BRIEF COMMUNICATION

SEDIMENTATION-DIFFUSION EQUILIBRIUM OF MONOMER-DIMER MIXTURES, STUDIED IN THE ANALYTICAL ULTRACENTRIFUGE

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ABSTRACT By generalizing the fundamental differential equation valid for a single ideal solute, it is usual to define, for a monomer-dimer nonideal mixture, an apparent molecular weight $M_{w,app} = (2RT/[1 - \rho\overline{V}]\omega^2)$ (d ln$c$/dr^2); RT has the usual meaning; ρ is the density of the solvent; $\overline{V}$ is the partial specific volume of the solute, assumed to be the same for the monomer and the dimer; w is the angular velocity of the rotor; c is the solute concentration at the radial position r in the cell. It is shown here that the above equation can be integrated in the case of a monomer-dimer nonideal mixture and that, after integration, we obtain the following relation between c and r: $([1 + 4Kc]^{1/2} - 1)/([1 + 4Kc_0]^{1/2} - 1])$ exp $(BM_m[c - c_0]) = \exp([\sigma_m/2]\,[r^2 - r_0^2])$; $\sigma_m = M_m(1 - \rho\overline{V})\omega^2/RT$ (M_m = molecular weight of the monomer); K is the monomer-dimer equilibrium constant; B is the second virial coefficient, assumed to be the same for the monomer and the dimer. As soon as M_m is known, the above equation permits the calculation of K and B, from the experimental curve $c(r)$. Moreover, the reversibility of the monomer-dimer equilibrium can be tested from this equation: it is necessary and sufficient that the values of K corresponding to different loading concentrations in the cell are identical.

My aim here is not to analyze nor to criticize the excellent paper of Harding (1985), but to take the opportunity of this publication, to present a complementary approach of the frequent problem of monomer-dimer mixtures, studied in the analytical ultracentrifuge, by means of the sedimentation-diffusion equilibrium technique.

Harding (1985) has mainly studied the case of nonideal polydisperse systems. Here, I shall consider the case of nonideal interacting systems. I shall assume that we are in the presence of a monomer-dimer reversible equilibrium and that the solute presents a thermodynamic nonideality. Let us call c the total concentration, c_m the concentration of the monomer and c_d the concentration of the dimer. We have the following equations:

$$c = c_m + c_d \tag{1}$$

$$c_d = Kc_m^2, \tag{2}$$

where K is the equilibrium constant. According to Adams and Fujita (1963) and by generalizing the equation valid for a single ideal solute, we can define an apparent molecular weight by

$$M_{w,app} = (2RT/[1 - \rho\overline{V}]\omega^2)\,([1/c]\,[dc/dr^2]). \tag{3}$$

By writing this equation, we assume that $\overline{V}_m = \overline{V}_d = \overline{V}$. Always according to Adams and Fujita (1963), we have

$$(1/M_{w,app}) = (1/M_w) + Bc. \tag{4}$$

B is the second virial coefficient, assumed to be the same for the monomer and the dimer ($B_m = B_d = B$). By calculating c_m and c_d from Eqs. 1 and 2, it is easy to show that

$$M_W = (c_m/c)M_m + 2(c_d/c)M_m = 2M_m/\left(1 + [1 + 4Kc]^{-1/2}\right) \tag{5}$$

from which we deduce, by combining with Eq. 4

$$M_{w,app} = 2M_m/\left(1 + [1 + 4Kc]^{-1/2} + 2BM_mc\right) \tag{6}$$

Let us now put

$$\sigma_m = M_m(1 - \rho\overline{V})\omega^2/RT \tag{7}$$

$$x^2 = 1 + 4Kc \tag{8}$$

By introducing Eq. 6 in Eq. 1 and by using the variable change (Eq. 8), it is easy to find the analytical relation between c and r

$$\{[(1 + 4Kc)^{1/2} - 1]/[(1 + 4Kc_0)^{1/2} - 1]\} \cdot \exp(BM_m(c - c_0)) = \exp([\sigma_m/2]\,[r^2 - r_0^2]), \tag{9}$$

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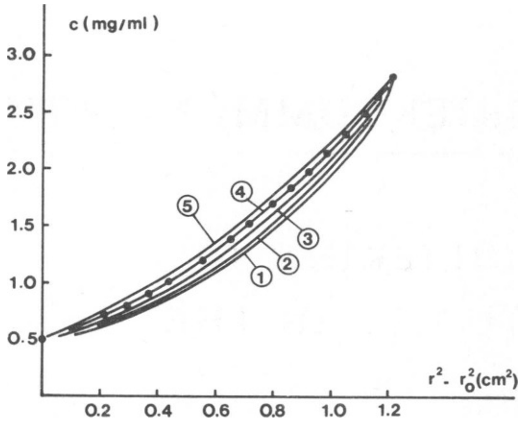

