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Boundary Spreading in Sedimentation Velocity Experiments. II. The Correction of Sedimentation Coefficient Distributions for the Dependence of Sedimentation Coefficient on Concentration[1,2]

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A method is presented for correcting a distribution of sedimentation coefficient for the dependence of sedimentation coefficient on concentration. This is, in general, an important correction and cannot be neglected. The method is applicable to uncorrected distributions obtained by the method of Signer and Gross, when diffusion is negligible, or by the method of Baldwin and Williams, when diffusion is not negligible. A considerable saving in time is achieved by the use of this correction as compared to extrapolating the uncorrected distributions to infinite dilution. The method requires knowledge of how the sedimentation coefficients depend on concentration.

Introduction

Several problems in chemistry and biochemistry await the development of a rapid and accurate method for determining the distribution of mass in a system. For example, the size and heterogeneity of the fragments produced by enzyme action on proteins and polysaccharides are of great interest to the biochemist, and, in polymer chemistry, the question of how the distribution of molecular weight affects the measured properties of a polymer must continually be faced.

Two instruments—both of them originally developed by Svedberg and his co-workers[4]—have been used to characterize mass heterogeneity; the equilibrium centrifuge, which yields a distribution of molecular weight, and the velocity ultracentrifuge, which gives a distribution of sedimentation coefficient. A distribution of molecular weight is more readily interpreted than one of sedimentation coefficient but the latter may be obtained without any *a priori* assumptions as to the nature of the distribution[5-8] and this is extremely difficult in the case of data from the equilibrium centrifuge.[9-11] Also the velocity ultracentrifuge experiment takes but a few hours in contrast to the situation with the equilibrium centrifuge, where two weeks may be required for a system to reach equilibrium.

The boundary gradient curves obtained with the velocity ultracentrifuge may be transformed directly into distributions of sedimentation coefficient, provided that the spread of the boundary has not been affected significantly either by diffusion or by the dependence of sedimentation coefficient on concentration. Signer and Gross[5] gave the following expression for transforming a boundary gradient curve into a distribution of sedimentation coefficient under these conditions

$$g(s) = \frac{dc}{dx}\,\omega^2 t x^3/c_0 x_0^2 \qquad (1a)$$

In this expression, ω is the angular speed of revolution, t is the time from the start of sedimentation, c_0 is the total concentration of the original solution, x_0 is the distance from the center of rotation to the meniscus, x is the distance from the center of rotation to a specified point in the boundary and dc/dx is the concentration gradient at this point. The weight fraction of material in the original solution of sedimentation coefficient s to $s + ds$ is given by $g(s)ds$.[12]

When the contribution of diffusion to the boundary spread is not negligible, $g(s)$ still may be obtained by extrapolation of an "apparent distribution" $g^*(s)$ against $1/t$ to infinite time.[6-8,14] Similarly, when the dependence of sedimentation co-

(1) Presented at the Symposium on Macromolecules, 13th International Congress of Pure and Applied Chemistry, Uppsala, Sweden, August, 1953.

(2) Based on a thesis submitted to the University of Oxford in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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(4) See T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940.

(5) R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 726 (1934).

(6) R. L. Baldwin and J. W. Williams, THIS JOURNAL, **72**, 4325 (1950).

(7) J. W. Williams, R. L. Baldwin, W. M. Saunders and P. G. Squire, *ibid.*, **74**, 1542 (1952).

(8) L. J. Gosting, *ibid.*, **74**, 1548 (1952).

(9) M. Wales, *J. Phys. Colloid Chem.*, **52**, 236 (1948).

(10) M. Wales, *ibid.*, **55**, 145 (1951).

(11) R. J. Goldberg, *ibid.*, **57**, 194 (1953).

(12) In general, the temperature and speed of rotation vary during sedimentation velocity measurements. This may be allowed for explicitly by rewriting (1a) as

$$g(s_{20}) = \frac{dc}{dx}\,x^3 \int_0^t \omega^2 \frac{\eta_{20}}{\eta_t}\,dt/c_0 x_0^2 \qquad (1b)$$

η is the solvent viscosity. This corresponds to rewriting the expression for the sedimentation coefficient, $s = \ln \frac{x}{x_0} \Big/ \omega^2 t$, as $s_{20} = \ln \frac{x}{x_0} \Big/ \int_0^t \omega^2 \frac{\eta_{20}}{\eta_t}\,dt$ and assumes that there is no significant error in setting $s_t\eta_t = s_{20}\eta_{20}$. If the sedimentation coefficient depends on concentration, the expression $s = \ln \frac{x}{x_0} \Big/ \omega^2 t$ gives an average s which will vary with t because the concentration decreases with time. Alberty[13] has discussed methods of calculating s which take this into account. At the highest concentration used in the experiments reported here, s changed by 2.5% from the beginning to the end of the centrifuge run. In general, conditions where this effect must be allowed for are not suitable for boundary-spreading measurements.

