PART III. FUNDAMENTAL THEORY OF SEDIMENTATION PROCESSES IN THE ULTRACENTRIFUGE

I. Basic Equations

The basic equation for describing the sedimentation of a single homogeneous solute in the sector-shaped cell under ultracentrifugal force was first presented by Lamm (20) as early as 1929. The most general form of this Lamm sedimentation equation may be written

$$\frac{\partial c}{\partial t} = \frac{\partial}{r\partial r}\left(Dr\frac{\partial c}{\partial r} - s\omega^2 r^2 c\right) \tag{1}$$

where c is the solute concentration at time t and position r measured from the center of rotation, ω is the angular speed of the rotor, and D and s are the diffusion and sedimentation coefficients of the solute, respectively. In deriving equation 1 it is assumed that the cell is shaped so that two planes defining its radial sides intersect on the axis of rotation. This geometry of the cell is necessary to keep the solution from convective flow when the rotor is in motion. The mathematical theory of sedimentation velocity experiments consists essentially of finding solutions to equation 1 under practical initial and boundary conditions, and thereby deriving methods which permit reliable evaluation of the sedimentation and diffusion coefficients of a given solute in a given solution from relevant ultracentrifugal measurements. Such solutions to equation 1 should form a basis for dealing with sedimentation processes in more complex systems involving two or more different solute components or a polydispersity with respect to molecular mass or shape or both.

In usual centrifugal experiments with a standard cell, the cell is filled initially with a uniform solution of a given concentration (say, c_0), and either the concentration distribution or the concentration gradient distribution produced in the cell when the rotor is in motion is measured as a function of time, using an appropriate optical technique. Therefore, the initial condition for a sedimentation velocity experiment of this type may be represented mathematically as follows:

$$c(r, 0) = c_0 \qquad (r_1 < r < r_2,\ t = 0) \tag{2}$$

where r_1 and r_2 are the positions of the meniscus and the bottom of the cell measured from the center of rotation, respectively.

When use is made of a synthetic boundary-forming cell which has recently come out as a new technique in ultracentrifugal experiments (18), initially the given solution is separated from the solvent by a sharp boundary formed midway between the meniscus and bottom, as is the case with the set-up in ordinary free-diffusion experiments on solutions. In this case, the initial condition to equation 1 may be represented by

$$c(r, 0) = \begin{cases} 0 & (r_1 < r < r_0,\ t = 0) \\ c_0 & (r_0 < r < r_2,\ t = 0) \end{cases} \tag{3}$$

where r_0 is the position of the initial sharp boundary formed between solution and solvent.

The boundary conditions to be given equation 1 are the same for both the standard and the synthetic boundary-forming cells. They are obtained from the physical requirement that no solute flow occurs across both the air–liquid meniscus and the bottom of the cell, leading to the equation:

$$D\frac{\partial c}{\partial r} - s\omega^2 rc = 0 \qquad (r = r_1 \text{ and } r_2,\ t > 0) \tag{4}$$

Either combination of the initial and boundary conditions, i.e., equations 2 and 4 or equations 3 and 4, suffices to determine uniquely a solution to the Lamm equation (equation 1) giving c as a function of time t and position r, provided that the forms or numerical values of s and D are given.

II. The Case in Which the Effect of Diffusion is Negligible

Before proceeding to discuss the basic solutions to the complete Lamm equation, it is convenient to consider its special case in which $D = 0$, i.e., the case in which diffusion is absent. Equation 1 then assumes the form:

$$\frac{\partial c}{\partial t} = -\frac{\partial}{r\partial r}(s\omega^2 r^2 c) \tag{5}$$

which is a partial differential equation of the first order in c. A simple kinetic consideration shows that substances of large molecular (or particle) size possess small diffusion coefficients and large sedimentation coefficients. Accordingly, equation 5 is expected to hold better for solutes having larger molecular size; strictly speaking, it should be valid only for a hypothetical limiting molecule of an infinitely large molecular weight.

