SEDIMENTATION EQUILIBRIUM OF POLYDISPERSE NONIDEAL SOLUTES: A COMPARISON OF THREE APPROXIMATIONS*

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Communicated September 15, 1969

Abstract.—In the evaluation of sedimentation equilibrium data for polydisperse nonideal solutions, extrapolation procedures are required for the determination of the true solute weight-average molecular weight and of the light-scattering second virial coefficient. In an attempt to decide just which concentration-dependent parameter should be used in making these extrapolations, Van Holde and Williams, J. Polym. Sci., 11, 243 (1953), and Fujita, J. Phys. Chem., 63, 1326 (1959), and ibid., 73, 1759 (1969), have derived relations between apparent and true weight-average molecular weights which, starting from approximate forms of the same differential equations, appear to give different working expressions. The present analysis of these results will demonstrate new and perhaps unexpected relations between them.

Further, these approximations are discussed in terms of certain of the available experimental data, the purpose being to clarify the conditions under which the behavior of a polydisperse nonideal solution in the ultracentrifuge may be treated as if a nonideal two-component system were involved.

Introduction.—For a two-component system at equilibrium in the ultracentrifuge, the data may be represented by the following equation:¹

\[(M^{app})^{-1} = M^{-1} + \frac{1}{2} B(c_b + c_m).\]  

(1)

The apparent molecular weight of the solute, \(M^{app}\), is the experimental quantity defined by

\[M^{app} = (c_b - c_m)/\lambda c_0.\]

In this expression, \(c_0\) is the initial concentration in g/dl, \(c_b\) and \(c_m\) are the actual equilibrium concentrations at the bottom and at the meniscus of the solution column, respectively, and \(\lambda\) is given by

\[\lambda = \frac{(1 - \bar{v}\rho_0)\omega^2(r_b^2 - r_m^2)}{2RT}.\]

The angular speed of the rotor is denoted by \(\omega\), \(\bar{v}\) is the partial specific volume of the solute, \(\rho_0\) is the solvent density, and \(r_b\) and \(r_m\) are the respective radial positions of the bottom and the top of the solution column. The true solute molecular weight is represented by \(M\).

According to equation (1), extrapolation of \((M^{app})^{-1}\) to infinite dilution in the concentration variable \(1/2(c_b + c_m)\) yields \(M^{-1}\) as the ordinate intercept and the second virial coefficient \(B\) as the limiting slope. The second virial coefficient
and the activity coefficient \( y \) for two-component systems are related by equation (2):

\[
\ln(y) = MBc + \ldots
\]

Both the solute molecular weight and the second virial coefficient may be determined by this extrapolation, so that two-component nonideal solutions present no interpretive difficulties as long as the single term in equation (2) adequately describes the solution nonideality.

For polydisperse solutes, the differential equations for sedimentation equilibrium become

\[
\lambda M_i c_i = dc_i/dx + M_i c_i \sum_{k=1}^{q} B_{ik} dc_k/dx + \ldots \quad i = 1, \ldots, q.
\]

Here, \( M_i \) is the gram molecular weight of the solute species \( i \), \( c_i \) is the concentration at \( r \) of species \( i \), and \( x \) is a dimensionless distance parameter,

\[
x = (r^2 - r_m^2)/(r_b^2 - r_m^2).
\]

The distance from the center of rotation to a point in the solution column is denoted by \( r \). The activity coefficient for species \( i \) has been written

\[
\ln(y_i) = M_i \sum_{k=1}^{q} B_{ik} c_k + \ldots \quad i = 1, \ldots, q.
\]

Because the nonideality couples the \( q \) equations (3), exact solution of them is difficult. The definition of the weight-average molecular weight, together with the conservation of mass statement:

\[
\int_{0}^{1} c_i dx = c_{i0} \quad i = 1, \ldots, q;
\]

in which \( c_{i0} \) is the initial concentration of solute species \( i \), allow equations (3) to be summarized in the following manner:

\[
(M_w^{app})^{-1} = (M_w)^{-1} + (M_w M_w^{app} \lambda c_0)^{-1} \sum_i \sum_k \int_{0}^{1} B_{ik} M_i c_i (dc_k/dx) dx.
\]