FIGURE 1 Example of application of Eq. 9 for a monomer-dimer nonideal mixture. The filled circles represent the experimental points, which could correspond to the following conditions. Loading concentration 1.5 mg/ml. $M_m = 10^5 \cdot \rho = 1.00$ g/ml. $\overline{V} = 0.74$ ml/g. Temperature 4°C. Rotor speed 10,000 rpm. Radial position of the meniscus $r_0 = 6.0$ cm and radial position of the cell bottom $r_b = 6.1$ cm (solution column length 1 mm). It is well-known that it is hazardous to deduce, from experimental values, two unknown parameters (K and BM_m) by the least-squares method. For this reason, I suggest the following procedure. (*a*) To smooth the experimental curve by using a usual routine. This smoothing gives the best values of $c(0)$ (meniscus) and $c(r_b^2 - r_0^2)$ (*bottom*). (*b*) To give to BM_m or K different values and to calculate K or BM_m for each value of BM_m or K and from the known values of $c(0)$ and $c(r_b^2 - r_0^2)$. (*c*) To draw the theoretical curves c vs. $(r^2 - r_0^2)$ for each value of BM_m or K and the calculated values of K or BM_m. (*d*) To select the values of BM_m and K, which seem to give the best fit to the experimental points. (*e*) To introduce these chosen values of BM_m and K in the computer and to use the least-squares method, by prescribing, for example, that the values of BM_m and K do not deviate by more than, say, 20% from the selected values. An example of application of this technique is given here. Curve 1 corresponds to a pure monomer ($K = 0$) and to $BM_m = -0.097$ ml/mg. Curve 2 corresponds to $BM_m = 0$ and $K = 0.17$ ml/mg. Curve 3 corresponds to $BM_m = 0.10$ ml/mg and $K = 0.75$ ml/mg. Curve 4 corresponds to $BM_m = 0.20$ ml/mg and $K = 5.0$ ml/mg. Curve 6 corresponds to a pure dimer ($K = \infty$) and $BM_m = 0.277$ ml/mg. It appears that curve 4 gives the best fit to the filled circles. The corresponding values of BM_m (0.20 ml/mg) and K (5.0 ml/mg) are then introduced in the computer and the least-squares method is used as suggested in point (*e*) above. After application of this technique, we finally find $K = 5.3$ ml/mg and $BM_m = 0.202$ ml/mg.

where r_0 is a reference radius, corresponding, for example, to the meniscus; $c_0 = c(r_0)$. The curve $c(r)$ is known experimentally from the Rayleigh interference fringe patterns for instance, and it is in principle possible to directly deduce, by the least-squares method, σ_m (i.e., M_m), K and B. However, it is extremely difficult to calculate with sufficient precision three unknown parameters from a single experimental curve $c(r)$. It is certainly better suited to directly measure M_m and to calculate σ_m. For that purpose, it is possible to modify the composition of the buffer (pH, ionic strength), as shown by Morel and Garrigos (1982). In fact, in most cases, K depends on these parameters and can reach the value zero (pure monomer) for suitable values of the pH and/or the ionic strength. If such a procedure cannot be used, it is always possible to reduce the loading concentration in the cell, so that the local concentration $c(r)$ is extremely low and that the solute is in the form of a pure ideal monomer. In this case, Eq. 9 reduces to the usual equation valid for a single ideal solute

$$\sigma_m/2 = \mathrm{d}\ln c/\mathrm{d}r^2. \qquad (10)$$

$\sigma_m/2$ can be deduced from the slope of the curve $\ln c$ vs. r^2. This parameter being measured, Eq. 9 allows us to calculate K and B by the least-squares method or by other methods (see Fig. 1).

In the particular case of an ideal monomer-dimer mixture ($B = 0$), Eq. 9 can be written

$$[(1 + 4Kc)^{1/2} - 1]/[(1 + 4Kc_0)^{1/2} - 1] = \exp[(\sigma_m/2)(r^2 - r_0^2)]. \qquad (11)$$

In the present case, it is extremely simple to compute K by the least-squares method.

Finally, note that Eq. 9 is particularly useful to determine whether a monomer-dimer mixture is in reversible equilibrium. In fact, by taking at least three loading concentrations, it is necessary and sufficient to show that the computed values of K are the same for all loading concentrations. If the values of K for the different loading concentrations are significantly different, one can conclude that there is no reversible equilibrium between the monomer and the dimer.

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