Time Dependence of Concentration Distributions in Sedimentation Analysis*

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ABSTRACT: Relations are developed for the determination of concentration-dependent sedimentation and diffusion coefficients by use of derivatives of concentration with respect to time in sedimentation analysis. The equation of continuity for the ultracentrifuge is shown to be simplified, under certain conditions, at a point in the liquid column where the velocities imparted by diffusion and transport are equal. A formal solution of this equation is presented.

Methodology for such determinations is proposed and the rationale behind application of time-lapse photography to molecular weight determinations is explicated.

Transport processes, such as electrophoresis, sedimentation, chromatography, and countercurrent distribution, are dynamic, yielding concentration distributions that are functions of time as well as space. Only in the calculation of mobilities, however, is the temporal aspect of such changes conventionally utilized, *e.g.*, in the calculation of sedimentation coefficients. While some of the advantages of utilization of the more subtle temporal concentration dependencies have been pointed out (Archibald, 1963), direct use of these has been restricted to only a few applications (Creeth, 1962; Charlwood, 1967). This is due in large part to experimental difficulties in the acquisition and evaluation of the relevant data. Thus, while the ultracentrifuge is an analog computer, continually displaying solutions of the continuity equation (Archibald, 1963), the conventional means of data acquisition and analysis preclude the sampling of more than a minute fraction of this constant flow of information from the optical systems of the instrument. This paper, the first in a series, proposes methodology to overcome the experimental difficulties and explores the theoretical aspects and advantages of such methodology in sedimentation analysis. A companion paper (Simpson and Bethune, 1970) details one experimental use of such methodology.

Methodology. There are, conventionally, three optical systems utilized to visualize concentration distributions in the ultracentrifuge. There are the interferometric systems which either display the concentration distribution or its derivative as a function of radial distance. These are, respectively, the Rayleigh (Svensson, 1950, 1951; Richards *et al.*, 1968) and the schlieren systems (Thovert, 1914; Philpot, 1938; Svensson, 1939, 1940, 1954; Forsberg and Svensson, 1954; Pickels, 1942; Wolter, 1950; Trautman and Burns, 1954). Data acquisition and storage are performed by photography. There are, alternatively, optical systems which depend on the absorptivity of the solution. The data output may be from a densitometered photograph or from a graphical display of the output of a scanning device (Schachman and Edelstein, 1966).

Any of the optical systems may be modified to enable collection and evaluation of the concentration distribution as a function of time. Thus, the photographic refractometric systems can be automated simply by time-lapse photography (Simpson and Bethune, 1970) followed by projection of the resultant film on a screen equipped with suitable rasters.

Utilization of this approach imposes a number of mechanical constraints on the equipment used. These comprise: (1) Reproducible and precise location of the film in the focal plane of the optical system. (2) Precise location of the film perforations (which regulate the distance the film advances between pictures) with reference to the optical axis (*i.e.*, the identical field is framed as successive photographs are taken). (3) Exposure intervals and film advance intervals must be highly reproducible. (4) The film perforations, which act as film locaters with reference to the optical fields of the camera and projector, viewed as a coupled pair, must be precisely spaced. (5) The film emulsion must undergo no distortion during processing. (6) The projector utilized must precisely reproduce the positioning of the film in the camera. (7) Distortion due to false motion or to optical artifacts must not be introduced by the projector. The first three are properties of the camera, the next two of the film, and the last two of the projection device.

As indicated elsewhere (Simpson and Bethune, 1970) commercial instrumentation and film are adequate to meet these demands. When projected over suitable rasters, any measurements desired may be made on either Rayleigh or schlieren patterns.

The approach has one advantage over any others visualized in that it is relatively inexpensive. It has the disadvantage that data from the experimental record, while continuous and readily evaluated in one reference frame (*i.e.*, the projection screen), must be monitored and evaluated by the experimenter, any desired computation being done subsequently. Moreover, examination of the record during the sedimentation run is generally difficult if a high-quality photographic record is desired.

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These difficulties can be overcome by use of a monitoring device which converts light to electric energy (*e.g.*, the ultraviolet scanner; Schachman and Edelstein, 1966) followed by digitalization of the signal and storage on magnetic tape allowing subsequent manipulation of the stored data by a computer, or by use of a dedicated "on line" computer (Spragg and Goodman, 1969).