Comparison with equation (1) shows that the treatment of polydisperse solutes is much more complicated than that for simple two-component systems, and it is not obvious how to determine \( M_w \), the true weight-average molecular weight of the solute, from the apparent weight-average molecular weight, \( M_w^{app} \), which is defined for polydisperse systems just as it was in the two-component case. Nevertheless, there have been efforts to generate an analogue to equation (1) which will allow determination of \( M_w \) and a nonideality parameter even when the nonideality is complicated by solute polydispersity.

In the development to follow, the approximations and developments of Van Holde and Williams,\(^4\) Fujita (1959),\(^5\) and Fujita (1969)\(^6\) will be analyzed and compared. This analysis will show relationships between these treatments which have not previously been known. Finally, these approximations will be discussed in terms of experimental evidence from the literature which will clarify
the conditions under which polydisperse nonideal solutes in the ultracentrifuge may be treated as if they were nonideal two-component systems.

Theory.—Van Holde and Williams were the first to recognize that an extrapolation of \((M^{\text{app}})^{-1}\) to infinite dilution should be made using a redefined concentration variable instead of the initial concentration \(c_0\). In condensed form, their starting equation,

\[
\lambda M_{\text{wr}} c = \frac{dc}{dx} + M_{\text{wr}} B c \frac{dc}{dx} + \ldots ,
\]  

contains the important assumption that

\[
B_{ik} = B \quad i, k = 1, \ldots , q .
\]  

Introducing the definition \((1/\lambda c)(dc/dx) = M^{\text{app}}_{\text{wr}}\), they transformed (8) into the following statement:

\[
M_{\text{wr}} = M^{\text{app}}_{\text{wr}} (1 - BM^{\text{app}}_{\text{wr}} c)^{-1}
= M^{\text{app}}_{\text{wr}} [1 + BM^{\text{app}}_{\text{wr}} c + (BM^{\text{app}}_{\text{wr}} c)^2 + \ldots ] .
\]  

Using the definition,

\[
M_{\text{w}} = \int_0^1 M_{\text{wr}} c dx / \int_0^1 c dx ,
\]  

and its analogue for the apparent molecular weight, they derived the equation

\[
(M^{\text{app}}_{\text{w}})^{-1} = (M_{\text{w}})^{-1} + B c_0^* Q
\]  

in which

\[
c_0^* = \frac{\left[ \int_0^1 M^{\text{app}}_{\text{wr}} c^2 dx \right]^2}{\int_0^1 M^{\text{app}}_{\text{wr}} c dx} ,
\]  

and

\[
Q = \left\{ \begin{array}{ll}
1 + B \int_0^1 M^{\text{app}}_{\text{wr}} c^2 dx / \int_0^1 M^{\text{app}}_{\text{wr}} c dx + \ldots ,
1 + B \int_0^1 M^{\text{app}}_{\text{wr}} c^2 dx / \int_0^1 M^{\text{app}}_{\text{wr}} c dx + \ldots ,
\end{array} \right.
\]  

If \(Q \approx 1\), then \((M^{\text{app}}_{\text{wr}})^{-1}\) may be plotted against \(c_0^*\) as indicated by equation (12) to yield \((M_{\text{w}})^{-1}\) as the ordinate intercept.

