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THE SEDIMENTATION EQUILIBRIUM OF HETEROGENEOUSLY ASSOCIATING SYSTEMS AND MIXTURES OF NON-INTERACTING SOLUTES: ANALYSIS WITHOUT DETERMINATION OF MOLECULAR WEIGHT AVERAGES

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Sedimentation equilibrium is first considered of a system in which a ligand of any size binds to an acceptor at p sites, the experimental result, obtained with either interference or absorption optics, being a distribution of total solute concentration as a function of radial distance. Theory illustrated by a numerical example, is presented which shows that this distribution may be analysed to give the activity of the unbound ligand as a function of total weight concentration. It is shown that this information may be used together with conservation of mass equations written in terms of the initial mixing composition to evaluate the equilibrium constant(s) relevant to the system. Correlation with composition evaluation by use of absorption optics (when possible) is also discussed. The procedure does not involve solution of simultaneous equations which are sums of exponentials nor differentiation of experimental results to obtain apparent weight-average molecular weights. It is general in that it leads to the evaluation of the activity of the species characterized by the smallest $M(1-\bar{v}\rho)$ product and, accordingly, is shown to be useful in the analysis of non-interacting as well as of interacting systems.

1. Introduction

In the study of the binding of a ligand A to a macromolecular acceptor B, involving a series of equilibria of the type $BA_{i-1} + A \rightleftharpoons BA_i$ ($i = 1,2,\ldots,p$), it is sometimes possible to utilize a membrane permeable only to A in the characterization of the system by equilibrium dialysis. However, when A is also a macromolecule, a suitable membrane may not be available to permit the direct determination of the equilibrium concentration of unbound A. Nichol and Winzor [1] have developed a frontal gel chromatographic method which permits the evaluation of this concentration for macromolecular mixtures of predetermined initial composition; but the series of experiments required for characterization of the thermodynamic parameters governing the system involves the use of relatively large quantities of material. This objection does not pertain to the method of sedimentation equilibrium, which in addition is potentially capable of elucidating thermodynamic non-ideality. Steinberg and Schachman [2] have pointed out that, with suitable systems, the concentration of unbound ligand and the composition of the mixture in terms of total (constituent) weight concentrations of A and B might be determinable at each radial distance with the use of absorption optics: they noted that in this sense a sedimentation equilibrium experiment was equivalent to a series of equilibrium dialysis experiments. It is clear, however, that many macromolecular–macromolecular interactions will not be characterized by spectra which permit such analysis. In these cases, analysis is required of the total solute concentration distribution obtained in sedimentation equilibrium either by the use of Rayleigh interference optics or absorption optics (if a suitable isosbestic point is available). Several theoretical treatments have been presented to guide this analysis: they rely on either solution of simultaneous equations which are sums of exponentials [3] (and hence on an assumed model) or use of apparent weight-average molecular weights [4] and second moments [5]. The last-named methods correlate with treatments based on the use of molecular weight averages determined by light scattering

[6] and osmotic pressure [7–9]. It is also possible to simulate the sedimentation equilibrium distribution of any heterogeneously associating system with assigned parameters [10–12]; but this procedure is more useful in checking and refining values of parameters than in searching for them.

In a previous communication [13], it was shown that sedimentation equilibrium results obtained with polymerizing systems could be analysed to yield the activity of the monomer as a function of total weight concentration without the need to solve simultaneous equations (sums of exponentials) or to differentiate the results to yield apparent weight-average molecular weights. It is the major purpose of the present work to extend this procedure to the examination of heterogeneously associating systems and it is shown that the activity of unbound A at each radial distance may indeed be evaluated by a simple and direct means from the distribution of total solute contration. Various methods are discussed for using these basic data to characterize the system in thermodynamic terms: these include correlation with composition evaluation by the use of absorption optics and (alternatively) the use of conservation of mass equations written in terms of the initial mixing composition. The treatment includes discussion of non-ideality effects and the analysis of mixtures of non-interacting solutes.

2. Basic theory

Consider the series of equilibrium reactions represented by $BA_{i-1} + A \rightleftharpoons BA_i$ $(i = 1, \ldots, p)$, where the relevant equilibrium constants on the weight-concentration scale are denoted by

$$K_i = a_{BA_i}/(a_{BA_{i-1}}\, a_A), \tag{1}$$

the symbol a denoting activity. At sedimentation equilibrium the distribution of each species j(= A, B, BA_i) is given by [14]

$$a_j(r) = a_j(r_F)\exp[\phi_j M_j(r^2 - r_F^2)], \tag{2a}$$

$$\phi_j = (1-\bar{v}_j\rho)\omega^2/2RT, \tag{2b}$$

where r and r_F are any radial distances between or at r_m and r_b, the meniscus and base of the cell, respectively; ω is the angular velocity, $\bar{v}_j$ the partial specific volume of species j, ρ the solution density (considered constant), R the gas constant and T the temperature of the sedimentation equilibrium experiment. It will be assumed that K_i is a constant independent of r, implying no volume changes on formation of complexes. The experimental record obtained by Rayleigh interference or absorption optics (if a suitable isosbestic point is available) is a plot of total weight concentration, $\bar{c}(r)$, versus r.