For the refractometric systems the requirement would be for an image converter situated at the focal plane of the optical system. A television camera, if of the correct specifications, would fit this prescription. These specifications, while taking a different form from those for a photographic apparatus, are designed to achieve the same purpose, fidelity of image reproduction.

Thus, (1) either the sensitivity of the camera must be high (since the light level is low in the Rayleigh optical system) or an integrating circuit must be incorporated. (2) The phosphor surface must be fine grained enough to allow definition of distances at which maximal intensity changes are detected within a preset error limit (*e.g.*, $\pm 5 \times 10^{-4}$ cm in 3.5 cm, that presently obtained using a comparator to evaluate the fringe pattern as recorded on a photographic plate). (3) The positioning of the electron scanning beam on the phosphor surface should be within the same limits as in 2. Indeed, it may be preferable to restrict the number of horizontal traces to some small integer (*e.g.*, one, which would allow much more highly defined positioning of the scanning beam). (4) Any distortion of the image imposed by the conversion device must be reproducible (*i.e.*, it can be included as part of the software for the computer ultimately used to manipulate the data).

A system of this general type would have a number of advantages over the time-lapse approach, including monitoring during the run (*i.e.*, by a television set) and selection and conversion of the data into a form suitable for direct manipulation by a computer.

The following theoretical development envisions utilization of the above means of data acquisition.

Theory. Archibald (1963) has elaborated three relations involving $\partial c/\partial t$ from the Lamm equation. First

$$\int_{r_1}^{r_2} r\frac{\partial c}{\partial t}\mathrm{d}r = \left[Dr\frac{\partial c}{\partial r} - s\omega^2 r^2 c\right]_{r_1}^{r_2} \tag{1}$$

which is exact, whatever the concentration dependence of s and D. There being only two unknowns in this linear equation, in principle, only two sets of values are required to obtain s and D. Archibald (1947) has given an extensive account of methods of evaluation using eq 1. In addition to methods described by Archibald (1947), an alternative is to perform the integration first from r_1 to r_2, then to $r_2 + \Delta r$. Thus

$$\left[Dr^2c\left(\frac{1}{rc}\frac{\partial c}{\partial r} - \frac{\omega^2 s}{D}\right)\right]_{r_1}^{r_2} = \int_{r_1}^{r_2} r\frac{\partial c}{\partial t}\mathrm{d}r$$

and

$$\left[Dr^2c\left(\frac{1}{rc}\frac{\partial c}{\partial r} - \frac{\omega^2 s}{D}\right)\right]_{r_1}^{r_2+\Delta r} = \int_{r_1}^{r_2+\Delta r} r\frac{\partial c}{\partial t}\mathrm{d}r$$

From these two simultaneous equations, s and D are immediately obtained.

The validity of this approach was checked utilizing calculated values of concentration, as a function of time and distance, similar to those obtained by Archibald (1947), using his values for the operating conditions. Direct numerical integration of the Lamm equation was performed (Dishon *et al.*, 1966) to obtain the numerical values rather than from the first eigenvalue of Archibald's analytical solution to the equation (Archibald, 1938). The values of the initial parameters were $s = 5.0$ S, $D = 7.5$ F. Those obtained by application of eq 2 were $s = 4.99$ S, and $D = 7.51$ F, both ± 0.01, when a random noise level equivalent to a plate reading error of 0.0005 cm was incorporated.

This direct method obviates the necessity of either selecting r_1 and r_2 to make

$$\int_{r_1}^{r_2} r\frac{\partial c}{\partial t}\mathrm{d}r = 0$$

or a prior estimate of $s\omega^2/D$, these being the techniques elaborated by Archibald (1947).

A second relation involving $\partial c/\partial t$ can be elaborated as follows (Archibald, 1963). If the concentration is expanded in Taylor's series as a function of r and t around a point, r_0, t_0, then

$$c(r,t) = c(r_0, t_0) - \left.\frac{\partial c}{\partial r}\right|_{r_0, t_0}(r - r_0) + \left.\frac{\partial^2 c}{\partial r^2}\right|_{r_0, t_0}(r - r_0)^2 + \ldots + \left.\frac{\partial c}{\partial t}\right|_{r_0, t_0}(t - t_0) + \ldots \tag{2}$$