Application of the definition of \(M^{\text{app}}_{\text{wr}}\) permits rearrangement of equation (13) to the following form:

\[
c_0^* = \frac{1}{2} (c_0 + c_m) \left[ 1 + \int_0^1 (M^{\text{app}}_{\text{wr}} - M^{\text{app}}_{\text{w}})(dc^2/dx) dx \right] .
\]
Recently, Fujita6 derived the expression

\[
(M_{w}^{\text{app}})^{-1} = (M_{w})^{-1} + \frac{1}{2} B(c_{0} + c_{m}) \left[ 1 + \int_{0}^{1} \frac{(M_{ur} - M_{w})(dc^{2}/dx)dx}{\int_{0}^{1} M_{w}(dc^{2}/dx)dx} \right]. \tag{16}
\]

The similarity of equation (16) to equation (12) with \( c_{0}^{*} \) defined by expression (15) suggests that the equation of Van Holde and Williams may be equivalent to this recent result of Fujita's. For this to be true, the following equations must hold:

\[
Q = \left[ \int_{0}^{1} \frac{M_{w}^{\text{app}}(dc^{2}/dx)dx}{\int_{0}^{1} M_{w}^{\text{app}}(dc^{2}/dx)dx} \right] \left[ \int_{0}^{1} \frac{M_{ur}(dc^{2}/dx)dx}{\int_{0}^{1} M_{w}(dc^{2}/dx)dx} \right] = 1 + \frac{\int_{0}^{1} (M_{ur} - M_{w})(dc^{2}/dx)dx}{\int_{0}^{1} M_{w}(dc^{2}/dx)dx}. \tag{17}
\]

Multiplication of equation (10) by \( c_{0} \), followed by integration with respect to \( x \), yields the equation

\[
M_{w}^{\text{app}} = M_{w} \left[ 1 + B \int_{0}^{1} M_{ur}^{\text{app}}c^{2}dx/\int_{0}^{1} M_{ur}^{\text{app}}cdx + \ldots \right]^{-1}. \tag{18}
\]

If expression (18) is inserted into the denominator of the middle member of (17), the factor

\[
\left[ 1 + B \int_{0}^{1} M_{ur}^{\text{app}}c^{2}dx/\int_{0}^{1} M_{ur}^{\text{app}}cdx + \ldots \right]^{-1}
\]

cancels the denominator of \( Q \). The product of the numerator of the middle expression in (17) with the numerator of \( Q \) may be consolidated by application of equation (10), so that equation (17) becomes

\[
\int_{0}^{1} M_{ur}(dc^{2}/dx)dx/\int_{0}^{1} M_{w}(dc^{2}/dx)dx = 1 + \int_{0}^{1} (M_{ur} - M_{w})(dc^{2}/dx)dx/\int_{0}^{1} M_{w}(dc^{2}/dx)dx. \tag{19}
\]

Since (19) is an identity, equation (16) is precisely equivalent to equation (12). Indeed, this is to be expected because the starting equation for the Van Holde-Williams treatment is identical to the equation resulting from the approximations used by Fujita\(^6\) in his latest work.

For a comparison of these equivalent representations of Van Holde and Williams and of Fujita to Fujita's earlier approximation,\(^5\) equation (16) may be written

\[
(M_{w}^{\text{app}})^{-1} = (M_{w})^{-1} + \frac{1}{2} B(c_{0} + c_{m})[1 + \Delta]. \tag{20}
\]
Fujita argued\(^6\) that deviation of \(\Delta\) from zero is to be ascribed primarily to solute polydispersity, and not so much to solution nonideality. This interpretation plus indications that \(\Delta\) should be small led him to approximate \(\Delta\) by using ideal expressions for the component concentrations in the ultracentrifuge cell:

\[
c_i = \frac{c_i^0 M_i \lambda \exp (M_i \lambda x)}{\exp (M_i \lambda) - 1}
\]

(21)

\[
dc_k/dx = \frac{c_k^0 M_k^2 \lambda^2 \exp (M_k \lambda x)}{\exp (M_k \lambda) - 1}.
\]

Use of these expressions allows \(\Delta\) to be written

\[
\Delta = (M_{w^2} \lambda^2/12)(M_z^2/M_w^2 - M_{\zeta}/M_w).
\]

(22)

Fujita has considered the newer treatment\(^6\) to be an improvement over his earlier result\(^2, 5\) since it required the replacement of both \((c_b + c_m)\) and \(\Delta\) by their values calculated on the basis of equations (21), while the newer analysis involved calculation of \(\Delta\) only with these equations.