The integral in (1b) is obtained by numerical integration and is first measured from an arbitrary time origin t_0; the complete integral is found by plotting $\ln x_p/x_0$ (where x_p denotes the position of the maximum gradient) against $\int_{t_0}^t \omega^2 \frac{\eta_{20}}{\eta_t}\,dt$ and extrapolating to the meniscus ($x_p = x_0$) to find $\int_0^{t_0} \omega^2 \frac{\eta_{20}}{\eta_t}\,dt$. For simplicity, the speed and temperature will be treated as constant throughout this paper, with the understanding that any variation may be allowed for in the manner indicated here.

When the refractive increment, Δn (the difference in refractive index between the original solution and dialysate in equilibrium with it) is directly proportional to c_0 and the proportionality constant is the same for all species present, $(dc/dx)/c_0$ may be replaced by $(dn/dx)/\Delta n$ in equations 1a and 1b. Even when the proportionality constants are not the same, doing this gives a well-defined distribution.[8]

(13) R. A. Alberty, THIS JOURNAL, **76**, 191 (1954).

(14) R. L. Baldwin, *Biochem. J.*, to be published.

efficient on concentration significantly affects the boundary spread, the distribution of sedimentation coefficient may be obtained by extrapolating curves of $g(s)$, as defined by equation 1, to infinite dilution.[15-17] However, this is a very time-consuming process and there is no theoretical guide for the form of the extrapolation to infinite dilution. In this paper, a method is presented for correcting the curve of $g(s)$ obtained at a single concentration for the dependence of s on c. Only the case of negligible diffusion is considered here but it is shown how the method may be applied, when diffusion is not negligible, to the extrapolated curve of $g(s)$.

Theory

To begin with, the situation in the ultracentrifuge will be described for the case when diffusion is negligible and s does not depend upon c. Sedimentation is observed by studying the boundary which leaves the meniscus, moving outward from the center of rotation. (For convenience, this direction will be called to the right.) The velocity of sedimentation is given by the field strength, w^2x, and the sedimentation coefficient, s: $dx/dt = s\omega^2x$. This expression may be integrated to show that the solute molecules originally at the meniscus, x_0, will have sedimented after a time t to a position given by $x = x_0e^{s\omega^2t}$. Consequently this gives the position of the boundary formed by species of sedimentation coefficient s; just as all molecules of this (and other) species were to the right of x_0 at the start of the experiment, so all of this species must be to the right of x at time t.

The value of dc/dx at this plane is a direct measure of the amount of the species which vanishes at x (or first appears, depending upon your point of view) because the concentration of each species is constant to the right of the plane where it vanishes. There is a change with time, but not with distance, in the concentration of each species. (Because of the dilution caused by sedimentation in a sector-shaped cell and a changing field, $c_t = c_0e^{-2s\omega^2t}$.[18]

Thus the boundary gradient curve is itself a distribution curve: it gives the concentration of a particular species *versus* its position in the boundary. (Although all species of lesser sedimentation coefficient are also present at this position, they do not contribute to dc/dx.) The curve of dc/dx *vs.* x is not a conventional distribution function because the area under the curve is $c_0e^{-2s\omega^2t}$; it is customary to normalize a distribution by requiring the area under the curve to be unity. In this case, if the distribution function, $g(x)$, is defined as $(dc/dx)e^{2s\omega^2t}/c_0$ then $\int g(x)dx = 1$.

In order to obtain $g(s)$ from $g(x)$ we need only make use of the general relation for changing variables of a distribution function[19]: $g(s)\,ds = g(x)dx$. By differentiating the expression for boundary position, $x = x_0e^{s\omega^2t}$, at a fixed value of ω^2t, dx/ds is found to be ω^2xt and $g(s) = g(x)(dx/ds) = (dc/dx)e^{2s\omega^2t}\omega^2xt/c_0$. This is identical with (1a) since $(x/x_0)^2 = e^{2s\omega^2t}$.