$$\bar{c}(r) = c_A(r) + c_B(r) + \sum_{i=1}^{p} c_{BA_i}(r). \tag{3}$$

Since the values of $\phi_A M_A$ and $\phi_B M_B$ may be found from separate studies on A and B alone, it is a simple matter to calculate, from the experimental record obtained with the interacting mixture, the following dimensionless parameters [13],

$$\Omega_A(r) = \frac{\bar{c}(r)\exp[\phi_A M_A(r_F^2 - r^2)]}{\bar{c}(r_F)}, \tag{4a}$$

$$\Omega_B(r) = \frac{\bar{c}(r)\exp[\phi_B M_B(r_F^2 - r^2)]}{\bar{c}(r_F)}. \tag{4b}$$

It follows that plots of $\Omega_A(r)$ versus $\bar{c}(r)$ and $\Omega_B(r)$ versus $\bar{c}(r)$ may be constructed and it is relevant to examine their behavior on extrapolation to infinite dilution. Combination of eqs. (2) and (4a) yields,

$$\lim_{\bar{c}(r)\to 0} \Omega_A(r) = \frac{a_A(r_F)}{\bar{c}(r_F)} \lim_{\bar{c}(r)\to 0} \frac{\bar{c}(r)}{a_A(r)}. \tag{5}$$

By application of l'Hôpital's rule, the latter limit equals $\lim_{\bar{c}(r)\to 0} \mathrm{d}\,\bar{c}(r)/\mathrm{d}\,a_A(r)$, which may be evaluated by reformulating eq. (3) as

$$\bar{c}(r) = \frac{a_A(r)}{y_A(r)} + \frac{a_B(r)}{y_B(r)} + a_B(r)\sum_{i=1}^{p} \frac{\{\prod^{i} K_l\}\, a_A^i(r)}{y_{BA_i}(r)}, \tag{6}$$

where y_j denotes the activity coefficient of species j. Differentiation of eq. (6) with respect to $a_A(r)$, on noting that the limit as $\bar{c}(r) \to 0$ of $a_j(r) = 0$, of $y_j(r) = 1$ and of $\mathrm{d}\ln y_j(r)/\mathrm{d}\,a_A(r)$ is not infinite, yields

$$\lim_{\bar{c}(r)\to 0} \frac{\mathrm{d}\,\bar{c}(r)}{\mathrm{d}\,a_A(r)} = 1 + \lim_{\bar{c}(r)\to 0} \frac{\mathrm{d}\,a_B(r)}{\mathrm{d}\,a_A(r)} + \lim_{\bar{c}(r)\to 0} \frac{\mathrm{d}\,a_B(r)}{\mathrm{d}\ln a_A(r)} \sum_{i=1}^{p} \{\prod^{i} K_l\}\, a_A^{i-1}(r). \tag{7}$$

The quantity $\mathrm{d}\,a_B(r)/\mathrm{d}\,a_A(r)$ may be written by differentiating $a_{BA_i}(r) = \{\prod^{i} K_l\}\, a_A^i(r)\, a_B(r)$ with respect

to $a_A(r)$ to give on rearrangement,

$$\frac{d a_B(r)}{d a_A(r)} = \left(\frac{d \ln a_{BA_i}(r)}{d \ln a_A(r)} - i \right) \frac{a_B(r)}{a_A(r)}$$

$$= \frac{a_B(r)}{a_A(r)} \left(\frac{\phi_{BA_i} M_{BA_i}}{\phi_A M_A} - i \right), \quad (8)$$

the latter equality following from the differentiated form of eq. (2). It may be seen from eq. (8) that the limit as $\bar{c}(r) \to 0$ of $d a_B(r)/d \ln a_A(r)$ equals zero and therefore, that the third term in eq. (7) equals zero. Eq. (7) becomes

$$\lim_{\bar{c}(r) \to 0} \frac{d \bar{c}(r)}{d a_A(r)} = 1 + \left(\frac{\phi_{BA_i} M_{BA_i}}{\phi_A M_A} - i \right) \lim_{\bar{c}(r) \to 0} \frac{a_B(r)}{a_A(r)}. \quad (9)$$

This is a useful formulation since with the use of eq. (2), we may write,

$$\lim_{\bar{c}(r) \to 0} \frac{a_B(r)}{a_A(r)} = \frac{a_B(r_m) \exp [r_m^2 (\phi_A M_A - \phi_B M_B)]}{a_A(r_m)}$$

$$\times \lim_{r^2 \to -\infty} \exp [r^2 (\phi_B M_B - \phi_A M_A)], \quad (10a)$$

$$= 0, \qquad \phi_B M_B > \phi_A M_A, \quad (10b)$$

$$= +\infty, \qquad \phi_B M_B < \phi_A M_A, \quad (10c)$$

$$= a_B(r_m)/a_A(r_m), \qquad \phi_B M_B = \phi_A M_A, \quad (10d)$$

since as $\bar{c}(r) \to 0$, $r^2 \to -\infty$. It follows by combination of eqs. (10), (9) and (5) that

$$\lim_{\bar{c}(r) \to 0} \Omega_A(r)$$

$$= \frac{a_A(r_F)}{\bar{c}(r_F)}, \qquad \phi_B M_B > \phi_A M_A, \quad (11a)$$

$$= +\infty \qquad \phi_B M_B < \phi_A M_A, \quad (11b)$$

$$= \frac{a_A(r_F)}{\bar{c}(r_F)} \left(1 + \frac{a_B(r_m)}{a_A(r_m)} \right), \quad \phi_B M_B = \phi_A M_A. \quad (11c)$$