Equation (20) is the result of a factoring operation followed by rearrangement based upon the property of associativity for addition. Finally, the ideal approximation for the concentration distribution was introduced to calculate \(\Delta\). The degree of approximation should not depend upon the particular stage in the rearranging process at which equations (21) are introduced. Also, factoring the quantity \((c_b + c_m)\) introduced a like term in the denominator of \(\Delta\), so that it is not obvious that Fujita improved upon his previous work.

To compare Fujita’s recent approximation to his earlier one, the initial concentration \(c_0\) is written in terms of the quantity \(1/2(c_b + c_m)\) for an ideal solution. From equation (21),

\[
dc^2 = 2 \sum_i \sum_k M_i M_k c_i^0 c^0_k \lambda^3 \exp [(M_i + M_k)\lambda x]/[\exp (M_i \lambda) - 1][\exp (M_k \lambda) - 1] dx.
\]

(23)

Application of the conservation of mass statement (6) yields

\[
\frac{1}{2}(c_b^2 - c_m^2) = c_0^2 \lambda \sum_i \sum_k M_i f_i f_k \left[ \frac{M_i M_k \lambda [\exp (M_i + M_k)\lambda - 1]}{(M_i + M_k) \exp (M_i \lambda) - 1][\exp (M_k \lambda) - 1]} \right]
\]

(24)

with \(f_i = c_i^0/c_0\). The Taylor’s series expansion of the term in brackets from Fujita’s monograph\(^2\) is introduced to yield:

\[
\frac{1}{2}(c_b^2 - c_m^2) = c_0^2 \lambda \sum_i \sum_k M_i f_k f_i (1 + \lambda^3 M_i M_k/12 + \ldots).
\]

(25)

The definitions of \(M_w\) and \(M_z\), together with the following expression which is valid for ideal solutions

\[
M_w = (c_b - c_m)/\lambda c_0
\]

(26)
allow (25) to be written

\[
\frac{1}{2}(c_b + c_m) = c_0(1 + \lambda^2 M_x M_w/12 + \ldots).
\] (27)

For \( \lambda \) sufficiently small, Fujita’s earlier approximation\(^5\) is

\[
(M_{w_{\text{app}}}^{-1}) = (M_w)^{-1} + B_{LS}c_0(1 + \lambda^2 M_x^2/12 + \ldots). \] (28)

In equation (28), the nonideal term has been calculated by using equations (21). Assumption (9) has been introduced only for evaluating the term in \( \lambda^2 \). Elimination of \( c_0 \) between equations (27) and (28) yields

\[
(M_{w_{\text{app}}}^{-1}) = (M_w)^{-1} + \frac{1}{2} B_{LS}(c_b + c_m)(1 + \Delta). \] (29)

Introduction of equation (9) into (29) yields equation (20), to terms in \( \lambda^4 \), with \( \Delta \) being given by equation (22). Consequently, Fujita’s latest result is equivalent to his own previous approximation\(^6\) when idealized concentration distributions are used to approximate \( \Delta \). With such an approximation, Fujita’s latest result\(^6\) is more restrictive than it need be, since (29) can be derived so that assumptions regarding the \( B_{LS} \) appear only in terms proportional to \( \lambda^2 \) and higher orders in \( \lambda \).

**Discussion.**—Of several experimental efforts which provide tests of these equivalent approximations, two are to be discussed. In one of them,\(^7\) the method of Van Holde and Williams\(^4\) was applied to the study of polyisobutylene in iso-octane at 25°C., and the resulting extrapolated values for \( M_{w_{\text{app}}} \) were compared to the corresponding quantities determined in ethyl n-heptanoate at 34°C., which is a theta solvent for this polymer. Discrepancies as large as 25% were detected in the higher molecular weight range (278,000–588,000 gm/mole), and consequently it was concluded that the method of Van Holde and Williams would be inaccurate except when applied to solutes of relatively low molecular weight.