Similarly, any $c(r)$ at t_0, given r_0, can be expressed as

$$c(r) = c(r_0) + \sigma(r - r_0) + \frac{\mu}{2}(r - r_0)^2 t \ldots$$

which is readily fitted to the data. Then, if s and D are not concentration dependent

$$\mu = \left.\frac{\partial^2 c}{\partial r^2}\right|_{r_0, t_0} = \frac{1}{D}\left.\frac{\partial c}{\partial t}\right|_{r_0, t_0} + \left[\frac{\omega^2 s r_0}{D} - \frac{1}{r_0}\right]\left.\frac{\partial c}{\partial r}\right|_{r_0, t_0} + \frac{2s\omega^2}{D}c(r_0)$$

This again allows determination of s and D, but only if any concentration dependence is absent. If it is not so, then the approach can be extended to yield

$$\mu = \frac{1}{D}\left(\left.\frac{\partial c}{\partial t}\right|_{r_0, t_0} + \omega^2 r_0 c(r_0, t_0)\frac{\partial s}{\partial r} + 2s\omega^2 c(r_0, t_0) + s\omega^2 r_0 \sigma\right) - \left(\frac{1}{D}\frac{\partial D}{\partial r} + \frac{1}{r_0}\right)\sigma$$

Alternatively, if the concentration dependence is explicitly expressed as

$$\begin{aligned} D &= D^0(1 - ac) \\ s &= s^0(1 - bc) \end{aligned} \tag{3}$$

where D^0 and s^0 are the limiting values of D and s, c being concentration and a and b constants, then

$$r_0 \frac{\partial c}{\partial t}\bigg|_{r_0,\, t_0} = D^0(\sigma + r_0\mu) + aD^0(-\sigma c(r_0) - r_0\sigma^2 - r_0c(r_0)\mu) + s^0(-\omega^2 r_0^2\sigma - 2\omega^2 r_0 c(r_0)) + bs^0(2\omega^2\, r_0\, \{c(r_0)\}^2 + 2\omega^2 r_0^2 c(r_0)\sigma)$$

The four unknowns are obtained directly by solution of a set of four simultaneous equations, at different values of t_0.

The third manipulation involves multiplication of the Lamm equation by r^n, followed by integration with respect to r. Then, if

$$I(n) = \int_{r_1}^{r_2} r^n c\mathrm{d}r$$

$$\frac{\partial}{\partial t}\{I(n+1)\} = n\omega^2 sI(n+1) + n^2 DI(n-1) - nD[r_2{}^n c(r_2) - r_1{}^n c(r_1)]$$

where again s and D are assumed constant. This may be written as

$$\int_{r_1}^{r_2} r^{n+1}\frac{\partial c}{\partial t}\mathrm{d}r = ns\omega^2 I(n+1) + n^2 DI(n-1) - nD[r_2{}^n c(r_2) - r_1{}^n c(r_1)]$$

which may be directly evaluated from Rayleigh fringe patterns, the only derivative involved being $\partial c/\partial t$.

While this evaluation depends on the constancy of s and D, Archibald (1963) observed that it should be possible to incorporate any concentration dependence of s and D in this expression.

If the concentration dependence is as expressed by eq 3, one approach is to define another integral

$$J(n) = \int_{r_1}^{r_2} r^n c^2 \mathrm{d}r$$

Then, if the same procedure, starting with the Lamm equation, is carried out in this case, after tedious algebra and numerous integrations by parts, it is found that

$$\frac{\partial}{\partial t}\{I(n+1)\} = n^2 D^0\left\{I(n-1) - \frac{a}{2}J(n-1)\right\} + ns^0\,\omega^2\{I(n+1) - bJ(n+1)\} - nD^0\left\{r_2{}^n c(r_2) - r_1{}^n c(r_1) - \frac{a}{2}r^n c(r_2) + \frac{a}{2}r_1{}^n c(r_1)\right\}$$

Here the full boundary condition

$$D^0\frac{\partial c}{\partial r} - aD^0 c\frac{\partial c}{\partial r} - s^0\omega^2 rc - bs^0\omega^2 rc^2 = 0$$

at $r = r_1$ and r_2, where r_1 and r_2 are the menisci, is utilized.

Again, this approach should be an excellent one, involving as it does only integrals involving the concentration, except for the derivative with respect to time.

