/RT) \tag{3}$$

for $P_1 + P_{i-1} \rightleftharpoons P_i$. For this model, the AK model, the equilibrium constant K_i is attenuated by an integer i ($i = 2, 3, ...$) for each successive step. In the AK model it is still assumed that the ΔH^0 values are the same for each step. The AK model was developed for a Type I and a Type III ideal self-association by Garland and Christian [5]. In addition their treatment was restricted to osmometric methods (membrane or vapor pressure osmometry) and used molar equilibrium concentrations, molar stoichiometric (formal) concentrations and the osmotic coefficient (which is the same as M_1/M_{na}, where M_1 is the molecular weight of the monomer).

Since nonideal effects have been reported in the study of the self-associations of nucleic acid bases and nucleosides in aqueous solutions [11–13], and since nonosmometric methods (sedimentation equilibrium and light scattering) are used to study these other self-associations, it seemed appropriate to remove these restrictions. The AK model may also apply to the self-association of some other solutes besides nucleosides and related compounds. Thus we have developed methods here for analyzing the four AK analogs of the SEK model indefinite self-associations already reported on [1]. In addition we have developed standard plots of η versus ξ to test for the Types I and II AK indefinite self-associations; these previously used quantities (see refs. [3], [14] and [15] for example) are defined by eqs. (14) and (13), respectively. For the Types III and IV AK indefinite self-associations we have developed analogs of the η versus ξ diagnostic plots.

2. Quantities needed for the analysis

In order to analyze the AK self-associations the following conditions will be assumed to apply to the self-associating species [1–4]: 1) The natural logarithm of the activity of any self-associating species i will be represented by

$$\ln y_i = iB_* M_1 c \quad (i = 1, 2, ...). \tag{4}$$

Here B_* is a constant whose value depends on the temperature and the solute-solvent combination. 2) The refractive index increments ($(\partial n/\partial c)_{T,P}$) of the associating species are equal. 3) The partial specific volumes ($\bar{v}$) or the density increments ($1000(\partial \rho/\partial c)_\mu$) of the associating species are equal. For self-association solutes that ionize, it will be assumed that the solutions are made up in the presence of supporting electrolyte and buffers and then dialyzed against the solvent (buffer) solution, so that the association species are defined according to the Vrij-Overbeek [16] or Casassa-Eisenberg [17] conventions. The subscript μ in the density increment ($1000(\partial \rho/\partial c)_\mu$) indicates this has been done, and that the diffusible solutes have the same chemical potential in the solution and in the buffer. The equilibrium constant(s) and the nonideal term will refer to species defined by these conventions.

Assumption one makes it simpler to analyze nonideal self-associations. It is the only one of the three assumptions needed in order to obtain M_{nc} or M_{na} from membrane or vapor pressure osmometry. The reasons for using this assumption, and its validity are described elsewhere [1–4,18]. The first two assumptions are needed in order to obtain M_{wc} or M_{wa} from sedimentation equilibrium experiments.

At constant temperature when a series of experiments at different concentrations have been carried out, one can make plots of M_1/M_{na} versus c or of M_1/M_{wa} versus c, and then use these plots to analyze the data. When M_{wa} (or M_{wc}) and c are the primary data, the following quantities are available [1,3,4,19]:

$$M_1/M_{wa} = M_1/M_{wc} + BM_1 c. \tag{5}$$

Here,

$$B = B_* + (\bar{v}/1000\, M_1), \tag{6}$$

and BM_1 is known as the nonideal term. An ideal self-association will be defined here as one for which $BM_1 = 0$.

$$\frac{M_1}{M_{\mathrm{na}}}=\frac{1}{c}\int_0^c \frac{M_1}{M_{\mathrm{wa}}}\,\mathrm{d}c=\frac{M_1}{M_{\mathrm{nc}}}+\frac{BM_1c}{2}, \tag{7}$$

$$\ln f_{\mathrm{a}}=\int_0^c\left(\frac{M_1}{M_{\mathrm{wa}}}-1\right)\frac{\mathrm{d}c}{c}=\ln f_1+BM_1c. \tag{8}$$

Here $f_1=c_1/c$ is the weight fraction of monomer, and f_{a} is its apparent value under nonideal conditions. When M_{na} (or M_{nc}) and c are the primary data, the following quantities are available [2]:

$$M_1/M_{\mathrm{na}}=M_1/M_{\mathrm{nc}}+BM_1c/2, \tag{9}$$

$$\frac{M_1}{M_{\mathrm{wa}}}=\frac{\mathrm{d}}{\mathrm{d}c}\left(\frac{cM_1}{M_{\mathrm{na}}}\right)=\frac{M_1}{M_{\mathrm{na}}}+c\frac{\mathrm{d}}{\mathrm{d}c}\left(\frac{M_1}{M_{\mathrm{na}}}\right)$$
$$=\frac{M_1}{M_{\mathrm{wc}}}+BM_1c, \tag{10}$$

$$\ln f_{\mathrm{a}}=\int_0^c\left(\frac{M_1}{M_{\mathrm{na}}}-1\right)\frac{\mathrm{d}c}{c}+\left(\frac{M_1}{M_{\mathrm{na}}}-1\right)$$
$$=\ln f_1+BM_1c. \tag{11}$$

For strong self-associations a plot of $(M_1/M_{\mathrm{wa}}-1)/c$ or $(M_1/M_{\mathrm{na}}-1)/c$ required for the evaluation of $\ln f_{\mathrm{a}}$ may not be too reliable at very low concentrations. Thus, it may be advantageous to make the plots from the lowest reliable concentration, denoted as c_*, and evaluate $\ln f_{\mathrm{a}}/f_{\mathrm{a}*}$, where $f_{\mathrm{a}*}$ is the value of f_{a} at c_*. In some cases, particularly with strong self-associations, it may be advantageous to use the quantity $\ln(f_{\mathrm{a}}/f_{\mathrm{a}*})$ which is defined by

$$\ln(f_{\mathrm{a}}/f_{\mathrm{a}*})=\int_{c_*}^c\left(\frac{M_1}{M_{\mathrm{wa}}}-1\right)\frac{\mathrm{d}c}{c}$$
$$=\int_{c_*}^c\left(\frac{M_1}{M_{\mathrm{na}}}-1\right)\frac{\mathrm{d}c}{c}+\left(\frac{M_1}{M_{\mathrm{na}}}-\frac{M_1}{M_{\mathrm{na}*}}\right)$$
$$=\ln(f_1/f_{1*})+BM_1(c-c_*). \tag{12}$$

The quantities M_{na}, M_{wa} and $\ln f_{\mathrm{a}}$ can be combined in various ways to analyze the data. Two very useful relations are given by the quantities ξ and η, which are defined by [1,2]

$$\xi=2M_1/M_{\mathrm{na}}-M_1/M_{\mathrm{wa}}=2M_1/M_{\mathrm{nc}}-M_1/M_{\mathrm{wc}}, \tag{13}$$

and

$$\eta=M_1/M_{\mathrm{wa}}-\ln f_{\mathrm{a}}=M_1/M_{\mathrm{wc}}-\ln f_1, \tag{14}$$

whenever eq. (4) applies. We will show how ξ, η and $\ln(f_{\mathrm{a}}/f_{\mathrm{a}*})$ can be used to analyze four types of attenuated equilibrium constant (AK) model indefinite self-associations.