When the sedimentation coefficient depends upon concentration, this interpretation of the boundary gradient curves requires considerable modification. The problem will be split into two parts by assuming for the moment that dc/dx still measures the amount of the species vanishing at x. The effect of the dependence of s upon c may then be described as a sharpening of the boundary caused by a change in concentration from zero at the far left of the boundary to $c_0e^{-2s\omega^2t}$ at the far right. (This will be referred to as the boundary sharpening effect.) The equation giving the position at which a species vanishes in the boundary is still $x = x_0e^{s\omega^2t}$, but now s is the sedimentation coefficient at c_x, c_x being the total concentration of all species at the plane where this species vanishes. c_x changes, of course, throughout the boundary and is different at every plane where a new species vanishes.

The concentration at x is known (since diffusion is negligible, c must be zero at x_0 and so c_x is simply $\int_{x_0}^{x}(dc/dx)dx$); if the dependence of sedimentation coefficient of a species upon c_x is known, its sedimentation coefficient at infinite dilution can be calculated from s and c_x. Then the correction for the dependence of s on c is simply once more a matter of changing variables in a distribution function; s_0, the sedimentation coefficient at infinite dilution, is substituted for s, the sedimentation coefficient at a particular concentration in the boundary and $g(s_0) = g(s)\,\frac{ds}{ds_0}$. This correction for the boundary sharpening effect is essentially the same as the one proposed by Jullander,[15] who considered the problem of transforming $g(s)$ to $g(M)$, the distribution of the molecular weight.

However, dc/dx no longer measures the amount of the species vanishing at x when the sedimentation coefficients of the various species are dependent upon the concentration. In fact, every species present at a plane in the boundary changes in concentration at that plane if its sedimentation coefficient varies with the total concentration. This effect, which was neglected by Jullander,[15] was treated quantitatively by Johnston and Ogston[20] for systems of two components; they showed that if the sedimentation coefficient of the slower moving component is greater in the absence than in the presence of the leading component, there will be a corresponding change in concentration of the trailing component. This may be thought of as the trailing component piling up behind the leading boundary because it moves more rapidly behind than in front of it, with the result that its concentration is greater behind the leading boundary than in front of it.

This effect also appears in a single boundary when the substance forming the boundary is heterogeneous. The situation seems insuperably complex in a multicomponent system until one realizes

(15) I. Jullander, *Arkiv. Kemi, Mineral., Geol.*, **21A**, No. 8 (1945).

(16) N. Gralen and G. Lagermalm, *J. Phys. Chem.*, **56**, 514 (1952).

(17) A. Fuhlbrigge, A. Haltner, Jr., W. M. Saunders, K. van Holde, J. A. Williams, J. W. Williams, Progress Report to the Office of the Surgeon General, Dept. of the Army, November 30, 1951. These experiments will be published in the usual way at a later time.

(18) T. Svedberg and H. Rinde, THIS JOURNAL, **46**, 2677 (1924).

(19) T. C. Fry, "Probability and its Engineering Uses," D. Van Nostrand Co., New York, N. Y., 1928, p. 188.

(20) J. P. Johnston and A. G. Ogston, *Trans. Faraday Soc.*, **42**, 789 (1946).

that this is simply a question of the concentration of a species depending upon its sedimentation coefficient: the problem of correction for the Johnston–Ogston effect then resolves itself into stating the sedimentation coefficient of a species as a function of the total concentration and of finding an expression for the rate of change of concentration of the species as its sedimentation coefficient changes. Then $(dc_i/ds_i)ds_i$ may be integrated numerically from the point at which i first appears in the boundary to the homogeneous solution at the far right, where its concentration is related to that of the original solution by $c_i = c_{0i}e^{-2s_i\omega^2t}$.

It is possible to find out from sedimentation velocity experiments how the sedimentation coefficient of a species depends on c_x; consequently it will be assumed here that this information is given and that s may be represented by $s_i = s_{0i} - f(c_x)$ where $f(c_x)$ is a known function of the total concentration at x.

A statement of the conservation of mass may be used to find an expression for dc_i/ds_i. Consider two planes moving at the rate r, which are always separated by the fixed, infinitesimal distance Δx. The amount of solute contained between these planes is $\int_x^{x+\Delta x} Ac\ dx$ or, since Δx is infinitesimal, $\Delta x Ac$, where A is the cross-sectional area. The rate of change with time of the mass in this lamella is equal to the inflow of solute, J_x, minus the outflow, $J_x + \Delta_x$

$$\frac{d}{dt}\{\Delta x A c\} = \Delta x \left\{c\frac{dA}{dt} + A\frac{dc}{dt}\right\} \qquad (2a)$$

$$c\frac{dA}{dt} + A\frac{dc}{dt} = -\frac{dJ}{dx} \qquad (2b)$$

In order to make the nature of the problem clear, sedimentation in a rectangular cell with constant field strength will be considered first and the complications, met in practice, of a sector-shaped cell and changing field will afterwards be taken into account. In a rectangular cell, the area is constant and since the proportionality constant does not enter into the final equation, it will be taken as unity. Equation 2b becomes