Another approach involves an interesting aspect of the Lamm equation that has apparently not been explicated before. Thus, if the equation is written as (assuming, initially, that s and D are not functions of concentration)

$$\frac{\partial c}{\partial t} = \left(\frac{D}{r} - s\omega^2\right)\frac{\partial c}{\partial r} + D\frac{\partial^2 c}{\partial r^2} - 2s\omega^2 c$$

There must exist some value of r, physically realizable, at which

$$s\omega^2 r = D/r \tag{4}$$

i.e.,

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial r^2} - 2s\omega^2 c \tag{4a}$$

since, for this value of r

$$\frac{D}{r} - s\omega^2 r = 0$$

The dimensions of D/r and $s\omega^2 r$ are $[1/t]$, *i.e.*, velocities. Therefore, let the value of r at which eq 4 is satisfied be denoted by r_v. The physical significance of r_v may be illuminated by consideration of the Svedberg equation and the situation existing at sedimentation equilibrium.

Thus, in the limit

$$M = \frac{s}{D}\frac{RT}{1 - v\rho}$$

Therefore

$$r_v{}^2 = \frac{RT}{M(1 - v\rho)\omega^2} \tag{5}$$

and, once r_v is determined, a molecular weight may be calculated. Additionally, the integrated sedimentation equilibrium equation is

$$M = \frac{2RT}{1 - v\rho}\frac{1}{\omega^2}\frac{1}{r_2{}^2 - r_1{}^2}\frac{\Delta c}{c^0} \tag{6}$$

and if

$$\begin{aligned} r_2 &= r_v + f\delta \\ r_1 &= r_v - (1 - f)\delta \end{aligned} \tag{7}$$

where δ is the length of the liquid column and f is the fractional distance from r_v to r_2, *i.e.*

$$f = \frac{r_2 - r_v}{\delta}$$

TABLE I: Speeds (in revolutions per minute) at Which a Given Molecular Weight Species Will Attain a Given Value of r_v, Together with Associated Values of $\Delta C/C_0$ at Two Values of δ.

	M				$\Delta C/C_0$	
r_v (cm)	100 $\times 10^{-4}$	1000 $\times 10^{-4}$	10,000 $\times 10^{-3}$	100,000 $\times 10^{-3}$	$\delta =$ 1.0	$\delta =$ 10.0
1	29.8	9.43	29.8	9.43	1.45	55.0
2	14.9	4.71	14.9	4.71	0.61	16.3
3	9.94	3.14	9.94	3.14	0.38	8.33
4	7.45	2.36	7.45	2.36	0.28	5.31
5	5.96	1.89	5.96	1.89	0.22	3.8
6	4.97	1.57	4.97	1.57	0.18	2.9
7	4.25	1.35	4.25	1.35	0.15	2.3
8	3.73	1.18	3.73	1.18	0.13	2.0
9	3.31	1.06	3.31	1.06	0.12	1.7
10	3.98	0.943	2.98	0.943	0.10	1.5

Then

$$M = \frac{2RT}{1 - v\rho}\frac{1}{\omega^2}\frac{1}{2r_v - \delta + 2f/\delta}\frac{\Delta c}{c^0} \tag{8}$$

Eliminating M from eq 5 and 8

$$\frac{1}{r_v^2} = \frac{2\Delta c}{\delta c^0(2r_v - \delta + 2f/\delta)}$$

or

$$\Delta c = \frac{\delta(2r_v - \delta + 2f/\delta)c^0}{2r_v^2}$$

Thus, it is desirable to obtain a physically realizable r_v, and simultaneously realize a maximal Δc since the number of values of $\partial c/\partial t$ obtainable is related at any point to the difference between the concentrations there at $t = 0$ and $t = \infty$. While r_v is a function only of size and radial velocity (eq 3), Δc is a function of r_v, the column height (δ), and its distribution around r_v (*i.e.*, f).

Table I summarizes values of rotor speed (in revolutions per minute) necessary to attain a given value of r_v, given a value of M, and associated values of δ for two values of $\Delta c/c^0$.

As examination of Table I demonstrates, physically realizable values of r_v, together with satisfactory values of Δc, are obtainable with present equipment for only a small range of molecular weights. The present range over which r_v can be measured is from 6 to 7 cm which would require a speed of ~1400 rpm for a molecular weight of 100,000. However, a liquid column situated at quite different radial distances from those presently obtainable could allow a much wider range of molecular weights to be covered.