3. Analysis of the AK model indefinite self-associations

3.1. Type I AK indefinite self-associations [3,5]

For this model it is assumed that all species are present; furthermore, it is assumed that the molar equilibrium constant between successive steps (see eq. (1)) is attenuated by a factor i ($i=2,3,\ldots$). Thus for any step described by eq. (1)

$$K_{(i-1)i}=[\mathrm{P}_i]/[\mathrm{P}_{(i-1)}][\mathrm{P}_1]=K/i$$
$$=K_i\quad \text{(as defined by Garland and Christian [5])} \tag{15}$$

and

$$[\mathrm{P}_i]=(K/i)[\mathrm{P}_{(i-1)}][\mathrm{P}_1]=(K^{i-1}/i!)[\mathrm{P}_1]^i. \tag{16}$$

The total concentration of the associating solute, P, in g/ℓ becomes

$$c=c_1+kc_1^2+(k^2/2)c_1^3+(k^3/2\cdot 3)c_1^4+\ldots$$
$$=c_1\exp(kc_1)=c_1\exp(x),$$
$$x=kc_1,\quad 0<kc_1<\infty. \tag{17}$$

Here

$$k=K/M_1.$$

The expressions for M_1/M_{nc}, M_1/M_{wc}, ξ and η become

$$\frac{M_1}{M_{\mathrm{nc}}}=\frac{1}{kc_1}[1-\exp(-kc_1)]=\frac{1}{x}[1-\exp(-x)], \tag{18}$$

$$M_1/M_{\mathrm{wc}}=1/(1+kc_1)=1/(1+x), \tag{19}$$

$$\xi=\frac{2M_1}{M_{\mathrm{na}}}-\frac{M_1}{M_{\mathrm{wa}}}=\frac{2}{x}[1-\exp(-x)]-\frac{1}{1+x}, \tag{20}$$

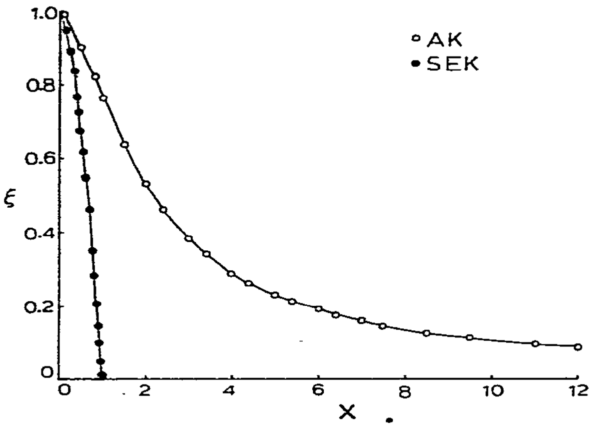

Fig. 1. Plot of ξ versus x for the Type I AK and Type I SEK models. An approximate value of x may be found if one knows the experimentally determined quantity ξ.

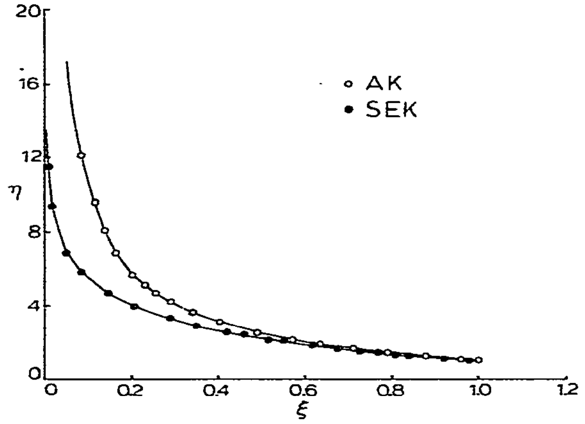

Fig. 2. Standard plot of η versus ξ for the Type I AK and Type I SEK models. Failure of the ξ and η values to fall on the specified curve indicates this type of self-association is not present.

and

$$\eta = (M_1/M_{wa}) - \ln f_a = 1/(1+x) + x. \qquad (21)$$

Note that $x = kc_1$ and $0 < x < \infty$. For the AK models it is *not* required that kc_1 be less than one; for the SEK models [1], it is required that $kc_1 < 1$ (for c_1 in g/ℓ).

If one has values of ξ available, then it is a simple matter to obtain x from ξ by successive approximations. This is also the case for the quantity η, defined by eq. (21). Both ξ and η are functions of x, so one can plot ξ or η versus x. Plots of ξ versus x are shown in fig. 1 for the Type I AK and SEK [1] models. Plots or tables of ξ or η versus x can be used as a guide in starting the successive approximations. One can also use the experimental data with standard plots of η versus ξ for the Type I AK and SEK model indefinite self-associations to test for the presence or absence of either of these self-associations; these standard plots are shown in fig. 2. Once x is known, then eq. (17) can be used to evaluate f_1, since

$$f_1 = \exp(-kc_1) = \exp(-x). \qquad (22)$$

The AK intrinsic equilibrium constant, k, can be obtained from a plot based on

$$x/f_1 = x/\exp(-x) \doteq kc. \qquad (23)$$

If the AK Type I indefinite self-association is the correct model, then the plot based on eq. (23) should give a straight line going through or close to the origin, and the slope of this line would be k. If the model is wrong, then a curved plot or a linear plot whose intercept is quite removed from the origin may be obtained. For this model, the nonideal term, BM_1, is obtained from a modification of eqs. (18) or (19). When eq. (19) is used, then

$$M_1/M_{wa} - 1/(1+x) = BM_1c. \qquad (24)$$

For a relatively large BM_1, a plot of the left hand side of eq. (24) versus c will have a slope of BM_1. With small BM_1 one may have to set up an array of values of $(M_1/M_{wa})_{CALCD}$ and $(M_1/M_{wa})_{OBSVD}$ and find the best value of BM_1, for which $(1/N)\Sigma_{i=1}^{N}\delta_i^2$ is a minimum, using procedures analogous to those described previously. Here

$$\delta_i = [(M_1/M_{wa})_{OBSVD} - (M_1/M_{wa})_{CALCD}]_i. \qquad (25)$$

If M_{na} values were the primary data, then δ_i would be calculated using M_1/M_{na} instead of M_1/M_{wa}.

If the quantity $\ln(f_a/f_{a*})$ is used in the analysis, then the appropriate equation for f_1 is given by

$$f_1 = \exp\{-[(M_1/M_{wa} - BM_1c)^{-1} - 1]\}. \qquad (26)$$

The analysis is performed in the same manner as described previously for the SEK Type I indefinite self-association.

3.2. Type II AK indefinite self-association

For this association it is assumed that all odd species higher than monomer are absent; it is still assumed that $K_{(i-2)i} = K/i$ ($i = 2, 4, 6, \ldots$). The total concentration of solute (in g/ℓ) becomes

$$\begin{aligned} c &= c_1 + kc_1^2 + (k^3c_1^4/2^2) + (k^5c_1^6/2^3 \cdot 4) + \ldots \\ &= c_1\,[1 + kc_1\,\{1 + (k^2c_1^2/2^2) + (k^4c_1^4/2 \cdot 2^4) \\ &\quad + (k^6c_1^6/2 \cdot 3 \cdot 2^6) + \ldots\}] \\ &= c_1\,[1 + 2(kc_1/2)\exp\{kc_1/2\}^2] \\ &= c_1\,[1 + 2x\exp(x^2)]. \end{aligned} \tag{27}$$

Here

$$x = kc_1/2 = kcf_1/2 \qquad (0 < x < \infty). \tag{28}$$

The equations for ξ and η become

$$\begin{aligned} \xi &= 2M_1/M_{\mathrm{nc}} - M_1/M_{\mathrm{wc}} \\ &= \frac{2 + (2/x)\exp(x^2 - 1)}{1 + 2x\exp(x^2)} - \frac{1 + 2x\exp(x^2)}{1 + (4x + 4x^3)\exp(x^2)} \end{aligned} \tag{29}$$

and

$$\begin{aligned} \eta &= M_1/M_{\mathrm{wc}} - \ln f_1 \\ &= \frac{1 + 2x\exp(x^2)}{1 + (4x + 4x^3)\exp(x^2)} + \ln[1 + 2x\exp(x^2)]. \end{aligned} \tag{30}$$