$$\frac{dc_i}{dt} = -\frac{d}{dx}\{c_i(v_i - r)\} \qquad (3a)$$

where v_i is the velocity of a particular solute component, relative to the cell, and r is the velocity of the frame of reference. If this is specified as the velocity of a plane in the boundary where c_i is constant, then $dc_i/dt = 0$ and

$$v_i\frac{dc_i}{dx} + c_i\frac{dv_i}{dx} - r\frac{dc_i}{dx} = 0 \qquad (3b)$$

$$\frac{dc_i}{dx}\left(v_i + c_i\frac{dv_i}{dc_i} - r\right) = 0 \qquad (3c)$$

Either $dc_i/dx = 0$ or $r = v_i + c_i\ dv_i/dc_i$. Provided that r is constant, it must be equal to the velocity of the species vanishing at this plane, *i.e.*, $r = (x - x_0)/t$, where x_0 marks the plane at which the boundary was formed.[21]

(21) In order to show that r is constant, consider a plane in the boundary where the total concentration is constant. The sedimentation coefficient of each species is assumed to depend only upon the total concentration and consequently is constant. The concentration of each species varies only with its sedimentation coefficient and so is constant. Since the same situation holds at a neighboring plane of constant total concentration dv_i/dc_i is also constant and therefore the value of r is constant.

Equation 3c may be solved for dc_i/dv_i

$$\frac{dc_i}{dv_i} = \frac{c_i}{r - v_i} \qquad (3d)$$

At first sight, this expression does not appear to be usable because c_i is unknown, although r and v_i are known. However, it is in fact possible to use this to find c_i at any point in the boundary as well as in the homogeneous solution. The method of doing this is discussed under the heading Calculations.

There still remains the problem of applying equation 2 to the case where the cell is sector shaped, so that the cross-sectional area is proportional to the distance from the center of rotation ($A = kx$) and the velocity of sedimentation is also proportional to the distance from the center ($dx/dt = s\omega^2x$). Writing equation 2b for all species, without specifying the rate of movement, r, of the frame of reference gives

$$\Sigma c_i\frac{d(kx)}{dt} + kx\Sigma\frac{dc_i}{dt} = -\frac{d}{dx}\{\Sigma c_i\ kx(s_i\omega^2x - r)\} \qquad (4a)$$

The proportionality constant, k, drops out and dx/dt is simply r.

$$cr + x\Sigma\frac{dc_i}{dt} = -\omega^2x^2\Sigma s_i\frac{dc_i}{dx} - \omega^2x^2\Sigma c_i\frac{ds_i}{dx} - 2\omega^2x\Sigma c_is_i + rx\Sigma\frac{dc_i}{dx} + cr \qquad (4b)$$

$$r = \left\{\Sigma\frac{dc_i}{dt} + 2\omega^2\Sigma c_is_i + \omega^2x\Sigma s_i\frac{dc_i}{dx} + \omega^2x\Sigma c_i\frac{ds_i}{dx}\right\}\Big/\frac{dc}{dx} \qquad (4c)$$

When the sedimentation coefficients of all species are independent of concentration (*i.e.*, all $ds_i/dx = 0$) then only the species vanishing at this plane gives rise to a non-zero dc/dx and $r = (\Sigma dc_i/dt + 2\omega^2\Sigma c_is_i)/dc/dx + \omega^2xs'$. This shows that, for the case of a sector-shaped cell and changing field, the natural analog of a plane at constant concentration is a plane where $dc/dt = -2\omega^2cs$ since the rate of motion, r, of this plane is the field strength, ω^2x, times s', the sedimentation coefficient of the species vanishing at this plane.

Taking r now as the rate of motion of a plane where $dc_i/dt = -2\omega^2c_is_i$ and returning to the case where ds_i/dx is not zero, we find that $r/\omega^2x = s_i + c_i(ds_i/dc_i)$, which is exactly equivalent to the expression for the constant field. However, there are new complications in the way of proving that r/ω^2x still equals s' and so this will be taken by analogy from the two cases discussed above; it is at least clear that r/ω^2x cannot differ very much from s'.