While the above discussion illuminates requirements for practical realization of r_v within the liquid column (it always exists as a point, but if speeds are too high, it may not lie within the column), no procedure has been indicated for evaluation. One approach is to consider the equation

$$\frac{\partial c}{\partial t} = a\frac{\partial^2 c}{\partial r^2} + bc + d \tag{9}$$

If this is evaluated as an $f(r)$, where $\partial c/\partial r \neq 0$, then, at $r = r_v$, $d = 0$. Thus, d will change sign as r passes through r_v, and by use at first of a coarse increment of Δr at which (9) is calculated, the Δr interval over which d changes sign can be determined. Then this interval may be scanned at a smaller Δr and so on, to achieve any predefined level of accuracy. This procedure is ideally suited to computer calculations.

Equation 4a itself is susceptible of solution. Only a formal solution will be given here to allow comparison with Archibald's solution of the Lamm equation (Archibald, 1938). Thus, if $c = c_r c_t$

$$c_r\frac{\partial c_t}{\partial t} = Dc_t\frac{\partial^2 c_r}{\partial r^2} - 2s\omega^2 c_r c_t$$

then

$$\frac{1}{c_t}\frac{\partial c_t}{\partial t} = \frac{D}{c_r}\frac{\partial^2 c_r}{\partial r^2} - 2s\omega^2 = -\beta$$

where β is a constant. Then

$$\frac{\mathrm{d}\ln c_t}{\mathrm{d}t} = -\beta;\ c_t = e^{-\beta t}$$

and

$$D\frac{\mathrm{d}^2 c_r}{\mathrm{d}r^2} - 2s\omega^2 c_r = -\beta c_r$$

Let

$$\alpha = \frac{\beta - 2s\omega^2}{D}$$

then

$$\frac{\mathrm{d}^2 c_r}{\mathrm{d}r^2} + \alpha c_r = 0$$

The roots of the auxiliary equation are then $\pm\sqrt{-\alpha}$ and a general solution is

$$c_r = ae^{r\sqrt{-\alpha}} + be^{-r\sqrt{-\alpha}}$$

where a and b are constants of integration. Since r_v may occur anywhere in the liquid column, the usual boundary conditions may be applied (Archibald, 1938).

$$\frac{\mathrm{d}c_r}{\mathrm{d}r} - \frac{s\omega^2 r c_r}{D} = 0,\ r = r_1, r_2$$

Then

$$ae^{r_1\sqrt{-\alpha}}\left(\sqrt{-\alpha} - \frac{s\omega^2 r_1}{D}\right) - be^{-r_1\sqrt{-\alpha}}\left(\sqrt{-\alpha} + \frac{s\omega^2 r_1}{D}\right) = 0$$

and

$$ae^{r_2\sqrt{-\alpha}}\left(\sqrt{-\alpha} - \frac{s\omega^2 r_2}{D}\right) - be^{-r_2\sqrt{-\alpha}}\left(\sqrt{-\alpha} + \frac{s\omega^2 r_2}{D}\right) = 0$$

Now these equations can have roots other than the trivial ones, $a = b = 0$, only if

$$\begin{vmatrix} e^{r_1\sqrt{-\alpha}}\left(\sqrt{-\alpha} - \frac{s\omega^2 r_1}{D}\right) & e^{-r_1\sqrt{-\alpha}}\left(\sqrt{-\alpha} + \frac{s\omega^2 r_1}{D}\right) \\ e^{r_2\sqrt{-\alpha}}\left(\sqrt{-\alpha} - \frac{s\omega^2 r_2}{D}\right) & e^{-r_2\sqrt{-\alpha}}\left(\sqrt{-\alpha} + \frac{s\omega^2 r_2}{D}\right) \end{vmatrix} = 0$$

Then there is a set of roots which define the set of α's. Let α_n designate a value of α, $0 \leq n \leq \infty$. Then b_n can, through eq 9, be defined as

$$b_n = \frac{-a_n e^{r_1\sqrt{-\alpha}}n(\sqrt{-\alpha_n} - s\omega^2 r_1/D)}{e^{-r_1\sqrt{-\alpha}}n(\sqrt{-\alpha_n} + s\omega^2 r_1/D)} \equiv J_n a_n$$

The a_n may be evaluated from

$$c(r, 0) = c_0$$

$$c_0 = \Sigma a_n(e^{r\sqrt{-\alpha_n}} + J_n e^{-r\sqrt{-\alpha_n}})$$

This may be multiplied on both sides by

$$e^{-r}(e^{r\sqrt{-\alpha_n}} + J_{ne}{}^{-r\sqrt{-\alpha_n}}) \equiv e^{-r}p_n$$

and integrated.