Note that η and ξ are each functions of x, so that plots or tables of η or ξ versus x can be constructed to aid in the successive approximations needed to solve eqs. (29) or (30) for x. Fig. 3 shows plots of ξ versus x for both the Type II AK and SEK [1] model indefinite self-associations. Standard plots of η versus ξ for both types of Type II self-associations are shown in fig. 4; these diagnostic plots can be used with the experimental data to test for the presence or absence of these two self-associations. Once x is obtained, then f_1 can be calculated from a modification of eq. (27) since

$$f_1 = 1/[1 + 2x\exp(x^2)]. \tag{31}$$

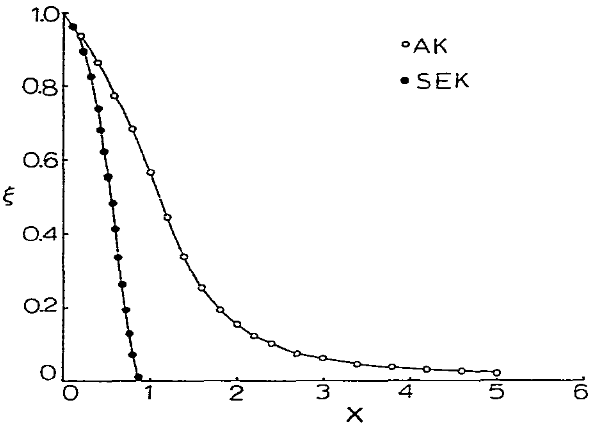

Fig. 3. ξ versus x for the Type II AK and Type II SEK models. If ξ is known then these curves may be used to determine an approximate value of x.

Then one obtains k from a plot based on

$$x/f_1 = kc/2. \tag{32}$$

The slope of a plot of x/f_1 versus c is $k/2$. If this model is correct, then a straight line going through or close to the origin is obtained. The wrong model would deviate from this behavior by giving curvature in the plot based on eq. (32) or by giving a straight line whose

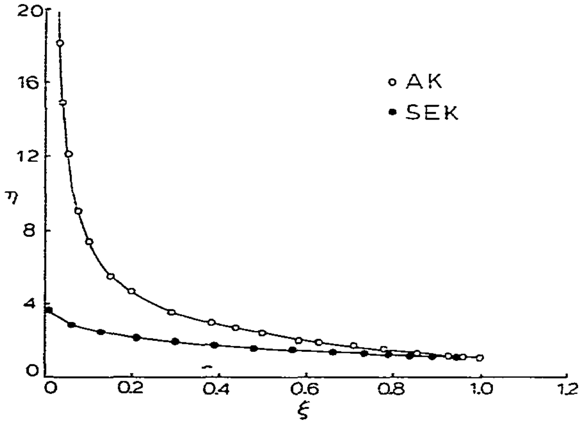

Fig. 4. Standard plot of η versus ξ for the Type II AK and Type II SEK models. Note that these are the characteristic curves for any Type II AK and SEK associations regardless of equilibrium constants or nonideal terms.

intercept deviates significantly from the origin. The nonideal term, BM_1, is obtained from

$$M_1/M_{wa} - M_1/M_{wc} = BM_1 c. \tag{33}$$

The appropriate form of M_1/M_{wc} is given by the last term in eq. (29).

If the quantity $\ln(f_a/f_{a*})$ is used in the analysis (see eq. (12)), then the approriate form of the equation to use is

$$\ln(f_a/f_{a*}) = \ln(x/x_*) - \ln(c/c_*) + BM_1(c - c_*). \tag{34}$$

Here

$$x = \tfrac{1}{2}kc_1 = \left\{\frac{(M_1/M_{wa} - BM_1 c)^{-1} - f_a \exp(-BM_1 c)}{2[1 - f_a \exp(-BM_1 c)]} - 1\right\}^{1/2}. \tag{35}$$

For the Type II AK indefinite self-association it is much easier to use the quantity ξ.

3.3. Type III AK indefinite self-association

This is a cooperative variant of the Type I association. Here it is assumed that $K_{12} \neq K/2$, but it is still assumed that $K_{(i-1)i} = K/i$ for $i = 3, 4, \ldots$. The total concentration of the associating solute is given by

$$\begin{aligned} c &= c_1 + 2k_{12}c_1^2 + k_{12}kc_1^3 \\ &\quad + (k_{12}k^2c_1^4/1 \cdot 3) + (k_{12}k^3c_1^5/1 \cdot 3 \cdot 4) + \ldots \\ &= c_1 + (2k_{12}c_1/k)[\exp(kc_1) - 1] \\ &= c_1[1 + (2y/x)(\exp(x) - 1)]. \end{aligned} \tag{36}$$

Here

$$y = k_{12}c_1 = k_{12}cf_1 = (K_{12}/M_1)cf_1, \tag{37}$$

and

$$x = kc_1 = ckf_1 = (K/M_1)cf_1. \tag{38}$$

The quantities ξ and η become

$$\begin{aligned} \xi = \frac{2M_1}{M_{nc}} - \frac{M_1}{M_{wc}} &= \frac{2[1 + (2y/x^2)(\exp(x) - 1 - x)]}{1 + (2y/x)[\exp(x) - 1]} \\ &\quad - \frac{1 + (2y/x)[\exp(x) - 1]}{1 + (2y/x)[\exp(x) + x\exp(x) - 1]} \end{aligned} \tag{39}$$

and

$$\begin{aligned} \eta &= M_1/M_{wc} - \ln f_1 \\ &= \frac{1 + (2y/x)[\exp(x) - 1]}{1 + (2y/x)[\exp(x) + x\exp(x) - 1]} \\ &\quad + \ln[1 + (2y/x)(\exp(x) - 1)]. \end{aligned} \tag{40}$$

Inspection of eq. (39) for ξ indicates that this can be converted to a quadratic equation in y. The root of this equation, which we will call $g(x, \xi)$, will contain terms in x and ξ. When $g(x, \xi)$ is inserted into eq. (40) for η, then since ξ and η are fixed, one has only one variable x, whose value can be obtained by successive approximations. This procedure would be repeated for every value of ξ and η that is used. Once x is known, then y is known from $g(x, \xi)$ and f_1 can be obtained from eq. (36). A plot of y/f_1 versus c will give a straight line (see eq. (37)) which should go through or close to the origin, if this be the correct model; the slope of the straight line would be k_{12}. Similarly a plot of x/f_1 versus c will give a straight line going through or close to the origin (see eq. (38)), if this be the correct model; for this case the slope would be k. Once x and y are known, then the nonideal terms can be evaluated from the expression for M_1/M_{wa} (cf. eq. (33)); the appropriate form of M_1/M_{wc} is given by the 1st term in eq. (40).