Calculations

Values of dc/dx are taken throughout the boundary at the constant interval Δx (see Fig. 1); suppose that fifteen such values are used. These are taken as representing fifteen components. The concentration of each is $\Delta x dc_i/dx$ and the sedimentation coefficient of each at the plane in the boundary where it vanishes is s_i'; this will of course be an average since values from $s_i' - {}^1/_2\Delta s$ to $s_i' +$

$^1/_2\Delta s$ are included in the lamella $x_i - {}^1/_2\Delta x$ to $x_i + {}^1/_2\Delta x$. Similarly the sedimentation coefficient of each component at an infinite dilution, s_{0i}, is also an average value and the component includes values from $s_{0i} - {}^1/_2\Delta s_{0i}$ to $s_{0i} + {}^1/_2\Delta s_0$.

The problem in correcting for the Johnston–Ogston effect is to find the correct concentration, c_i or $\Delta x dc_i/dx$, of each component in the homogeneous solution—that is, the total concentration in the homogeneous solution of all species which vanish in the boundary with sedimentation coefficients from $s_i' - {}^1/_2\Delta s$ to $s_i' + {}^1/_2\Delta s$. This concentration is readily converted into the weight fraction of the i^{th} component in the original solution ($g(s_i)\Delta s$ or $g(s_{0i})\Delta s_0$) by dividing by c_0, the total concentration of the original solution, and multiplying by $e^{2s_i\omega^2 t}$, where s_i is the sedimentation coefficient of the i'th component in the homogeneous solution. The problem in correcting for the boundary-sharpening effect is to find s_{0i} and Δs_0: the species which are crowded together in Δs still represent the same weight fraction when they are spread out over the greater interval Δs_0, and so $g(s_{0i}) = g(s_i)(\Delta s_i/\Delta s_{0i})$.

Consider now the situation at the first plane in the boundary: only component 1 is present and so $\Delta x(dc/dx)_{x1}$ represents c_1. On moving to the second plane, at x_2, the total concentration increases by Δc and the sedimentation coefficient of component 1 consequently decreases by an amount $(\Delta s_1)_{x_2-x_1}$. If $s = s_0 - kc$ then $(\Delta s_1)_{x_2-x_1} = -k(\Delta c)_{x_2-x_1}$. The concentration of component 1 decreases by the amount $(\Delta c_1)_{x_2-x_1} = (c_1)_{x_1}(\Delta s_1)_{x_2-x_1}/(r/\omega^2 x - s_1)_{x_2}$. As a result, the concentration of component 2 is greater than $\Delta x(dc/dx)_{x_2}$ by $-(\Delta c_1)_{x_2-x_2}$. ($r/\omega^2 x$ is taken as the sedimentation coefficient of the species vanishing at x_2; it may be found by the method outlined in footnote 12 for finding s from $\ln(x/x_0)$.)

The same process is repeated in moving on to plane 3: c_1 is decreased by $(\Delta c_1)_{x_3-x_2} = (c_1)_{x_2}(\Delta s_1)_{x_3-x_2}/(r/\omega^2 x - s_1)_{x_3}$, c_2 is decreased by $(\Delta c_2)_{x_3-x_2}$ and c_3 is greater than $\Delta x(dc/dx)_{x_3}$ by $-(\Delta c_1 + \Delta c_2)_{x_3-x_2}$. This process is continued step by step until the concentration in the homogeneous solution of each component is known.

Once the concentrations in the homogeneous solution are known and converted to weight fractions of the original solution, there remains only the problem of finding s_0 and ds/ds_0 values. It is assumed that the relation between s and c_x is known. The concentration at x is simply $\int_{x_0}^{x}(dc/dx)dx$—trapezoidal integration is convenient and sufficiently accurate—and so the process of finding s_0 of the species vanishing at x is straightforward. Finally, ds/ds_0 can be obtained either by plotting s against the corresponding s_0 and reading the slope, or else simply by tabular differentiation.

When diffusion is not negligible and $g(s)$ must be obtained by extrapolation to infinite time[6-8,14] an appropriate curve of dc/dx *vs.* x may be obtained by calculating dc/dx from $g(s)$, at a time corresponding to the middle of the centrifuge run, by means of equation 1. Alternatively a curve of dc/ds *vs.* s will serve as well and is easier to obtain: it is found by multiplying $g(s)$ by the dilution factor, $e^{-2s\omega^2 t}$, and the original concentration, c_0.[21a]

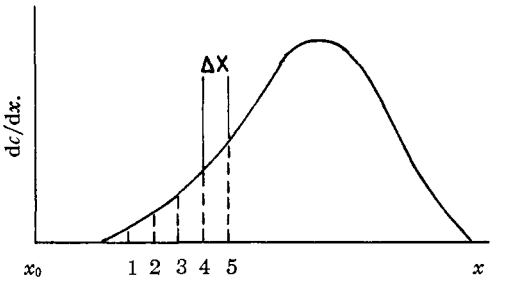

Fig. 1.—A representative boundary gradient curve. The position of the meniscus is marked by x_0. The concentration is constant to the right of the boundary; this is the region referred to in the text as the homogeneous solution.