In relation to eq. (11c) it is first noted that the term appearing in eq. (9), $[(\phi_{BA_i} M_{BA_i}/\phi_A M_A) - i]$ equals one when $\phi_B M_B = \phi_A M_A$ and $\bar{v}_{BA_i} = (M_B \bar{v}_B + i M_A \bar{v}_A)/M_{BA_i}$, the latter being the condition for no volume changes. Secondly, eq. (11c) may be viewed in terms of a system containing a single polymerizing solute where $\phi_A = \phi_B$, $M_A = M_B$ and hence $a_A(r_m) = a_B(r_m)$. In this case, the limit becomes $2 a_A(r_F)/\bar{c}(r_F)$ which agrees with that obtained previously [13] for such systems: in fact, the extrapolation yields the total activity of monomer at r_F represented here by reason of nomenclature as $2 a_A(r_F)$. If $\phi_B M_B \neq \phi_A M_A$, then for a given system it follows that either eq. (11a) or eq. (11b) must pertain. In the present instance of multiple binding of A to B, it is realistic to specify that $\phi_B M_B > \phi_A M_A$, where upon eq. (11a) applies and the plot of $\Omega_A(r)$ versus $\bar{c}(r)$ may be extrapolated to a defined limit at infinite dilution. With the specification that $\phi_B M_B > \phi_A M_A$, it follows by entirely analogous reasoning based on eq. (4b) that

$$\lim_{\bar{c}(r) \to 0} \Omega_B(r) = +\infty, \qquad \phi_B M_B > \phi_A M_A. \quad (11d)$$

Clearly, the useful plot when $\phi_B M_B > \phi_A M_A$ is one of $\Omega_A(r)$ versus $\bar{c}(r)$, which permits on application of eq. (11a) the determination of $a_A(r_F)$ and hence of $a_A(r)$ at all r, using eq. (2).

3. Equilibrium constant evaluation using $a_A(r)$

The mass of species j in the cell of thickness b and sector angle θ is given by [4,12],

$$Q_j = c_j(r_m) \alpha_j, \quad (12a)$$

$$\alpha_j = \theta b [\exp (\phi_j M_j z) - 1]/2 \phi_j M_j, \quad (12b)$$

$$z = r_b^2 - r_m^2. \quad (12c)$$

Although Q_j does not equal the amount of j introduced into the cell initially, it does follow from consideration of conservation of mass that,

$$\bar{Q}_A^0 = Q_A + \sum_{i=1}^{p} \frac{i M_A Q_{BA_i}}{M_{BA_i}}, \quad (13a)$$

$$\bar{Q}_B^0 = Q_B + \sum_{i=1}^{p} \frac{M_B Q_{BA_i}}{M_{BA_i}}, \quad (13b)$$

where $\bar{Q}_A^0$ and $\bar{Q}_B^0$ are the total amounts of A and B, respectively, introduced into the cell initially: $\bar{Q}_A^0$ and $\bar{Q}_B^0$ define the composition of the original mixture and are known to the experimenter. Eqs. (13a) and (13b) may be written in terms of Q_A and Q_B as follows. First, eqs. (1) and (12a) are employed to formulate the ratio [10]

$$\frac{Q_{BA_i}}{Q_{BA_{i-1}} Q_A} = K_i \frac{\alpha_{BA_i}}{\alpha_{BA_{i-1}} \alpha_A}. \quad (14)$$

The assumption implicit in eq. (14) is that $y_{BA_i}/y_{BA_{i-1}} y_A = 1$ which has been discussed by Adams [9]. By rewriting eq. (14) for $i = 1,2$ and so on, it may readily be induced that,

$$Q_{BA_i} = \frac{\{\prod^i K_l\} \alpha_{BA_i} Q_A^i Q_B}{\alpha_B \alpha_A^i}. \quad (15)$$

Substitution of eq. (15) into eq. (13) gives, on noting from eq. (12a) that $Q_A/\alpha_A = c_A(r_m)$

$$\frac{\bar{Q}_A^0 - c_A(r_m)\alpha_A}{\bar{Q}_B^0} = \frac{\sum_{i=1}^{p}{}' \frac{iM_A \{\prod^i K_l\} \alpha_{BA_i} c_A^i(r_m)}{M_{BA_i} \alpha_B}}{1 + \sum_{i=1}^{p} \frac{M_B \{\prod^i K_l\} \alpha_{BA_i} c_A^i(r_m)}{M_{BA_i} \alpha_B}} \quad (16)$$

In the discussion of eq. (16) consider first the simplest case, $B + A \rightleftharpoons BA$ ($p = 1$), for which eq. (16) becomes on dividing numerator and denominator by $V_{cell} = \theta bz/2$

$$K_1 = \frac{M_{BA}\, \beta_B [\bar{c}_A^0 - c_A(r_m)\beta_A z^{-1}]}{\beta_{BA} c_A(r_m)[M_A \bar{c}_B^0 - M_B \bar{c}_A^0 + M_B c_A(r_m)\beta_A z^{-1}]}, \quad (17a)$$

$$\beta_j = \frac{2\alpha_j}{\theta b} = \frac{\exp(\phi_j M_j z) - 1}{\phi_j M_j}. \quad (17b)$$