One can construct analogs of the standard plots of η versus ξ for the Type III AK indefinite [1] self-association. At fixed values of ξ, one can make standard plots or tables of η versus x (remember $0 \leqslant x < \infty$), and these plots or tables can be used to aid in the successive approximations. The same procedures could be used with a Type II SEK indefinite self-association; for this case the values of x are limited to $0 \leqslant x < 1$. The pertinent equations for the Type III SEK model are given in the appendix. Fig. 5 shows some standard plots for various values of ξ for both Type III models; in this figure we have cutt off the values of x for the AK model at $x = 2$ for ease of comparison. The quantity $g(x, \xi)$ needed for use in eq. (40) is given by

$$\begin{aligned} g(x, \xi) \equiv y &= \frac{1}{2(2\alpha\gamma - \beta^2 - \beta\gamma\xi)} \\ &\quad \times \{-[2(\alpha + \gamma - \beta) - (\beta + \gamma)\xi] \end{aligned}$$

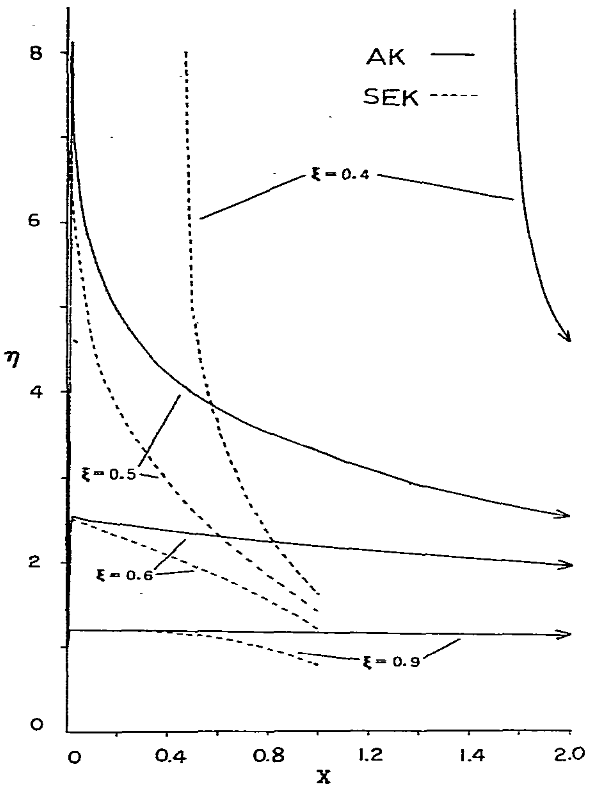

Fig. 5. Standard plot of η versus x at several ξ values for the Type III AK and Type III SEK models. Solid lines show the AK model; dashed lines represent the SEK model for which $0 \leqslant x < 1$.

$$\pm ([2(\alpha+\gamma-\beta)-(\beta+\gamma)\xi]^2 - 4(2\alpha\gamma-\beta^2-\beta\gamma\xi)(1-\xi))^{1/2}\}. \tag{41}$$

Here

$$\alpha = (2/x^2)[\exp(x) - 1 - x],$$

$$\beta = (2/x)[\exp(x) - 1],$$

$$\gamma = (2/x)[(x+1)\exp(x) - 1].$$

When the quantity $\ln (f_a/f_{a*})$ is used, then one notes that

$$\ln (f_a/f_{a*}) = \ln [(x/c)/(x/c)_*] + BM_1(c - c_*). \tag{41a}$$

The equation for x is given by

$$x = \frac{2 - f_1 - (M_{wc}/M_1)}{1 - (M_1/M_{nc})} = \frac{2 - f_a \exp(-BM_1c) - (M_1/M_{wa} - BM_1c)^{-1}}{1 - (M_1/M_{na} - BM_1c/2)} \tag{41b}$$

Thus to be able to use $\ln (f_a/f_{a*})$ one must know the values of f_a and of f_{a*} since they will be needed in the equations for x and x_*, respectively. If the value of f_a is needed here, then it is simpler to use the quantities η and ξ as described above, since one evaluates x directly from them.

3.4. Type IV AK indefinite self-association

This is a cooperative variant of the Type II AK indefinite self-association. Here it is assumed that $K_{12} \neq K/2$, but it is still assumed that $K_{(i-2)i} = K/i$ for $i = 4, 6, \ldots$. The total concentration of the self-associating solute (in g/ℓ) is given by

$$\begin{aligned} c &= c_1 + 2k_{12}c_1^2 + k_{12}^2 k c_1^4 \\ &\quad + (k_{12}^3 k^2 c_1^6/4) + (k_{12}^4 k^3 c_1^8/4 \cdot 6) + \ldots \\ &= c_1 \left[1 + 2k_{12}c_1 \left(1 + \frac{z^2}{2} + \frac{z^4}{2^2 \cdot 2!} + \frac{z^6}{2^3 \cdot 3!} + \ldots \right) \right] \\ &= c_1 [1 + 2k_{12}c_1 \exp(z^2/2)] = c_1 [1 + 2y \exp(z^2/2)] \end{aligned} \tag{42}$$

Here

$$y = K_{12}\overline{C}_1 = k_{12}c_1, \tag{43}$$

$$z^2 = K_{12}K\overline{C}_1^2 = k_{12}kc_1^2, \tag{44}$$

$$z = (k_{12}k)^{1/2}c_1 = k_* c_1, \tag{44a}$$

$$k_{12} = K_{12}/M_1, \quad k = K/M_1, \quad \overline{C}_1 = C_1/M_1. \tag{45--47}$$

The equations for ξ and η become

$$\xi = \frac{2M_1}{M_{nc}} - \frac{M_1}{M_{wc}} = \frac{2\{1 + (2y/z^2)[\exp(z^2/2) - 1]\}}{1 + 2y\exp(z^2/2)} - \frac{1 + 2y\exp(z^2/2)}{1 + y[4 + 2z^2]\exp(z^2/2)}$$

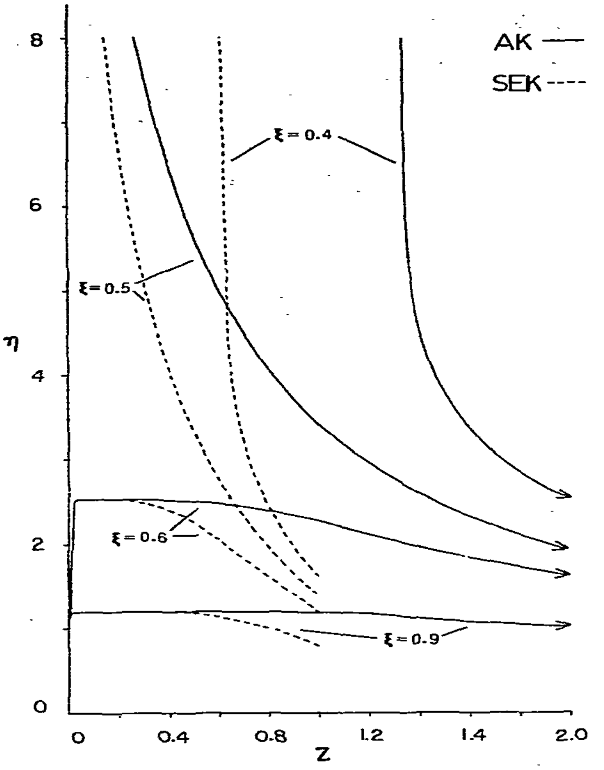

Fig. 6. Standard plot of η versus z at several ξ values for the Type IV AK and Type IV SEK models. Solid lines show the AK model; dashed lines represent the SEK model for which $0 \leqslant z < 1$. An initial guess for z is found by generating a η versus z curve at a particular ξ value. Since there is a unique value for ξ and η at each concentration, a value for z may be read from the curve.

and

$$\eta = M_1/M_{wc} - \ln f_1 = \frac{1 + 2y\exp(z^2/2)}{1 + y(4 + 2z^2)\exp(z^2/2)} + \ln[1 + 2y\exp(z^2/2)]. \qquad (49)$$