$$c_0\int e^{-r}p_n \mathrm{d}r = \Sigma a_n \int p_m p_n e^{-r}\mathrm{d}r$$

But p_n is a solution of

$$\frac{\mathrm{d}^2 p_n}{\mathrm{d}r^2} + \alpha_n p_n = 0$$

Therefore

$$p_n = -\frac{1}{\alpha_n}\frac{\mathrm{d}^2 p_n}{\mathrm{d}r^2}$$

and

$$\int p_m p_n e^{-r}\,\mathrm{d}r = \frac{1}{\alpha_n}\int p_n \frac{\mathrm{d}^2 p_n}{\mathrm{d}r^2}e^{-r}\mathrm{d}r$$

from which, by integration by parts

$$\int p_m p_n\, e^{-r}\mathrm{d}r = 0, m \neq n$$

Then

$$a_n = \frac{c_0\int e^{-r}p_n \mathrm{d}r}{\int e^{-r}p_n \mathrm{d}_r}$$

and finally the formal solution is

$$c(r, t) = c_0\sum\left\{\frac{\int e^{-r}p_n \mathrm{d}r}{\int e^{-r}p_n{}^2\mathrm{d}r}\, p_n e^{-\beta_n t}\right\}$$

A second class of problems, involving molecular weight measurements at sedimentation equilibrium, can also be investigated by the above techniques utilizing the interferometric optical systems.

In such utilization the data can be manipulated in a number of different ways, some of which involve determination of the initial concentration, while others do not (Creeth and Pain, 1967). The most accurate of these latter techniques is apparently that of LaBar (1965), which involves establishment of a conventional low-speed equilibrium, followed by an acceleration to a higher speed, at which the meniscus is depleted of the kinetic unit. The fringe displacement is plotted against time and extrapolated, if necessary, to infinite time. The total fringe displacement, after division by the fringe spacing, gives the absolute fringe number at the meniscus in the equilibrium run. Thus, this technique allows direct determination, at a given point, of the absolute refractometric concentration of the molecule of interest in the actual solution in which the molecular weight experiment has been carried out.

However, with small molecules, or in solvents containing high concentrations of a third component, such as guanidinium chloride, high fields must be imposed to deplete the meniscus. Inevitably, large blanks are generated by distortion of the cell and windows (Simpson and Bethune, 1970). We have demonstrated the advantages of utilizing the converse process, *i.e.*, imposition of the high field initially, followed by a rapid drop to a much lower field, at which equilibrium is established by back-diffusion.

This approach encompasses all the advantages of the LaBar procedure, and additionally allows the whole process of concentration measurement to be achieved in minimal concentration gradients of the added component, as well as obviating the field imposed distortion of cell components. It was observed (Simpson and Bethune, 1970) that, with the cells used, optical artifacts relaxed completely in 1800 sec.

To determine the relaxation rate of both solvent components and of proteins, the high-speed equilibrium concentration distribution was calculated using the Lansing and Kraemer equation (1935)

$$c/c_0 = \frac{(z_2 - z_1)e^z}{e^{z_2} - e^{z_1}} \tag{10}$$

where $z = \omega^2 sr^2/D$ and $z_i = \omega^2 sr_i{}^2/D$, $i = 1, 2$, and 1 and 2 refer to the two ends of the liquid column.

This calculated concentration distribution was then fitted by a least-squares procedure to

$$\ln c = \ln a + bh, q < h \leq L$$
$$c = 0, 0 \leq h \leq q \tag{11}$$

where $L = r_2 - r_1$ and q is the value of h at which $C/C_0 = 1 \times 1.0^{-4}$. The fit to this equation is excellent. This can be illustrated by consideration of two different cases (Table **II**), where the values of assigned s and D are first used in the Svedberg equation

$$M = \frac{s}{D}\frac{RT}{1 - v\rho}$$

to calculate a molecular weight appropriate for those values of s and D (line 1). Then using the aggregate of data generated directly from eq 10 the molecular weights are calculated from

$$M = \frac{2RT}{1 - v\rho\omega^2}\frac{1}{}\frac{\mathrm{d}\ln c}{\mathrm{d}r^2}$$

yielding the second line.