Strictly speaking, the problem of obtaining $g(s_0)$ when neither the effects of diffusion nor those of concentration dependence of s are negligible should be solved by taking all three factors (heterogeneity, diffusion and concentration dependence) into account simultaneously. However, the difficulties of doing this appear prohibitive at this time. The present approach of taking into account two factors at a time should be a satisfactory approximation.

Discussion

Figure 2 shows curves of $g(s)$ *vs.* s for the same dextran sample measured at varying concentrations; these were kindly made available to me before publication by Professor J. W. Williams.[17] The influence of diffusion on the spread of the boundary was not negligible and so these were obtained by extrapolation to infinite time of an "apparent distribution."[6-8] In order to provide a clearer basis of comparison, these curves have been normalized so that $\int_0^\infty g(s)ds = 1$. This amounted to a correction of 4%, on the average, to the area of each curve of $g(s)$ *vs.* s and a correction of 8% in the case of the curve extrapolated to infinite dilution. The distribution is defined in such a way that the area under the curve is 1 but errors of extrapolation may cause the final curve obtained experimentally to differ from this.

The following two expressions were used to represent the dependence of s on c_x, in testing the correction for the dependence of $g(s)$ on concentration: $s_i = s_{0i}(1 - 0.2c)$ and $s_i = s_{0i}(1 - 0.06s_{0i}c)$, where c is in g./100 ml. and s is in Svedberg units. (Although the composition, as well as the total

(21a) (Footnote added in proof.) Since there is a dilution with time, extrapolation to infinite time could also remove some of the concentration dependence effects. However, as long as the changes brought about by the dilution with time are of the same order as the experimental uncertainty, such an effect would not be expected. Another problem in making these calculations for systems in which the effects of concentration dependence are large is the possibility of transport by convection. Harrington and Schachman[21b] have shown that it occurs in two-solute systems as a consequence of the change with time in the Johnston–Ogston effect. Trautman, *et al.*,[21c] have given a quantitative treatment of this for two-solute systems. The author would like to thank Dr. Trautman for making his manuscript available before publication and for discussion of this article.

(21b) W. F. Harrington and H. K. Schachman, THIS JOURNAL, **75**, 3533 (1953).

(21c) R. Trautman, V. N. Schumaker, W. F. Harrington and H. K. Schachman, *J. Chem. Phys.*, in press.

concentration, undoubtedly affects s_i, no attempt was made to allow for this because the necessary experimental information was not available.) The former represents the dependence of $\bar{s}$, the weight-average sedimentation coefficient, on c for the data of Fig. 2.[22] The latter satisfactorily represents the results obtained by E. F. Woods for a series of dextran samples studied in this Laboratory.[23] There may be differences in sedimentation behavior between these and the sample considered here, because of the difference in sources, so that it is difficult to say which of the two ways used to represent the dependence of s on c is preferable.

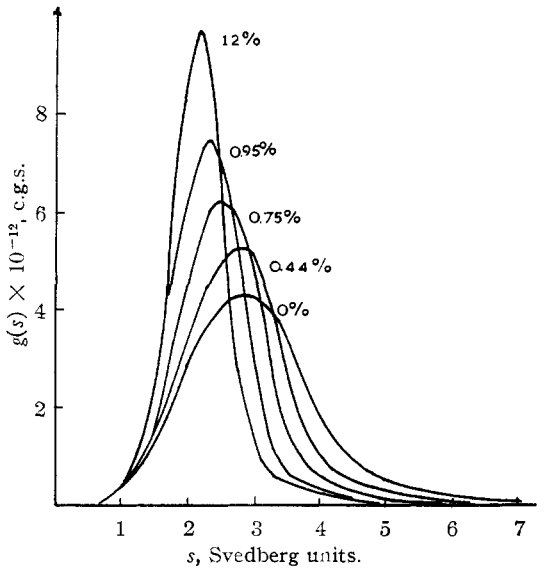

Fig. 2.—$g(s)$ measured, without correction for the dependence of s on c, at varying concentrations of the same dextran sample. The curve marked 0% was obtained by extrapolation. These curves were kindly made available by Dr. J. W. Williams.[17]

In order to estimate the reliability of the curve extrapolated to infinite dilution (the one labeled 0% in Fig. 2) another way of carrying out this extrapolation was tried. The extrapolated curve of Fig. 2 was obtained by plotting $g(s)$ against c at fixed values of s; the extrapolations were fairly linear in this case.[24] Figure 3 shows this curve and another obtained by plotting s against c at fixed values of the ratio $g(s)$ to $g(s)_{max}$.[25] Also shown in this figure is a curve in which the correction to infinite dilution has been made by calculation (taking $s_i = s_{0i}(1 - 0.2c)$ rather than by extrapolation; the curve labeled 0.44% in Fig. 2 is the starting curve for this calculation. The calculated curve agrees with either of the extrapolated curves better than they agree with each other.