Note that eq. (48) can be converted to a quadratic equation in y. The root of this equation can be called $\phi(z, \xi)$, since it contains terms in z and ξ. At a fixed value of ξ one can insert $\phi(z, \xi)$ into eq. (49) to convert it to one equation in one unknown z; this procedure is repeated at other values of ξ. Again one can construct analogs of the standard plots of η versus z. At fixed values of ξ one can make standard plots or tables of η versus z, and these plots or tables can be used to aid in the successive approximations. Similar procedures can be used with the Type IV SEK model, although here the values of z^2 are limited to $0 \leqslant z^2 < 1$. The pertinent equations for the Type IV SEK model are given in the appendix. Fig. 6 shows some of the standard plots of η versus z for various values of ξ for the Type IV AK and the Type IV SEK models. For ease of comparison the values of z are plotted only to $z = 2$, since for the SEK model the values of z extend only to $z = 1$. The quantity $\phi(z, \xi)$ needed for eq. (49) is given by

$$y = \phi(z, \xi) = \frac{1}{2(\alpha\gamma - \beta^2 - \beta\gamma\xi)} \times \{-(\alpha + \gamma[2 - \xi] - \beta[2 + \xi]) \pm [(\alpha + \gamma[2 - \xi] - \beta[2 + \xi])^2 - 4(\alpha\gamma - \beta^2 - \beta\gamma\xi)(1 - \xi)]^{1/2}\}. \qquad (50)$$

Here

$$\alpha = (4/z^2)[\exp(z^2/2) - 1],$$

$$\beta = 2\exp(z^2/2). \qquad \gamma = [4 + 2z^2]\exp(z^2/2).$$

Once z is known, so is y. The quantities k, k_*, BM_1 can be obtained using procedures analogous to those used for a Type III AK indefinite self-association.

When the quantity $\ln(f_a/f_{a*})$ is used, then one notes that

$$\ln(f_a/f_{a*}) = \ln[(z/c)/(z/c)_*] + BM_1(c - c_*). \qquad (51)$$

The equation for z is given

$$z = \left\{\frac{(M_1/M_{wa} - BM_1c)^{-1} - f_a\exp(-BM_1c)}{1 - f_a\exp(-BM_1c)} - 2\right\}^{1/2}.$$

4. Tests with simulated examples

In order to test the validity of the relations, two simulated Type III AK examples were chosen; the values for k_{12}, k and BM_1 for these two examples are listed in table 1. Example A was based on the data of Garland and Christian [5,7] for the self-association of 6-methylpurine in water at 25°C over a concentration

Table 1
Comparison of Type III AK simulated parameters with results of model tests

Example	Model	k_{12} (ℓ/g)	k (ℓ/g)	BM_1 (ℓ/g)	Comments
A	III AK	0.0596	0.0426	0.01	k_{12}, k from [5]. BM_1 picked as a representative value.
A* [a)]	III AK	0.0653	0.0363	0.01	III AK and III SEK models both fit the Example A* data equally well.
A*	III SEK	0.0659	0.0121	0.01	III AK and III SEK models both fit the Example A* data equally well.
B	III AK	0.596	0.426	0.01	k_{12}, k picked to be 10 times larger than those in Example A.
B* [b)]	III AK	0.587	0.421	0.01	III AK model gave the best fit of all models tested with Example B* data.

[a)] Represents Example A data which was randomized and smoothed.
[b)] Represents Example B data which was randomized and smoothed.

range of 0 to 88.2 g/ℓ. Their values of k_{12} and k (see their table II) were converted to ℓ/g, and a small nonideal term was added by us in the form of BM_1. Simulated values of M_1/M_{wa} versus c were generated for a Type III AK association using these k_{12}, k and BM_1 values. For Example B, also a Type III AK model, the values of k_{12} and k were increased by a factor of ten, while the value of the nonideal term, BM_1, was held constant. Here again the tests were performed over a concentration range of 0 to 88.2 g/ℓ. Tests with perfect data showed that one could recover the original values of k_{12}, k, and BM_1 for either example from the plots of M_1/M_{wa} versus c and the quantities ξ and η.

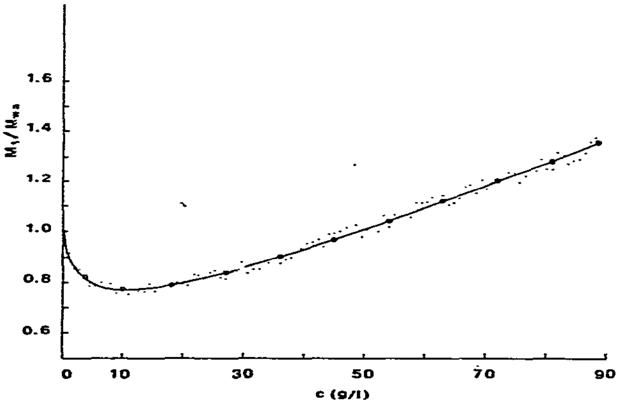

Fig. 7. Smoothed plot of M_1/M_{wa} versus c for Example A*. Included are the noisy data (±3% random error) before smoothing (•) and representative values of the original Example A perfect data (o).

For a more realistic test, 3% error was inputed randomly to the M_1/M_{wa} versus c data for both examples, using procedures analogous to those described previously [1]. These data were smoothed in both cases according to previously described procedures and the new, smoothed M_1/M_{wa} versus c values became Example A* and Example B*. Values of M_1/M_{na}, $\ln f_a$, ξ and η were obtained from the smoothed data for use in the model tests. Fig. 7 shows the smoothed plot of M_1/M_{wa} versus c for Example A*. This plot also shows the noisy data before smoothing (•) and the original Example A perfect data (o). It is evident from this figure that the smoothed plot shows relatively little deviation from the perfect data. Analogous plots for Examples B and B* are shown in fig. 8. The values of k_{12}, k and BM_1 obtained from the smoothed data for both examples are listed in table 1.

We also tested to see if these noisy Type III AK data (Examples A* and B*) would fit any of the other AK or SEK type indefinite self-associations. Figs. 9a and 9b show plots to test for the Type I AK and SEK indefinite self-associations, respectively. For the AK model, the methods based on the quantity ξ (see section 3.1) were used. For the SEK model the procedures based on the quantity ξ (see section 3.1 of ref. [1]) were also used. If either model were correct, then the appropriate plot should give a straight line going through or close to the origin, and the curvature in

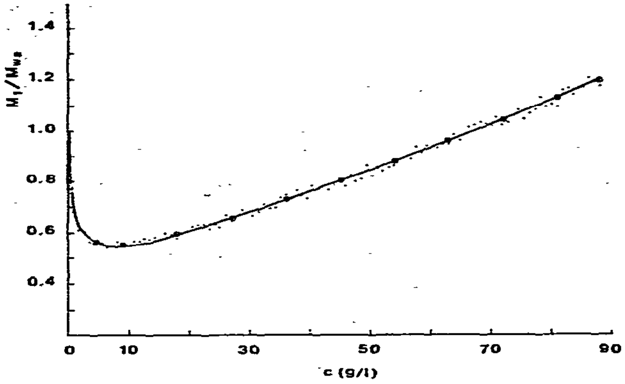

Fig. 8. Smoothed M_1/M_{wa} versus c curve for Example B*. Also shown are the noisy data (±3% random error) before smoothing (·) and representative values of the original Example B perfect data (○).

these plots indicates that these models fail. Figs. 10a and 10b show the corresponding diagnostic plots used to test for a type II indefinite self-association; the quantity ξ was used in all cases. For the AK model the procedures described in section 3.2. were followed, and for the SEK model the procedures described in section 3.2 of ref. [1] were followed. If either model were correct, then the appropriate plot would give a straight line going through or close to the origin; the linear plot for Example A* would suggest this association might be present.