Lastly, using the same relation, the molecular weights are calculated from the data generated by the linear approximation, eq 11. The error in the less favorable case is 0.2%, in the more favorable 0.02%.[1]

To obtain the relaxation rates, the Fourier series solution to the restricted diffusion equation (Harned, 1947) was then employed, with eq 11 in the form $C(h) = ae^{bh}$ characterizing the initial distribution. Then

$$c(r,t) = A_0 + \sum_{j=1}^{\infty} A_j e^{-j^2\pi\ Dt/L} \cos\frac{j\pi h}{L} \tag{12}$$

TABLE II

	s (S)	D (F)	s (S)	D(F)
Assumed Values:	1.0	15.0	2.0	7.0
	Calcd mol wt[a]			
(a) Svedberg equation	6485		27,793	
(b) *L* and *K*	6485		27,796	
(c) Least squares	6471		27,734	

[a] $\omega^2 = 5.035 \times 10^7$ sec^{-2}, $\bar{V} = 0.25$ ml/g, $T = 293$°K, and $\rho = 1.0$ g/ml.

where

$$A_0 = \frac{1}{L}\int_0^L c\mathrm{d}h$$

and

$$A_j = \frac{2}{L}\int_0^L c \cos\frac{j\pi h}{L}\mathrm{d}h$$

Then

$$A_0 = \frac{1}{L}\int_0^L ae^{bh}\mathrm{d}h$$
$$= \frac{a}{L}\int_q^L e^{bh}\mathrm{d}h$$

and

$$A_j = \frac{2}{L}\int_0^L ae^{bh} \cos\frac{j\pi h}{L}\mathrm{d}h$$
$$= \frac{2a}{L}\int_q^L e^{bh} \cos\frac{j\pi h}{L}\mathrm{d}h$$

where

$$L = r_2 - r_1$$

Then

$$A_0 = \frac{a}{bL}(e^{bh} - e^{bq})$$

and since A_0 is the only time-independent term, this must represent the final concentration everywhere in the liquid column. Since it is calculated for a zero field, it must be uniform and equal to that obtaining before the field was applied to concentrate the macromolecular component in the end of the liquid column. That is

$$c_0 = \frac{a}{bL}(e^{bh} - e^{bq})$$

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[1] That this is predictable can be discerned by examination of eq 1, if written as

$$c = a'e^{b'r^2}$$

where

$$a' = \frac{z_2 - z_1}{e^{z_2} - e^{z_1}}c_0$$
$$b' = \frac{\omega^2 s}{D}$$

But r can be written as

$$r = r_1 + h, \qquad 0 \leq h \leq L$$

and eq 11 becomes

$$c = a'e^{b'(r_1+h)^2}$$
$$\approx ae^{bh}$$

where

$$a = a'e^{b'r_1^2}$$
$$b = 2b'r_1$$

the error being of the order $r^{b'h^2}$. If short columns are used the range of h is about 3 mm, and for a protein component that is sedimented well down in the cell the concentration ratio is below 1×10^{-4} over most of this range. Fitting, therefore, occurs only over a small portion of $r_2 - r_1$.

The A_j terms are conveniently evaluated using the identities

$$2\,i \sin x = e^{ix} - e^{-ix}$$

$$2 \cos x = e^{ix} + e^{-ix}$$

Then

$$A_j = \frac{2b}{L}\,c(L) - \frac{2}{L}\,c(q)\left\{b \cos\frac{j\pi q}{L} - \frac{j\pi}{L}\sin\frac{j\pi q}{L}\right\}$$

and

$$c(r,t) = c_0 + \sum\left\{\frac{2bc(L)}{L} - \frac{2c(q)}{L}\left[b\cos\frac{j\pi q}{L} - \frac{j\pi}{L}\sin\frac{j\pi q}{L}\right]\right\}\cdot \{e^{-j^2\pi Dt/L}\}\left\{\cos\frac{j\pi h}{L}\right\}$$

and, at $h = 0$

$$c(0,t) = c_0 + \sum\left\{\frac{2b}{L}(L) - \frac{2}{L}c(q)\left[b\cos\frac{j\pi q}{L} - \frac{j\pi}{L}\sin\frac{j\pi q}{L}\right]\right\}\cdot \{e^{-j^2\pi Dt/L}\}$$

Then, for given values of s and D, the initial concentration was calculated as a function of r and t, *cf.* eq 12, assuming a zero field, until the concentration distribution yielded by eq 10 for a lower field was approached. For urea or guanidinium chloride, relaxation was 99% complete in 1800 sec, while for a protein with an s of 1.0 S and a diffusion coefficient of 15 F, a concentration difference equivalent to 0.02 fringe appeared at the meniscus only after 3600 sec, with a column length of 0.3 cm. If a protein has a molecular weight of ~7000 (*i.e.*, roughly corresponding to the values assumed above), the optical artifacts are relaxed completely in less than one-half the time necessary for any detectable amount of protein to appear at the meniscus.