More recently, Utiyama et al.\(^8\) have investigated the sedimentation equilibrium behavior of mixtures of two polystyrene fractions in 2-butane at 25°C. Although their main concern was the use of \( \lambda \) as an experimental variable (see also the disclosures of Albright and Williams\(^9\)), they also discussed the application of the ultracentrifuge to studies of polydisperse nonideal solutes at constant values of \( \lambda \). In reference to the earlier results of Mandelkern et al., they stated: “The difficulty in the extrapolation of \( 1/M_{\text{app}} \) to \( c_0 = 0 \) reported by Mandelkern, et al., for a high molecular weight sample of polyisobutylene in isooctane is simply due to the fact that the value of \( M\lambda \) used in their experiment was too large.” They presented a figure which showed that extrapolation of \( (M_{w_{\text{app}}}^{-1}) \) to \( c_0 = 0 \) yielded the same ordinate intercept as they had obtained by taking into account the \( \lambda \) dependence. Finally, they displayed a plot of \( (M_{w_{\text{app}}}^{-1}) \) as a function of \( c_0(1 + \lambda^2 M_x^2/12) \) to show the advantage of the plot using the reciprocal of the apparent weight-average molecular weight, and from the slopes of such plots they evaluated \( B_{LS} \) for several different mixtures of the polystyrene fractions.
Although Mandelkern et al. did not list the experimental conditions used in their test of the Van Holde and Williams equation, their times reported for the attainment of sedimentation equilibrium do suggest that they used long solution columns in their experiments. Thus it seems probable that \( M_w \lambda \) was indeed too large. Utiyama et al. apparently believed that the extrapolation used by Mandelkern et al. was performed by using the concentration variable \( c_0 \). Actually, the extrapolation was performed with the Van Holde and Williams variable \( c_0^* \) which already contains a \( \lambda \)-dependent term, as can be seen from equation (15). Had they used proper experimental conditions, Mandelkern et al. probably could have obtained a close approximation to the true weight-average molecular weight by using the available theory.

The plots of \( (M_w^{\text{app}})^{-1} \) versus \( c_0(1 + \lambda^2 M_w^2/12) \) used by Utiyama et al. to obtain \( B_{LS} \) could not be used ordinarily, since data for \( M_w \) would not usually be available. Comparison of \( c_0(1 + \lambda^2 M_w^2/12) \) with the expression for \( 1/2(c_0 + c_m) \) given in equation (27) shows that

\[
  c_0(1 + \lambda^2 M_w^2/12) = \frac{1}{2}(c_0 + c_m)[1 + \Delta]
\]

so that the extrapolation variable which they used may be well approximated by \( 1/2(c_0 + c_m) \) when \( M_w \lambda \) is sufficiently small or the molecular weight distribution is sufficiently narrow. Thus Fujita's main point in his recent article\(^6\)—that \( 1/2(c_0 + c_m) \) is useful variable for extrapolating \( (M_w^{\text{app}})^{-1} \) to infinite dilution—follows not only from the disclosures of Van Holde and Williams\(^4\) but also from his previous approximation.\(^5\) This variable is superior to \( c_0 \) because it automatically accounts for some of the dependence of \( M_w^{\text{app}} \) upon \( \lambda \). Equation (29) shows that an approximation of \( B_{LS} \) may be obtained as the initial slope of a plot of \( (M_w^{\text{app}})^{-1} \) versus \( 1/2(c_0 + c_m) \). Approximation (9) has been introduced only in the correction term for this equation, although the ideal approximations to the concentration distributions appear in the nonideal term as a whole.

\* This work was supported in part by a research grant from the United States Public Health Service (GM 14963).
\( \dagger \) Recipient of a National Aeronautics and Space Administration Traineeship, 1964–1967.

6 Ibid., 73, 1759 (1969).