To test the Types III and IV AK indefinite self-associations, the methods using η and ξ described in sections 3.3 and 3.4, respectively, were used. The tests for the Types III and IV SEK indefinite self-associations also involved the quantities η and ξ, and the appropriate equations for these models are describ-

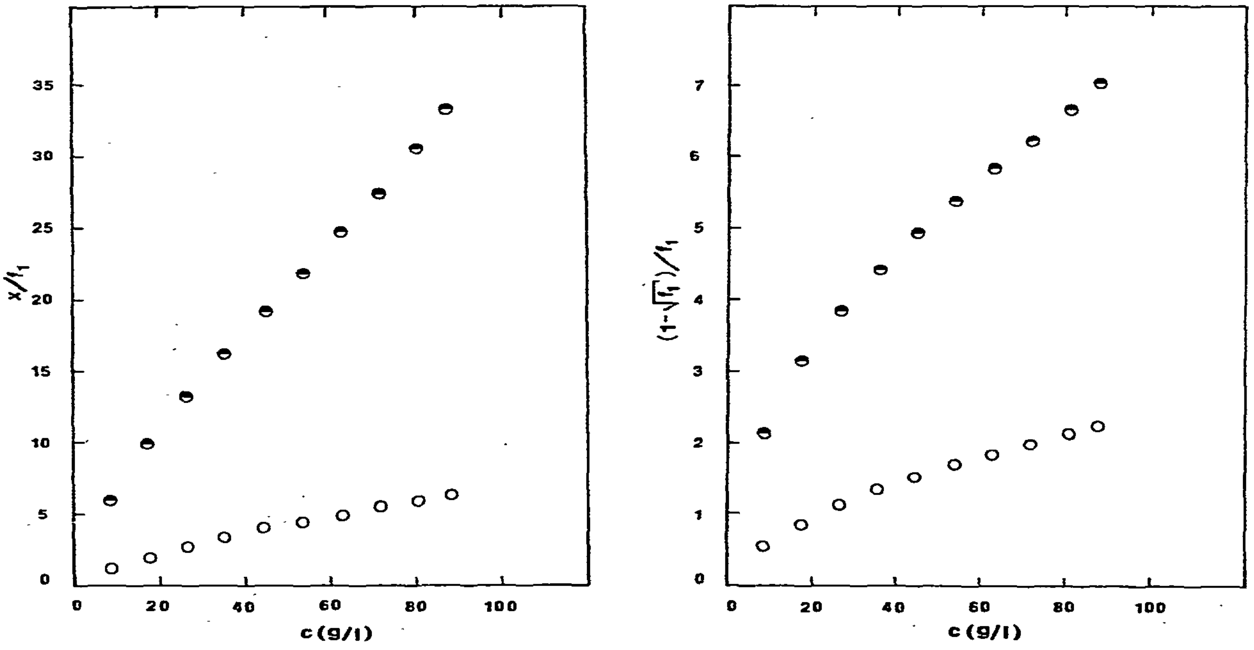

Fig. 9. a. Diagnostic plot for a Type I AK model. Failure of Example B* (○) data to give a straight line that passes through or close to the origin indicates a Type I AK association is absent for that case. Even though Example A* (○) apparently gave the desired straight line, this model was ruled out on the basis of the variance. See table 2. For an explanation of the quantities involved see section 3.1. b. Diagnostic plot to test for the presence of a Type I SEK association. The observed curvature of the two cases shown rules out a Type I SEK association. See section 3.1 of ref. 1 for an explanation of the quantities used. The curves shown were obtained from the Example A* (●) and B* (○) data.

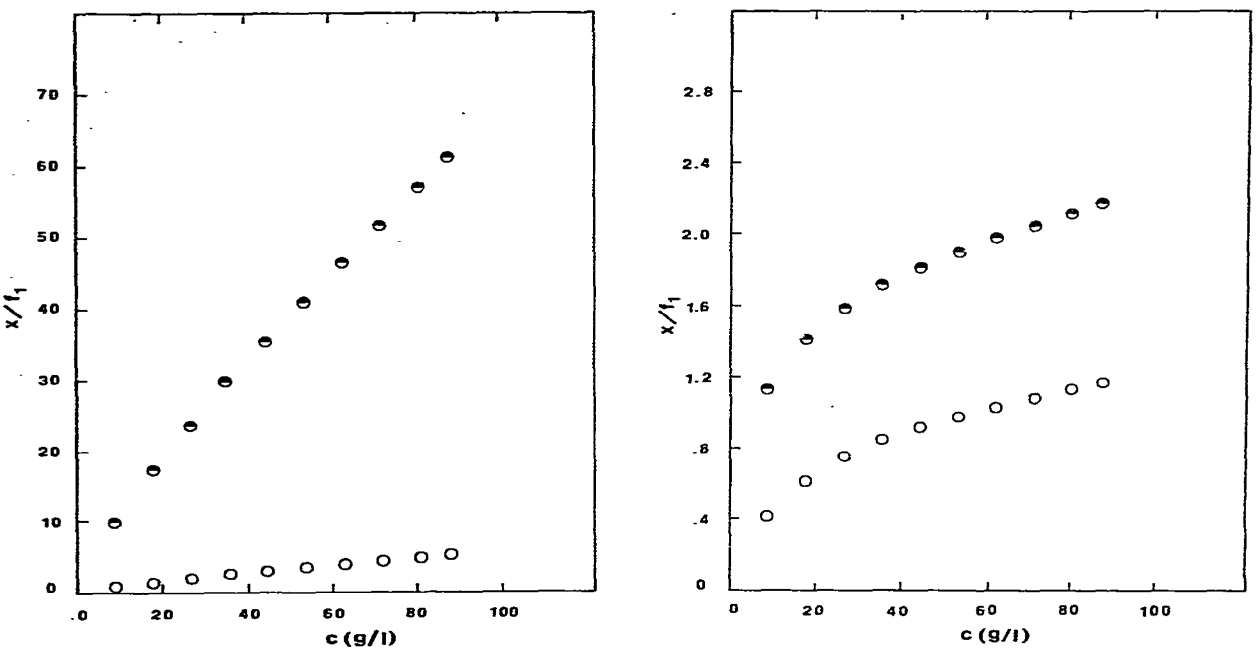

Fig. 10. a. Diagnostic plot for a Type II AK association. Failure of these plots to give a straight line that passes through or close to origin indicates the absence of a Type II AK association. The plots of the Example A* (o) and B* (●) data, however, suggest that a Type II AK association might be present but this model was ruled out on the basis of the variance for both cases. See table 2. Section 3.2 describes the quantities used in evaluating the Type II AK model with both Example A* and B* data. b. Diagnostic plot for a Type II SEK association. Neither example is described by this model as is evidenced by the lack of a straight line passing through or near the origin. An explanation of the values plotted may be found in section 3.2 of ref. [1]. Note the obvious curvature in the plots for both Example A* (o) and Example B* (●).

ed in the appendix. The results of these tests are listed in table 2. For example A* the Type III AK and the Type III SEK gave the best fits as judged by the variance. Here one could not distinguish between these models. With example B*, on the other hand, we achieved complete discrimination between the AK and SEK models, and the best fit, as judged by the variance, was given by the Type III AK model. The values of k_{12}, k and BM_1, and the variance for all these tests are listed in table 2. The variance, defined by

$$\text{Variance} = \frac{1}{N} \sum_{i=1}^{N} \left[\left(\frac{M_1}{M_{\text{wa}}} \right)_{\text{calcd}} - \left(\frac{M_1}{M_{\text{wa}}} \right)_{\text{obsvd}} \right]_i^2 \qquad (53)$$

was used as a criterion for the goodness of fit. Here N was the number of data points used and the subscripts CALCD and OBSVD refer to calculated and observed values of M_1/M_{wa} at data value i. In order to obtain the variance, the $(M_1/M_{\text{wa}})_{\text{CALCD}}$ values were based on values of k_{12}, k and BM_1 that were obtained from linear regression. For the Type III AK model, modifications of eqs. (37) and (38) plus the appropriate form of eq. (33) were used. For the Type IV AK model, modifications of eqs. (43) and (44) plus the appropriate form of eq. (33) were employed.