A. The Radial Dilution Law

Equation 5 can be integrated readily using the method of characteristics, even when s depends on concentration c or r or a combination of both (10). In this case, however, the fulfillment of one of the boundary conditions (equation 4) must be abandoned, since equation 5 is of the first order with respect to the space coördinate r. It is physically legitimate in this case to discard the boundary condition at the bottom of the cell (10). Under this condition the solution of equation 5 subject to conditions 3 and 4 is obtained to give[1]

$$\begin{aligned} c(r, t) &= 0 \quad \text{for} \quad r_0 < r < r_*,\ t > 0 \\ c(r, t) &= c_0 \exp(-2\omega^2 st) \quad \text{for} \quad r_* < r, \quad t > 0 \end{aligned} \tag{6}$$

Here r_* is a function of t determined by the relation:

$$r_* = r_0 \exp(\omega^2 st) \tag{7}$$

In deriving the above solution, it has been assumed for simplicity that s does not depend on any factors such as solute concentration c and hydrostatic pres-

[1] Exactly the same solution is obtained with the initial condition 2 for the standard cell, in which case r_0 in all subsequent equations should be replaced by r_1.

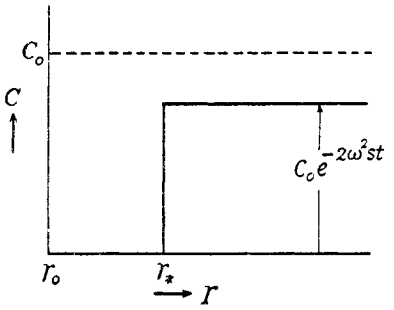

FIG. 1a

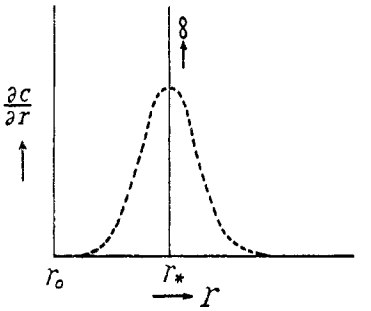

FIG. 1b

FIG. 1a. Concentration distribution in the cell when the effect of diffusion is absent.
FIG. 1b. Concentration gradient distributions in the cell when the effect of diffusion is absent (solid line) and when either diffusion or a spread in sedimentation coefficient distribution is present (dashed line).

sure p. Figures 1a and 1b show the concentration distribution and the concentration gradient distribution, respectively, calculated from the above solution. The concentration distribution forms a step-function, as might be expected from the condition that the back-flow of solute due to diffusion is absent. The discontinuous point r_* moves towards the cell bottom as time goes on in accordance with equation 7; it represents an infinitely sharp separation between solution and solvent. It should be noted here that, as the second equation of equation 6 shows, the concentration in the solution phase decreases exponentially with increasing time. This comes out of the geometrical condition that the cell has a sector shape and the field varies with r. By combining the second of equation 6 with equation 7 one finds the well-known relationship:

$$cr_*^2 = c_0r_0^2 \tag{8}$$

which indicates that the product of the concentration in the solution phase and the square of the radius to the solution–solvent separation remains constant during the course of an ultracentrifugal run. This fact is called the "square dilution effect" of the sector-shaped cell. It can be shown that this law holds for systems in which s depends on c (26), but it no longer does with s which varies with pressure (10). Figure 1b shows an infinitely sharp concentration gradient curve, corresponding to the discontinuous step in the concentration distribution diagram. Actually, concentration gradient curves observed in practical systems are more or less spread over a range about the maximum gradient, as shown schematically in figure 1b by a dashed line. This is mainly due to two causes: (*1*) that the effect of diffusion is not completely negligible, and (*2*) that actual systems often involve a distribution of sedimentation coefficient (and diffusion coefficient as well), particularly in systems of macromolecular solutes, either naturally occurring or synthetic. Spread in the sedimentation boundary produced by the latter cause has been discussed in detail in prior sections of this article.