Figure 4 shows a more severe test of the theory. The corrected curves based on $g(s)$ measured at the highest (1.2%) and the lowest (0.44%) concentrations are shown. Both ways of representing the dependence of s on c were tried. The agreement of the two curves calculated from $g(s)$ at 0.44% is quite satisfactory, the agreement of the two curves from the higher concentration is less satisfactory.

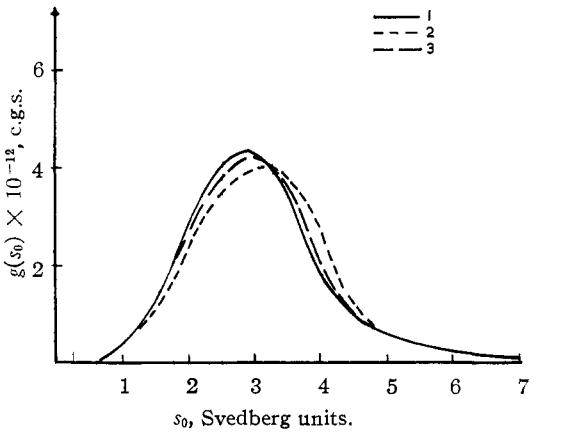

Fig. 3.—Comparison of curves for infinite dilution. No. 1 is the extrapolated curve of Fig. 2. No. 2 was obtained by extrapolation of s *versus* c at fixed values of $g(s)/g(s)_{max.}$ and No. 3 has been calculated from the curve marked 0.44% in Fig. 2.

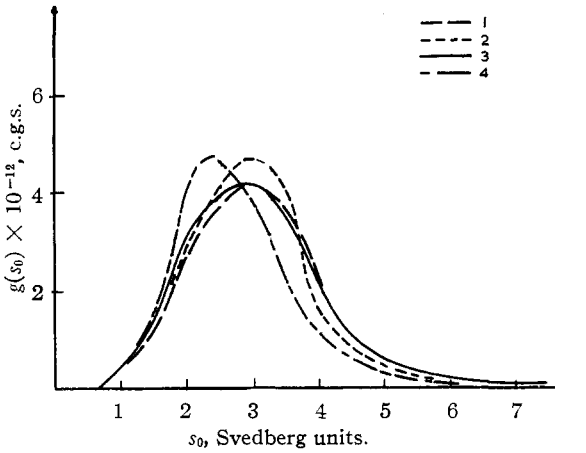

Fig. 4.—Comparison of curves corrected for the dependence of s on c. In calculating 1 and 2, the dependence of s on c was represented by $s_i = s_{0i}(1 - 0.2c)$, in 3 and 4 by $s_i = s_{0i}(1 - 0.06s_{0i}c)$. Nos. 1 and 3 were calculated from the curve marked 0.44% in Fig. 2, Nos. 2 and 4 from the curve marked 1.2%.

Finally, Fig. 5 shows the magnitude of the correction and also the relative importance of the Johnston–Ogston and boundary-sharpening effects; the curve of $g(s)$ at 1.2% was used and the dependence of s on c represented by $s_i = s_{0i}(1 - 0.2c)$. The curves which Jullander[15] obtained after making a correction for the boundary-sharpening effect but not for the Johnston–Ogston effect are compared, in his paper, with ones extrapolated to infinite dilution (obtained by extrapolating separately the three parameters of a distribution function). Comparison of these with Fig. 5 of this paper shows that correction for the Johnston–Ogston effect would bring his two sets of curves closer together.

(22) The sedimentation coefficient corresponding to the maximum concentration gradient may be measured with greater precision but does not necessarily refer to the same species in sedimentation measurements made at different concentrations. A method of measuring the weight-average sedimentation coefficient in the homogeneous phase has been described.[14]

(23) A. G. Ogston and E. F. Woods, to be published.

(24) J. W. Williams, personal communication.

(25) This method of extrapolation was suggested by B. G. Dick.