5. Discussion

In the preceding section we showed with two simulated examples the problems that can arise when analyzing for a Type III AK indefinite self-association. The virtues of our new procedures involving the quantities η and ξ for the Types III and IV AK model in-

Table 2
Analysis of Type III AK simulated data with 3% random error

Example	Model Tested	k_{12} (ℓ/g)	k (ℓ/g)	BM_1	Variance	Comments
A*	IAK	N/A	0.078 ±0.001	0.0109 ± 0.0001	1.41×10^{-4}	Rejected on the basis of variance
	IIAK	N/A	0.124 ± 0.001	0.0123 ± 0.0001	5.30×10^{-4}	Rejected on the basis of variance
	IIIAK	0.0653 ± 0.0001	0.0363 ± 0.0006	0.00980 ± 0.00001	2.15×10^{-6}	III AK and III SEK models both fit the data equally well
	IVAK	0.0723 ± 0.0001	0.055 ± 0.006	0.0112 ± 0.0001	3.29×10^{-5}	Rejected on the basis of variance
A*	ISEK	N/A	0.0295 ± 0.0007	0.0123 ± 0.0001	5.00×10^{-4}	Rejected on the basis of variance and diagnostic plot
	IISEK	N/A	0.0167 ± 0.0006	0.0101 ± 0.0002	2.63×10^{-2}	Rejected on the basis of variance and diagnostic plot
	IIISEK	0.0659 ± 0.0002	0.0121 ± 0.0002	0.00997 ± 0.00001	2.14×10^{-6}	III AK and III SEK models both fit the data equally well
	IVSEK	0.0733 ± 0.0001	0.015 ± 0.002	0.0118 ± 0.0001	5.20×10^{-5}	Rejected on the basis of variance
B*	IAK	N/A	0.397 ± 0.005	0.0106 ± 0.0001	1.26×10^{-4}	Rejected on the basis of variance and diagnostic plot
	IIAK	N/A	0.733 ± 0.008	0.0124 ± 0.0002	3.07×10^{-3}	Rejected on the basis of variance
	IIIAK	0.587 ± 0.001	0.421 ± 0.002	0.00999 ± 0.00001	3.39×10^{-6}	Gave the best fit
	IVAK	0.756 ± 0.002	0.147 ± 0.003	0.0108 ± 0.0001	7.69×10^{-5}	Rejected on the basis of variance
B*	ISEK	N/A	0.094 ± 0.003	0.0121 ± 0.0001	9.36×10^{-4}	Rejected on the basis of variance and diagnostic plot
	IISEK	N/A	0.032 ± 0.002	0.0043 ± 0.0005	1.35×10^{-1}	Rejected on the basis of variance and diagnostic plot
	IIISEK	0.637 ± 0.008	0.119 ± 0.001	0.0106 ± 0.0001	5.10×10^{-6}	Rejected on the basis of variance
	IVSEK	0.778 ± 0.003	0.033 ± 0.001	0.0115 ± 0.0001	1.32×10^{-4}	Rejected on the basis of variance

definite self-association are the following: 1) We do not have to depend on the quantities c and ξ only and use them in an iterative procedure [1] to determine the two equilibrium constants from equations involving three unknowns (c_1, k_{12} and k). 2) Here we deal with one equation in one unknown (the quantity x for a Type III or the quantity z for a Type IV AK model) from which we can then extract the more precise parameters, the equilibrium constants k_{12} and k. 3) One does not have to determine the nonideal term BM_1 first [1]. This is usually the least precise experimental quantity, and if it were determined first, then a small error in BM_1 could have a much larger, incommensurate effect on the values of k and

k_{12}. This is why the quantities η and ξ were used. These same principles can be applied to the Types III and IV SEK indefinite self-associations or to any model involving two equilibrium constants, such as a monomer–dimer–n-mer self-association. When osmometric experiments are performed, M_{na} is the primary data, and one may want to use the quantity ν, where [15]

$$\nu = 2M_1/M_{na} - \ln f_a = 2M_1/M_{nc} - \ln f_1 \qquad (54)$$

instead of η, since for these experiments ν may be a more precise quantity than η. The quantity $\ln f_a$ may be evaluated from M_{na} by means of eq. (11). Thus one could use tables or graphs of ν versus x (or ν versus z) at constant ξ in the analysis of Type III (or Type IV) AK or SEK indefinite self-associations. It should be noted that we were able to show that example B* was best described by the Type III AK indefinite self-association (see table 2) instead of the other seven types (three other AK and four SEK) of indefinite self-associations. However, with Example A* we found two models (Types III AK and SEK) that could equally well describe the observed self-association. We believe that this was due to a combination of weak equilibrium constants and the random error. With perfect data (Example A) we were able to distinguish between these two models.

It should be noted that the values of k_{12}, k and BM_1 obtained from the simulated data with 3% error input randomly did differ from the true values (see table 1). The differences were greater with Example A*, the weaker self-association, than they were with Example B*, the stronger self-association. For both Examples A* and B*, the values of k showed the greatest difference between the observed and true values, whereas the k_{12} values were closer to the true values and seemed to be less affected by the random error. Most surprisingly, the values of BM_1 were reasonably close to the true values. When we tried eqs. (42) and (43) for $\ln(f_a/f_{a*})$ and solved first for BM_1 and then used this value of BM_1 to obtain k_{12} and k, we found that small errors in BM_1 produced quite large errors in k and k_{12}. Thus we abandonned this method, which was chronologically the first one, and we used methods involving η and ξ exclusively for the analysis of the Types III or IV AK or SEK indefinite self-associations.

For Example A* it appeared that the Types I and II AK indefinite models might describe the self-association, since plots of x/f_1 versus c (see refs. [23] and [32]) appeared to give a straight line (see figs. 9a and 10a). The values of k and BM_1 obtained from this choice were used to regenerate the M_1/M_{wa} versus c data. The calculated variances were much larger than those found for the Types III AK and SEK models. So, even though we used plots which should give a straight line going through or close to the origin to test for various models, the ultimate criterion is really how well a model agrees with the observed data, and the variance is used as a criterion for the goodness of fit. The lower the variance, the better the fit. With weak self-associations one might find two models that would describe the experimental data equally well; this is evident in tables 1 and 2 of the Garland and Christian paper [5] and is also evident from our results.