From equation 7 it follows that

$$\ln r_* = \ln r_0 + \omega^2st \tag{9}$$

Thus, it is required that a plot of ln r_* vs. t forms a straight line with a slope of $\omega^2 s$. This affords a means of evaluating directly the sedimentation coefficient from experiment. Strictly speaking, this method of evaluation of s could be valid only in cases where boundary spreading due to diffusion is completely negligible, but, as has been shown by Goldberg (13), it may be used correctly if the square root of the second moment of the gradient curve is chosen as r_*, even when the boundary curve shows a spread due to diffusion. It can be shown that equation 9 holds with sufficient accuracy also for systems with s varying with concentration, provided that the concentration dependence is not too strong (1). In this case, the slope of the resulting straight line leads to a value of s corresponding to the initial concentration of the solution. Thus, one may determine the concentration dependence of s from a series of measurements started with different initial concentrations. It has been shown recently that this procedure of determining s as a function of c may also be applied for systems involving effects of diffusion (11). In this connection it is instructive to note that this method has been frequently used by previous investigators in this field, in the absence of reliable theoretical foundation for the validity of its use in practical systems where the spreading of the sedimentation pattern due to diffusion is apparent.

III. The Approximate Solution Due to Faxén

Faxén (9) was the first among those who have given solutions to the Lamm equation. Although his solution is merely an approximate solution derived under some restrictions, its remarkable simplicity has provided us, since the early days in Uppsala, with a practically unique method for analyzing sedimentation velocity data on a great variety of macromolecular systems.

A close examination (12) of his original analysis reveals that it is essentially based on two assumptions that

$$2D/\omega^2 s r_0^2 \ll 1 \tag{10}$$

$$2\omega^2 st \ll 1 \tag{11}$$

Besides these, his analysis assumes both sedimentation and diffusion coefficients to be independent of solute concentration and of any other factors. Assumption 10 implies that Faxén's solution may be applied only for systems in which D is small enough or s is large enough or there is a combination of both. More precisely speaking, the contribution from diffusion should be of a practically (not completely) negligible order in comparison with that due to ultracentrifugal force. Thus it is realized that his solution is essentially that for solutes of large enough molecular size. Mathematically, it must be a first-order refinement to the solution discussed in the preceding paragraph, where D was assumed completely zero. Approximation 11 suggests that t must be sufficiently small, because from condition 10 the product $s\omega^2$ should not be too small. Thus Faxén's solution may be useful only for the very early stages of the velocity ultracentrifugation of solutes of sufficiently large molecular size.

In addition to these restrictions, Faxén replaced the set of auxiliary conditions

3 and 4 by the so-called "infinite cell" condition such that

$$c(r, 0) = \begin{cases} 0 & (-\infty < r < r_0) \\ c_0 & (r_0 < r < \infty) \end{cases} \tag{12}$$

It is shown that this replacement is a good enough approximation, so far as the above two conditions 10 and 11 are satisfied. It should be noted that condition 12 can be derived as a special case for the conditions which obtain in the synthetic boundary-forming cell.

Under these restrictions one finds a solution of equation 1 in the form:

$$\frac{c}{c_0} = \frac{e^{-\tau}}{2}[1 - \Phi(\xi)] + \frac{e^{-\xi^2}}{\sqrt{y}}\left[\frac{\epsilon e^{-\tau}}{16\pi}(1 - e^{-\tau})\right]^{1/2} + \text{higher terms} \tag{13}$$

where $\Phi(\xi)$ is the error integral and other symbols are the dimensionless quantities defined by

$$\begin{aligned} \tau &= 2\omega^2 st \\ y &= (r/r_0)^2 \\ \xi &= [1 - (ye^{-\tau})^{1/2}]/[\epsilon(1 - e^{-\tau})]^{1/2} \\ \epsilon &= 2D/s\omega^2 r_0^2 \end{aligned} \tag{14}$$

In terms of these quantities assumptions 10 and 11 are written

$$\epsilon \ll 1 \quad \text{and} \quad \tau \ll 1 \tag{15}$$

The second term on the right-hand side of equation 13 is actually of the order of $(\epsilon\tau)^{1/2}$, so that it may be neglected, together with following higher terms, in comparison with the first term, except in the region where ξ is sufficiently large. Thus one obtains from equation 13 with a satisfactory approximation:

$$\frac{c}{c_0} = \frac{e^{-\tau}}{2}[1 - \Phi(\xi)] \tag{16}$$

A number of higher-order terms left undetermined in Faxén's original analysis have been calculated by Gosting (14) in connection with a study by Williams and Baldwin (6, 30, 31) on the boundary spreading due to molecular heterogeneity. It should be remarked, however, that Faxén's solution is originally based on the two fundamental assumptions, $\epsilon \ll 1$ and $\tau \ll 1$, so that contributions from such higher terms are practically negligible.