Since from separate studies $\phi_A M_A$, (hence β_A) and $\phi_B M_B$ (hence β_B) are available, it follows that M_{BA} and β_{BA} are readily calculable. In addition, $\bar{c}_A^0$, $\bar{c}_B^0$ and z are dictated by the experimenter, which leaves the single unknown $c_A(r_m)$ for the calculation of K_1. This is the quantity which is available from the $\Omega_A(r)$ versus $\bar{c}(r)$ curve since $a_A(r_F)$ may be related by eq. (2) to $a_A(r_m)$ which at low $\bar{c}(r_m)$ approximates $c_A(r_m)$. Indeed if the approximation is of concern, the relation between $y_A(r)$ and $a_A(r)$ may be found in a separate sedimentation equilibrium experiment conducted with A alone, since in this case the limit of $\Omega_A(r)$ as $c_A(r) \to 0$ is $a_A(r_F)/c_A(r_F)$ and eq. (2) applies. In fact such a study would lead to the determination of B_{AA} defined by $\ln y_A(r) = B_{AA}\, c_A(r)$, whereas in the study of the interacting mixture, it is more likely as Adams [9] pointed out that $\ln y_A(r) = B_{AA}\, \bar{c}_A(r) + B_{AB}\, \bar{c}_B(r)$, the activity coefficient being a function of the composition of the mixture written in terms of the total (constituent) weight concentrations of the reactants. One simple approach to this problem is to assume that $B_{AA} = B_{AB}$ whereupon $c_A(r_m) = a(r_m)/\exp[B_{AA}\bar{c}(r_m)]$. This is likely to be a reasonable approach in the study of interacting globular protein systems at low concentrations. A more detailed analysis in terms of different values for B_{AA} and B_{AB} would clearly require definition of $\bar{c}_A(r_m)$ and $\bar{c}_B(r_m)$, which may be available for certain systems by the use of absorption optics. Alternatively, the analysis in terms of $B_{AA} = B_{AB}$ could be used to obtain by numerical simulation first estimates of $\bar{c}_A(r_m)$ and $\bar{c}_B(r_m)$, whereupon different values of B_{AB} (such as that calculated from charge and molecular covolume contributions) could be explored.

Consider next the situation where $p > 1$, but the sites are equivalent and independent so that a single intrinsic binding constant on the molar scale, K_B ℓ/mole, suffices to describe the system. It follows that [15], when K_i is expressed as ℓ/g,

$$K_i = \frac{M_{BA_i}(p - i + 1) K_B}{i M_A M_{BA_{i-1}}}, \quad (18a)$$

$$\prod^i K_l = \frac{M_{BA_i}[p(p-1)\ldots(p-i+1)]\, K_B^i}{i!\, M_A^i M_B}. \quad (18b)$$

Substitution of eq. (18b) into eq. (16) gives on rearrangement,

$$\psi + \sum_{i=1}^{p} \{K_B^i \alpha_{BA_i} c_A^i(r_m)[p(p-1)\ldots(p-i+1)] \times (\psi M_B - iM_A)/M_B \alpha_B\, i!\, M_A^i\} = 0. \quad (19a)$$

$$\psi = [\bar{c}_A^0 - c_A(r_m)\beta_A z^{-1}]/\bar{c}_B^0. \quad (19b)$$

All quantities in eq. (19) are experimentally available, as noted earlier for the simpler $B + A \rightleftharpoons BA$ case, except for K_B and p. It is therefore possible to assign a range of integral values to p and to solve the polynomial eq. (19) for corresponding values of K_B. These sets may be tested on the following basis. Eq. (3) may be rewritten as

$$c_B(r) = \frac{\bar{c}(r) - c_A(r)}{1 + \sum_{i=1}^{p} \{\prod^i K_l\} c_A^i(r)}, \quad (20)$$

whereupon with the aid of eq. (18b) apparent $c_B(r)$

values may be calculated for each set $\{p, K_B\}$: the appropriate set is that for which the values of $c_B(r)$, corrected for non-ideality if necessary, conform to eq. (2).

In more complicated cases where the p sites are either non-equivalent and/or dependent, there are p individual values of K_i and thus eq. (16) cannot be simplified with the use of eq. (18). For a given value of p, however, eq. (16) does relate the unknowns (K_i) to experimentally determinable quantities. It would be required for these cases, therefore, to perform a series of sedimentation equilibrium experiments with mixtures of different and known initial mixing composition ($\bar{c}_A^0$ and $\bar{c}_B^0$ being suitably varied): for each experiment eq. (16) provides one equation in a set which may be solved to yield the values of K_i. Evidently, experimental error would render such an analysis extremely difficult when p was large.