With respect to the Type I AK model, the equations we developed for c and for ϕ, the osmotic coefficient (or M_1/M_{nc} under ideal conditions), are comparable to those developed by Garland and Christian [5]. Division of our eq. (17) for c by M_1 leads to

$$C_F = c/M_1 = (c_1/M_1)\exp(kc_1)$$
$$= \overline{C}_1 \exp(K\overline{C}_1) \qquad (55)$$

since $k = K/M_1$, $C_F = c/M_1$ and $\overline{C}_1 = c_1/M_1$. This is the same as Garland and Christian's eq. (3). Similarly, our equation for $\phi = M_1/M_{nc}$ is

$$\phi = M_1/M_{nc} = (1/kc_1)[1 - \exp(-kc_1)]$$
$$= (1/K\overline{C}_1)[1 - \exp(-K\overline{C}_1)]. \qquad (56)$$

The division of eq. (56) by eq. (55) for C_F leads to

$$C_A = C_F\phi = (1/K)[1 - \exp(-K\overline{C}_1)], \qquad (57)$$

which is the same as Garland and Christian's eq. (5). For the Type III AK indefinite self-association, our procedures are comparable, except that their eq. (7) should read

$$C_A = C_F\phi = \overline{C}_1 + 2(K_2/K^2)[\exp(K\overline{C}_1) - 1 - K\overline{C}_1]. \qquad (58)$$

Only four examples of AK indefinite self-associations have been considered here, since these may be the simplest and most frequently encountered ones. The methods used here can be applied to develop equations for analyzing other potential AK self-associations.

In figs. 2 and 4 we have compared the η and ξ plots for the Types I and II AK and SEK indefinite self-associations, respectively. It is evident from these plots that there is a region of overlap of the models near $\xi = 1$. This would correspond to very low concentrations, or to a situation where a weak self-association is present. In this latter case, it might be virtually impossible to distinguish between the appropriate AK or SEK models. For the former case, one could overcome this problem by carrying out experiments at higher concentrations where the plots for the AK or SEK models diverge, provided enough material is available. For the latter case, if one cannot get out of the region of overlap by doing experiments at higher solute concentrations, then it may be virtually impossible to distinguish between the models.

In fig. 5 we have shown η versus x plots at constant ξ for the Types III AK and SEK models and the plots of η versus z at constant ξ are shown in fig. 6 for Types IV AK and SEK models. In both figures the values of x or z have been limited to a maximum value of 2 for the AK models for ease in comparing these models with their appropriate SEK analogs. It is evident that there is quite a difference in these plots. For the Type III models at $\xi = 0.9$ (see fig. 5) the plots of η versus x overlap for both models until a value of $x = 0.4$ is attained; beyond this value they diverge. It is quite evident that this plot for the AK model shows a very slight inclination from the horizontal, so the values of x obtained in this region (a region of low concentration or a region encountered with a weak self-association) may be less precise than those obtained at lower values of ξ. It is evident from fig. 5 that as ξ gets smaller the region of overlap tends to vanish; there is hardly any overlap of the two models when one gets to $\xi = 0.6$. The comparison plots for the Type IV models are shown in fig. 6. Note that in this case there is still significant overlap at $\xi = 0.6$. So in some cases it may be difficult or virtually impossible to distinguish between the two Type IV models. If the values of x or z should be $\geqslant 1$, then one cannot have an SEK self-association. It is interesting to note from fig. 5 that as ξ gets smaller, there is a narrower range of acceptable values of x for a Type III AK model. On the other hand, for the Type IV AK model there is a wider range of acceptable values of z at the lower ξ values (see fig. 6).

We have followed a traditional, historical approach [1–4] in the analysis of these various indefinite self-associations; here we have used quantities available or derived from the experimental data to obtain unique equations in one unknown with the various models. This approach avoids the problem of a baggy fit that arises when one tries to determine two or more unknowns by an iterative procedure. The reasons for using our approach have been discussed previously [1]. Perhaps our experience will stimulate others to test for the presence of these AK indefinite self-associations, as well as to devise newer and better methods for analyzing them.

Acknowledgement

This work was supported by grants from the National Institute of General Medical Science (GM 23877) and from the Robert A. Welch Foundation (A-485).

Appendix

The purpose of this appendix is to show how the quantities ξ and η can be used in the analysis of the Type III and the Type IV SEK indefinite self-associations. The procedures are similar to those used with the Type III and the Type IV AK indefinite self-associations (see sections 3.3 and 3.4, respectively) in that one shows that ξ is a quadratic equation in y, and then the root of this equation is inserted into the appropriate equation for η to obtain an equation in one unknown (x or z). Here values of x or z for these SEK models are restricted to an interval from zero to one, whereas the upper limit in the AK models can approach infinity. The pertinent equations for $\ln f_a$, M_1/M_{wa}, M_1/M_{na}, ξ and η for these SEK models are based on relations that were developed previously.

A. Type III SEK indefinite self-association [1]

The pertinent relations for this self-association are as follows (for c in g/ℓ):

$$c = c_1 [1 + y(2 - x)/(1 - x)^2]. \tag{A1}$$

Here

$$y = k_{12}c_1, \qquad (0 \leqslant y < \infty)$$

$$x = kc_1, \qquad (0 \leqslant x < 1)$$

$$k_{12} = K_{12}/M_1, \qquad k = K/M_1.$$

The quantities ξ and η are defined by

$$\xi = \frac{2(1 + [y/(1-x)])}{1 + [y(2-x)/(1-x)^2]} - \frac{1 + y(2-x)/(1-x)^2}{1 + y(4-3x+x^2)/(1-x)^3}, \qquad \text{(A2)}$$

and

$$\eta = M_1/M_{\mathrm{wc}} - \ln f_1 = \frac{1 + y[(2-x)/(1-x)^2}{1 + y[(4-3x+x^2)/(1-x)^3]} + \ln[1 + y(2-x)/(1-x)^2]. \qquad \text{(A3)}$$

Eq. (A2) can be rearranged into a quadratic equation in y, and the root of this equation $y = f(x, \xi)$ can be inserted into eq. (A3) to obtain an equation in one unknown, $x = kc_1$. Since ξ is known, one can construct tables or plots of η versus x to aid in the successive approximations needed to obtain x; remember that $0 \leqslant x < 1$. Once x is known, then $y = k_{12}c_1$ is also known from the equation for $y = f(x, \xi)$, and the f_1 can be obtained from eq. (A1). Thus the analysis is done in the same manner as was done with the analysis for the Type III AK indefinite self-association in section 3.3.

B. Type IV SEK indefinite self-association [1]

The pertinent relations for this self-association are as follows (for c in g/ℓ):

$$c = c_1[1 + 2y/(1-z^2)^2]. \qquad \text{(B1)}$$

Here

$$y = k_{12}c_1, \qquad z^2 = kk_{12}c_1^2 = k_*^2c_1^2, \qquad (0 \leqslant z^2 < 1),$$

$$\xi = \frac{2M_1}{M_{\mathrm{nc}}} - \frac{M_1}{M_{\mathrm{wc}}} = \frac{2[1 + y/(1-z^2)]}{1 + [2y/(1-z^2)^2]} - \frac{1 + [2y/(1-z^2)^2]}{1 + [4y(1+z^2)/(1-z^2)^3]}, \qquad \text{(B2)}$$

and

$$\eta = M_1/M_{\mathrm{wc}} - \ln f_1 = \frac{1 + [2y/(1-z^2)^2]}{1 + [4y(1+z^2)/(1-z^2)^3]} + \ln[1 + 2y/(1-z^2)^2]. \qquad \text{(B3)}$$

It is evident that eq. (B2) is quadratic in y, and the root of this equation can be designated as $y = g(z, \xi)$. This root can be inserted into eq. (B3) to give an equation in one unknown z, which can be solved by successive approximations. Since ξ is known, it is possible to construct plots or tables of η versus z at constant values of ξ to aid in the successive approximations; for this model the values of z are restricted to an interval between zero and one. Once z is known, then y is known from the equation for $y = g(z, \xi)$, and f_1 can be obtained from eq. (B1). The rest of the analysis is done in the same manner as was done with the Type IV AK indefinite self-association in section 3.4.

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