These results show that the correction gives a reliable distribution of $g(s_0)$ *vs.* s_0 when applied to a curve of $g(s)$ in which the effects of the dependence of s on c are not severe but nevertheless cannot be neglected. The value of the correction is that it effects a considerable saving of time, in comparison with extrapolation to infinite dilution, and perhaps a slight increase in accuracy. When diffusion cannot be considered negligible and $g(s)$ must be obtained by extrapolation to infinite time, two days' work are required to obtain a curve of $g(s)$ at a single concentration. Four such curves are required for extrapolation to infinite dilution, only one is required for the correction to infinite dilution. The time required to apply the correction is one half day.

The chief source of error likely to be encountered in using this procedure is an inadequate knowledge of how the dependence of s upon c_x is to be represented. When only a single sample of a substance is available, it is possible to determine no more than how the weight-average sedimentation coefficient varies with the total concentration and it does not necessarily follow that all species present will show this same behavior. Even when fractions are available, so that the variation with concentration can be studied as a function of s_0, there is the further problem that s_i in general depends not only upon the total concentration, c_x, but also upon the composition at x.

Considering this and both the magnitude and complexity of the correction, as shown by Fig. 5, (complex in the sense that not only the position but also the shape of the curve must be altered by the correction), the agreement of the curves in Fig. 4 calculated from the two concentrations is probably satisfactory, from the standpoint of testing the theory. From the experimental standpoint of obtaining the best distribution, the curve of $g(s)$ measured at the lowest workable concentration should be used for finding $g(s_0)$.[26]

(26) The problem of correcting apparent diffusion coefficients for the dependence of s on c may be approached in a similar fashion: such treatment is reported elsewhere.[14]

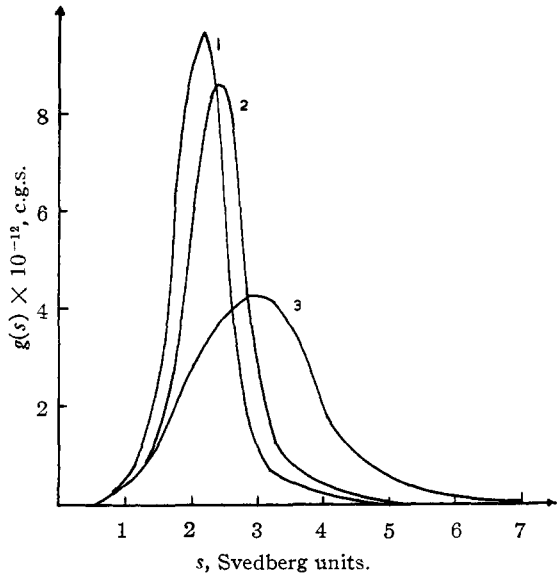

Fig. 5.—Relative magnitudes of the Johnston–Ogston and the boundary-sharpening effects. No. 1 is the curve marked 1.2% in Fig. 2, No. 2 has been corrected for the Johnston–Ogston effect and No. 3 has been corrected for both the boundary-sharpening and Johnston–Ogston effects.

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MADISON, WISCONSIN

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY][1]

Composition and Hydration of Protein Crystals in Salt Solutions[2]

BY T. L. MCMEEKIN, M. L. GROVES AND N. J. HIPP

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Values are reported for the compositions and densities of β-lactoglobulin, hemoglobin and chymotrypsin DP crystals in a number of salt solutions. β-Lactoglobulin crystals suspended in ammonium sulfate solutions differ markedly from hemoglobin and chymotrypsin crystals. The hydration of the protein, as calculated on the assumption that salt in the protein crystal is associated with the same amount of water as in the suspending medium, leads to values for protein hydration which are in good agreement with the relative vapor pressure water content curve except in cases where there is an apparently extensive combination of protein with salt.

The water content of large protein crystals can be determined readily by loss in weight on drying.[3] Estimates of the water in protein crystals have also been made by means of density and X-ray data.[4,5] The results of these measurements show the β-lactoglobulin and horse methemoglobin crystals contain about 0.8 g. of water per g. of protein in the absence of salt. If, however, the water content of these protein crystals is estimated by the method of

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(2) Presented in part before the 120th Meeting of the American Chemical Society at New York, N. Y., September, 1951; and also in part at the Meeting of Federation of American Societies for Experimental Biology, New York, N. Y., April, 1952.

(3) T. L. McMeekin and R. C. Warner, THIS JOURNAL, **64**, 2393 (1942).

(4) D. Crowfoot, *Chem. Revs.*, **28**, 215 (1941).

(5) M. F. Perutz, *Trans. Faraday Soc.*, **XLIIB**, 187 (1946).