4. Numerical illustration

In order to discuss further the suggested plots of $\bar{c}(r)$ versus $\Omega_j(r)$, defined in eq. (4), it is convenient to refer to a particular system. The system was chosen to approximate that encountered with a mixture of ovalbumin and lysozyme in an environment where a 1:1 complex was formed [1,11]. The following values of the relevant parameters were selected; $M_A = 14\,400$, $M_B = 45\,000$, $\bar{v}_A = \bar{v}_B = \bar{v}_{BA} = 0.72$ ml/g, $K_1 = 7.33$ ℓ/g, $T = 25°C$ and $y_A = y_B = y_{BA} = 1$. A sedimentation equilibrium distribution of $\bar{c}(r)$ versus r was computed [10–12] employing $\bar{c}_A^0 = 0.1152$ g/ℓ, $\bar{c}_B^0 = 0.36$ g/ℓ, $\rho = 1$ g/ml, $\omega = 28\,000$ rpm, $r_m = 6.9$ cm and $r_b = 7.2$ cm. Eqs. (4a) and (4b) were used to construct the plots shown in figs. 1A and 1B, respectively. For each, values of $r_F = 7.16$ cm and $\bar{c}(r_F) = 1.16$ g/ℓ were selected which corresponded to a position in the $\bar{c}(r)$ versus r plot where the fringe density was slightly less than the measurable value [16] of 200 fringes/cm.

In fig. 1A the extrapolation suggested by eq. (11a) is shown in the inset. The assessed limiting value of $\Omega_A(r)$ was 0.112, which corresponds to $a_A(r_F) = c_A(r_F) = 0.13$ g/ℓ and to a value of K_1 calculated from eqs. (2) and (17) of 7.3 ℓ/g in agreement with the assigned value. In addition to illustrating the ease by which $a_A(r)$ may be found, fig. 1A also illustrates that the plot of $\Omega_A(r)$ versus $\bar{c}(r)$ is not complicated by the existence of critical points. This may be established for the more general case $BA_{i-1} + A \rightleftharpoons BA_i$ (all $y_j = 1$) by differentiating $\Omega_A(r) = c_A(r_F)\,\bar{c}(r)/\bar{c}(r_F)\,c_A(r)$ with respect to $\bar{c}(r)$ with the result that,

$$d\,\Omega_A(r)/d\,\bar{c}(r) = 0, \quad \text{only if}$$

$$d\,c_A(r)/d\,\bar{c}(r) = c_A(r)/\bar{c}(r). \tag{21a}$$

Since from eq. (2) $d\,c_j(r)/d(r^2) = \phi_j M_j c_j(r)$, it follows from eq. (1) that,

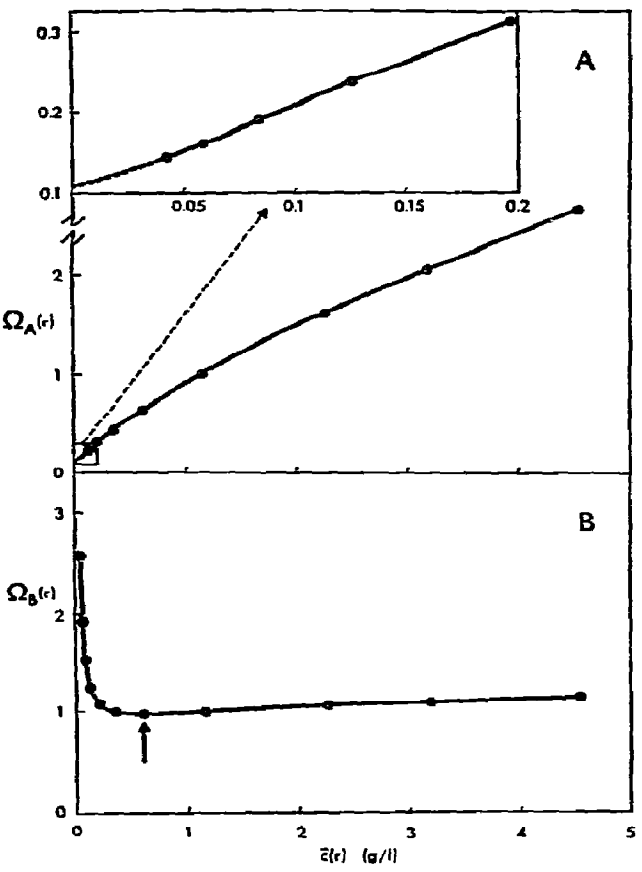

Fig. 1. Computer simulated results referring to the sedimentation equilibrium of the system B + A ⇌ BA with the parameters reported in the text.

(A) A plot of $\Omega_A(r)$ defined by eq. (4a) versus the total weight concentration, $\bar{c}(r)$. The inset shows the required extrapolation to infinite dilution.

(B) A plot of $\Omega_B(r)$ defined by eq. (4b) versus $\bar{c}(r)$ from the same sedimentation equilibrium results. The arrow denotes the position of the predicted minimum.

$$d c_A(r)/d \bar{c}(r) = \phi_A M_A c_A(r)/(\phi_A M_A c_A(r) + \phi_B M_B c_B(r) + \sum_{i=1}^{p} \phi_{BA_i} M_{BA_i} c_{BA_i}(r)). \quad (21b)$$

Combination of eq. (21b) with the condition in eq. (21a) for the existence of a critical point leads to,

$$c_B(r) = \frac{\sum_{i=1}^{p} c_{BA_i}(r)(\phi_A M_A - \phi_{BA_i} M_{BA_i})}{\phi_B M_B - \phi_A M_A}. \quad (21c)$$

Since from eq. (11a) $(\phi_B M_B - \phi_A M_A) > 0$ and $(\phi_A M_A - \phi_{BA_i} M_{BA_i}) < 0$ for all i, it follows that the condition in eq. (21c) is that $c_B(r) < 0$. Thus, it is clear that the condition cannot be met and that no critical points occur in the plot of $\Omega_A(r)$ versus $\bar{c}(r)$.