Application of this technique, however, requires that the Rayleigh pattern be monitored constantly, which has been accomplished successfully using time-lapse photography (Simpson and Bethune, 1970).

Discussion

The possibility of coupling data gathering systems in transport experiments with computers allows the ready investigation of the dynamics of such processes. To permit full utilization of the derived experimental data requires elaboration of the theoretical aspects of the temporal dependence of concentration distributions in such processes. The theory, however, is general and is independent of the means of data acquisition.

Archibald (1947, 1963) indicated, for sedimentation analysis, certain directions along which such developments might proceed. The most useful of these would appear to be utilization of integrals of the form

$$\xi(n,v) = \int_{r_1}^{r_2} r^n c^v \mathrm{d}r$$

since data from the interferometric optical system can be used, without differentiation along a space axis.[2]

The development of the concept of the position r_V, determination of which allows an estimate of molecular weights, constitutes another direction along which theoretical developments can proceed. The continuity equation at this point is much simplified, and, after the variables are separated, can be compared with Archibald's (1938) solution of the full equation. There the solution of the radially dependent portion of the equation involves hypergeometric series, and determination of the eigenvalues constitutes such a problem that application of the solution to actual problems has rarely been attempted. The eigenvalues for the formal solution of the simpler equation at the point r_V, however, are defined by a much simpler equation involving only the sum of two exponentials, or, alternatively, a sine and a cosine term. Thus, while the formal solution resembles that of Archibald, application of it will constitute a much simpler arithmetic problem.[3]

The methodology proposed has been applied to molecular weight determinations at sedimentation equilibrium, utilizing a continuous photographic record to determine refractometric concentrations (Simpson and Bethune, 1970). Concentration of a macromolecular component at the periphery of the cell is accomplished by initial application of high fields, followed by relaxation of the macromolecular components, through diffusion, to a condition of sedimentation equilibrium at a much lower field. This procedure is especially useful when high concentrations of a third small component are present, since the macromolecule diffuses into an essentially uniform solvent and the concentration can be determined, to a high degree of accuracy, at any point in the cell.

It has been proved possible to obtain an estimate of such relaxation times by calculating a concentration distribution at high speed, for given values of s and D, and fitting these to a simple exponential (eq 11). This function is then employed to describe the initial concentration distribution required for application of the restricted diffusion equation to describe the relaxation at a low field. This procedure will yield a lower limit for such a relaxation process, since in itself it contains no terms involving sedimentation. Thus, the time required for a given relaxation of the concentration of a macromolecule under conditions closely approximating those established by Casassa and Eisenberg (1964) allows unambiguous measurement of molecular weights, even in mixed-solvent systems.

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[2] While all of the equations developed in the theoretical section are in differential form, these will typically be replaced by finite difference forms in the experimental situation. The development of suitable algorithms for computation to accomplish this with maximal accuracy is being achieved by generating, for a given choice of s, D, ω, and column position, tables of concentrations as a function of time and space. The exact values of the various derivatives are obtained either from Mason and Weaver's (1924) solution of the continuity equation for a rectangular cell in a uniform field or Archibald's (1938) solution to the Lamm equation. The algorithms are then used to generate the various derivatives from the concentration distributions, and these are compared with the exact derivatives. The regions of best fit then can be used to define the characteristics of the data gathering system, constituting an interesting application of *a priori* specification of an instrument.

[3] The generalization of the relations derived in this paper to systems undergoing interactions in transport experiments will form the subject of another paper.

In this respect the direct application of this methodology complements the automatic split-beam photoelectric scanner (Schachman and Edelstein, 1966), which allows determination of low absolute concentrations. Here advantage is taken of the present high accuracy of the interferometric system, and allows exploration of higher concentration ranges, of practical advantage in investigation of chemically reacting macromolecular systems.

Glossary of Symbols: a, b, d, constants; c, concentration; c_0 or c^0, initial concentration; Δc, concentration difference between the ends of a liquid column at menisci positions r_1 and r_2; D, diffusion coefficient; M, molecular weight; R, gas constant; r, radial distance from center of rotation; s, sedimentation coefficient; t, time; v, partial specific volume; ρ, density; and ω, centrifugal field ((rev/sec) $\times$ 2π).

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