Fig. 1B illustrates two major points. First, in accord with eq. (11d), the limiting value of $\Omega_B(r)$ as $\bar{c}(r) \to 0$ is undefined (tending to $+\infty$). Secondly, a critical point (minimum) does exist in the plot. It may readily be shown that the analog to eq. (21c) is

$$c_A(r) = \frac{\sum_{i=1}^{p} c_{BA_i}(r)(\phi_{BA_i} M_{BA_i} - \phi_B M_B)}{\phi_B M_B - \phi_A M_A}, \quad (22)$$

which is the condition of existence of a critical point in the plot of Ω_B versus $\bar{c}(r)$. Clearly, it is met since it requires only that $c_A(r) > 0$: indeed, for the system B + A $\rightleftharpoons$ BA appropriate to fig. 1B, eq. (22) may be rearranged with the aid of eq. (1) to define the abscissa position of the critical point in terms of

$$c_B(r_c) = \frac{\phi_B M_B - \phi_A M_A}{K_1(\phi_{BA} M_{BA} - \phi_B M_B)}, \quad (23)$$

which permitted its location in fig. 1B.

The observation in eq. (11d) and fig. 1B that extrapolation of the available plot of $\Omega_B(r)$ versus $\bar{c}(r)$ does not lead to a useful result, prompted the enquiry whether an alternative formulation might be found to evaluate $a_B(r)$ directly. For the B + A $\rightleftharpoons$ BA system under discussion, fig. 1A has provided a means of obtaining $c_A(r)$ and hence a plot may be constructed of $\Omega_B^*(r)$ versus $\bar{c}^*(r)$ where

$$\bar{c}^*(r) = \bar{c}(r) - c_A(r) = c_B(r) + c_{BA}(r), \quad (24a)$$

$$\Omega_B^*(r) = \frac{\bar{c}^*(r) \exp[\phi_B M_B(r_F^2 - r^2)]}{\bar{c}^*(r_F)} = \frac{\bar{c}^*(r)\, a_B(r_F)}{\bar{c}^*(r_F)\, a_B(r)}, \quad (24b)$$

$$\lim_{\bar{c}^*(r) \to 0} \Omega_B^*(r) = \frac{a_B(r_F)}{\bar{c}^*(r_F)} \lim_{\bar{c}^*(r) \to 0} \frac{d\bar{c}^*(r)}{d a_B(r)}. \quad (24c)$$

It may be shown by differentiating eq. (24a) with respect to $a_B(r)$ that even for the non-ideal case (all $y_j \neq 1$),

$$\lim_{\bar{c}^*(r) \to 0} \frac{d\bar{c}^*(r)}{d a_B(r)} = 1, \quad \text{and}$$

$$\lim_{\bar{c}^*(r) \to 0} \Omega_B^*(r) = \frac{a_B(r_F)}{\bar{c}^*(r_F)}. \quad (25)$$

At first sight, this result might suggest that application of fig. 1A to find $a_A(r) = c_A(r)$, together with the use of eqs. (25) and (2) to find $a_B(r) = c_B(r)$ and hence $c_{BA}(r) = \bar{c}(r) - c_A(r) - c_B(r)$, provides a useful means of evaluating K_1 as an alternative to the use of eq. (17) previously described which is based on a knowledge of the initial mixing composition. The relative merits of the two approaches, however, must be judged on the ability to determine the value of $\Omega_B^*(r)$ at infinite dilution by extrapolation. Fig. 2 presents the plot of $\Omega_B^*(r)$ versus $\bar{c}^*(r)$ appropriate to

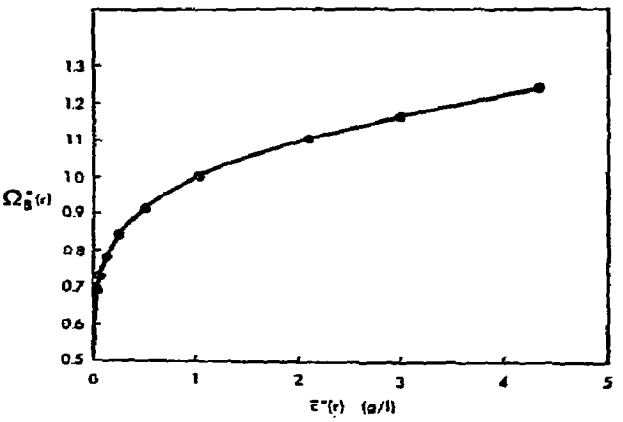

Fig. 2. Sedimentation equilibrium data for the same system as considered in fig. 1, but plotted as $\Omega_B^*(r)$ defined by eq. (24b) as a function of $\bar{c}^*(r)$ defined by eq. (24a).

the system selected to compute fig. 1 with use of the same $(\bar{c}(r_F), r_F)$ point. The plot exhibits no critical points, as may readily be shown mathematically, and in contrast to fig. 1B does not tend to $+\infty$ on infinite dilution. However, there is no doubt that extrapolation from the lowest $\bar{c}^*(r)$ point, chosen to correspond to a 50μ fringe displacement, to the correct value of $\Omega_B^*(r) = 0.51$ at infinite dilution would be hazardous in practice. Accordingly while the subtractive method embodied in eqs. (24) and (25) is theoretically sound, its application in practice is likely to be inferior to that involving evaluation of $a_A(r)$ and the use of the initial mixing composition.

5. Discussion

The first step in the analysis of sedimentation equilibrium distributions obtained with mixtures of two constituents and of known initial compositions is to establish whether or not a chemical interaction occurs between the species. The quantity $M_{W\,cell}$ [17] may be evaluated for each experiment and, provided $\bar{c}_A^0/\bar{c}_B^0$ has been held fixed, an increase of $M_{W\,cell}$ with $(\bar{c}_A^0 + \bar{c}_B^0)$ is indicative of an interaction even though $M_{W\,cell}$ does not equal the weight-average molecular weight of the original solution. Alternatively, it is possible using eqs. (2) and (12) and the known initial compositions of the original solutions to calculate the sedimentation equilibrium distribution of any experiment on the basis that no interaction has occurred [10,11]: these may be compared with the experimental results to decide the question. The latter procedure has been illustrated in fig. 1 of ref. [11]. Once the existence of an interaction has been established, the basic point which emerges from this work is that the equilibrium activity of one reactant A [that with the lower value of $M(1-\bar{v}\rho)$] may be determined as a function of total solute concentration by direct analysis of the total concentration distribution obtained in sedimentation equilibrium using eq. (11a). The finding parallels that previously reported [13] where similar treatment of sedimentation equilibrium results obtained with polymerizing systems led to the specification of the activity of monomer as a function of total weight concentration. The present work continued by relating the determined value of $a_A(r_F)$ to $a_A(r_m)$ and hence to the initial mixing composition via eqs. (12)–(16). Relations were thereby formulated which permit the evaluation of equilibrium constants for systems where one or more binding sites on the acceptor B are encountered. The approach is quite general and would apply to systems, such as ovalbumin–lysozyme [1,11], where spectral methods are incapable of distinguishing between unbound reactants and complexes and indeed between individual reactants. For simplicity of presentation, the analysis has been discussed in terms of one experimentally obtained sedimentation equilibrium distribution (fig. 1). It is stressed, however, that a single experiment encompasses only a restricted range of composition which varies with radial distance and, accordingly, in testing possible models and in investigating non-ideality effects, it is important to conduct and analyse several experiments performed employing different angular velocities or mixtures of different initial composition. Although the composition variation with radial distance prevents the construction of overlapping plots of $\Omega_A(r)$ versus $\bar{c}(r)$, which was possible for a single interacting solute [13], it is possible to correlate the results of the series of experiments as follows. As a first step, the results of a single experiment would be analysed to yield as described, for example, K_1 (eq. 17a) or K_B (eq. 19). It is then possible to test the indicated model and associated equilibrium constant by using established procedures [10–12] to simulate the sedimentation equilibrium distributions for the other chosen initial compositions and angular velocities. Comparisons may be made on the basis of either $(\bar{c}(r), r)$ points or $(a_A(r), \bar{c}(r))$ values, the latter being obtainable from the $\Omega_A(r)$ analysis for each experiment independent of the model. If a model involving a single equilibrium constant is indicated, eqs. (17a) and (19) may be employed to obtain an average value of the constant from a series of experiments. In relation to systems governed by more than one equilibrium constant, it is timely to comment on the use of absorption optics. With those systems exhibiting a large spectral shift on binding, it may be possible to determine $c_A(r)$ directly, rendering unnecessary for these particular systems the present approach [2]. With others, it may only be possible to evaluate with the use of suitable isosbestic points the composition of the mixture at each radial distance in terms of $\bar{c}_A(r)$ and $\bar{c}_B(r)$, the total (constituent) weight concentrations of reactants. Combination of this information with the values of $c_A(r)$

found by the present method [relying only on a record of $\bar{c}(r)$ versus r] would permit evaluation of the binding function, $[\bar{c}_A(r) - c_A(r)]/\bar{c}_B(r)$ as a function of $c_A(r)$: interpretation of the resultant binding curve, which averages the data from several experiments, in terms of equilibrium constants relevant to various models involving binding sites of diverse kind has been discussed extensively elsewhere [18].

It could also be noted that the present method is applicable to the analysis of mixtures of two non-interacting solutes. Thus, Jeffrey and Pont [19] have shown that sedimentation equilibrium experiments of the meniscus-depletion design [20] conducted with such a mixture give a good estimate of the product $M_A(1-\bar{v}_A\rho)$ for the smallest component A in the mixture. It is therefore possible to employ eq. (4) in the construction of a plot of $\Omega_A(r)$ versus $\bar{c}(r)$. Eq. (5) continues to apply and leads with the use of l'Hôpital's rule to the conclusion that the limit of $\Omega_A(r)$ as $\bar{c}(r) \to 0$ equals $a_A(r_F)/\bar{c}(r_F)$. In contrast to the behaviour of an interacting system, the amount of a non-interacting solute in the cell at sedimentation equilibrium equals the amount of that species introduced. Thus eqs. (2) and (12a) may be used directly to calculate Q_A^0, provided it is assumed that $a_A(r_m) = c_A(r_m)$. Subtraction of Q_A^0 from the total amount of solute introduced into the cell gives Q_B^0, thereby defining the composition of the original mixture of two non-interacting solutes. Moreover subtraction of $c_A(r)$ values, available from the determined $c_A(r_F)$, from $\bar{c}(r)$ values yields $c_B(r)$ as a function of r: this subtractive method does not suffer from the limitation discussed in fig. 2 pertaining to a reacting system. A plot of $\ln c_B(r)$ versus r^2 would yield a straight line of slope $M_B(1-\bar{v}_B\rho)\omega^2/2RT$, provided the mixture were indeed non-interacting. Thus, a means has been provided of defining a naturally occuring mixture of two non-interacting solutes and of detecting chemical interaction between the species, if it occurs, by curvilinearity of the plot. In this context, also, it would be desirable to analyse several experiments conducted with different dilutions of the original mixture or angular velocities. For the case of a non-interacting mixture, eqs. (2) and (12) could be used directly to correlate the results of different experiments in the form of $\bar{c}(r)$ versus r plots. Cases where chemical interaction occurs between two species in a naturally occurring mixture of unknown composition, while detectable, are evidently more difficult to characterize completely. For such systems involving the formation of a 1:1 complex, a first estimate of $c_B(r)$ and hence of K could be obtained by plotting $\Omega_B^*(r)$ versus $\bar{c}^*(r)$; but due to the uncertainty in the extrapolation (fig. 2), the value of the equilibrium constant would need refinement on the basis of simulations [10,11] in relation to a series of experimental results. Clearly, for more complicated interactions between two species in a naturally occurring mixture or in the study of such mixtures comprising more than two solutes (chemically interacting or not), it would be desirable to separate the mixture so that selected combinations of the components could be examined, as described, using mixtures of known composition.

In conclusion, it appears that the formulation of sedimentation equilibrium results in terms of the $\Omega(r)$ function is useful in the characterization of a wide variety of systems (non-interacting, polymerizing or heterogeneously associating). Two further points may be made in this connection. First, it may readily be shown for even more complicated systems involving a combination of the aforementioned classes (such as the heterogeneous association of a ligand with a polymerizing acceptor) that use of the appropriate $\Omega(r)$ function leads to the activity of the species with the lowest $M(1-\bar{v}\rho)$ product as a function of total concentration. Secondly, it is noted that no restriction has been placed on the magnitudes of $\phi_A M_A$ and $\phi_B M_B$ with the result that the method is applicable to the study of mixtures of small molecules, which like certain macromolecular mixtures may not be amenable to study by equilibrium dialysis. For any system, the method offers the advantage that it is unnecessary to differentiate the experimental results, in contrast to previous suggestions [4,5]. The further interpretation, in terms of relevant equilibrium constants (or their counterpart, the composition of a non-interacting mixture), of the results obtained with the $\Omega(r)$ function is difficult only if the number of species present is large, a limitation shared by all other established analysis procedures.

References

[1] L.W. Nichol and D.J. Winzor, J. Phys. Chem. 68 (1964) 2455.

[2] I.Z. Steinberg and H.K. Schachman, Biochemistry 5 (1966) 3728.
[3] P.W. Chun and S.J. Kim, J. Phys. Chem. 74 (1970) 899.
[4] L.W. Nichol and A.G. Ogston, J. Phys. Chem. 69 (1965) 4365.
[5] E.T. Adams, Jr., Ann. N.Y. Acad. Sci. 164 (1969) 226.
[6] R.F. Steiner, Arch. Biochem. Biophys. 49 (1954) 71.
[7] R.F. Steiner, Biochemistry 7 (1968) 2201.
[8] R.F. Steiner, Biochemistry 9 (1970) 1375.
[9] E.T. Adams, Jr., A.H. Pekar, D.A. Soucek, L.H. Tang, G. Barlow and J.L. Armstrong, Biopolymers 7 (1969) 5.
[10] G.J. Howlett, P.D. Jeffrey and L.W. Nichol, J. Phys. Chem. 74 (1970) 3607.
[11] G.J. Howlett and L.W. Nichol, J. Biol. Chem. 248 (1973) 619.
[12] G.J. Howlett and L.W. Nichol, J. Biol. Chem. 247 (1972) 5681.
[13] B.K. Milthorpe, P.D. Jeffrey and L.W. Nichol, Biophys. Chem. 3 (1975) 169.
[14] R.H. Haschemeyer and W.F. Bowers, Biochemistry 9 (1970) 435.
[15] I.M. Klotz, Arch. Biochem. 9 (1946) 109.
[16] E.G. Richards, D.C. Teller and H.K. Schachman, Biochemistry 7 (1968) 1054.
[17] E.T. Adams, Jr., Proc. Nat. Acad. Sci. 51 (1964) 509.
[18] L.W. Nichol and D.J. Winzor, in: Migration of interacting systems (Oxford University Press, London, 1972).
[19] P.D. Jeffrey and M.J. Pont, Biochemistry 8 (1969) 4597.
[20] D.A. Yphantis, Biochemistry 3 (1964) 297.