From equation 16 one obtains an approximate equation for the concentration gradient distribution in the form:

$$\frac{\partial(c/c_0)}{\partial(r/r_0)} = \{e^{-(3/2)\tau}[\pi\epsilon(1 - e^{-\tau})]^{1/2}\}e^{-\xi^2} \tag{17}$$

Since the third equation in equation 14 may be rewritten

$$\xi = [\epsilon\,(1 - e^{-\tau})]^{-1/2}\,[1 - e^{-\tau/2}(r/r_0)] \tag{18}$$

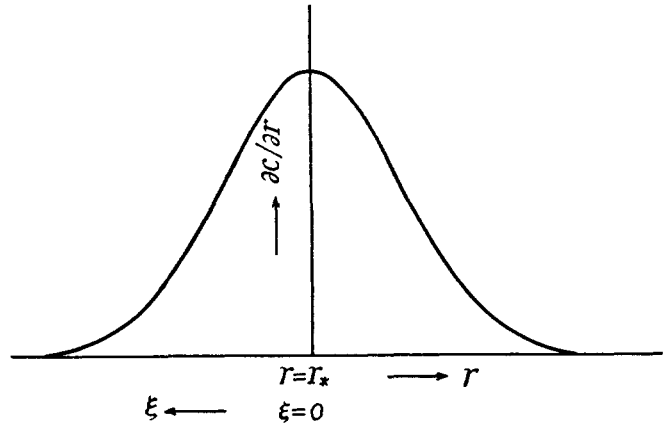

FIG. 2. Concentration gradient curve predicted from Faxén's solution with both s and D independent of concentration; the sedimentation is from left to right. The boundary pattern forms a Gaussian curve.

it is expected from equation 17 that measured sedimentation boundaries, $\partial c/\partial r$, should form a Gaussian curve (figure 2) when the coördinate origin of space variable r is displaced to a point r_* satisfying the relation:

$$1 - e^{-\tau/2}(r_*/r_0) = 0 \tag{19}$$

It is convenient to refer to r as the physical cell coördinate and to ξ as the reduced cell coördinate. Equation 19 indicates that the origin of the reduced cell coördinate, r_*, moves with the "reduced" time τ in accordance with a relation:

$$\ln r_* = \ln r_0 + \tau/2 \tag{20}$$

or

$$\ln r_* = \ln r_0 + \omega^2 st \tag{21}$$

Hence a plot of $\ln r_*$ against time t results in a straight line, and one can evaluate the required sedimentation coefficient from its slope. It is important to note that equation 21 is identical in form with equation 9, which was derived by assuming D to be zero. This implies that, so far as the basic assumptions involved in Faxén's solution are fulfilled, the displacement rate of the maximum position of a sedimentation boundary curve is solely determined by the value of s, irrespective of the value of the diffusion coefficient.[2]

Let us denote by A the area that a given sedimentation boundary encloses above the base line. It readily follows from equation 16 by letting $\xi \to -\infty$ that

$$A = c_0 \exp(-\tau) \tag{22}$$

Since, as is immediately understood from equations 18 and 19, $\xi > 0$ corresponds to $r < r_*$—namely, the solvent side—and $\xi < 0$ does to the solution side, the

[2] It is readily shown that the maximum of a sedimentation boundary curve given by Faxén's solution accords with the origin of the reduced cell coördinate if the contributions in equation 13 arising from the second- and higher-order terms are neglected.

quantity A represents physically the solute concentration at a place sufficiently far in front of the boundary between solvent and solution. The maximum height, H, of the boundary curve given by equation 17 is obtained by putting in it $\xi = 0$, giving

$$H = (c_0/r_0)\, e^{-(3/2)\tau}\, [\pi\epsilon\, (1 - e^{-\tau})]^{-1/2} \tag{23}$$

The "height-area ratio," H/A, is then obtained by combining equations 22 and 23 to give

$$\left(\frac{H}{A}\right)^2 = \frac{e^{-\tau}}{r_0^2\, \pi\epsilon(1 - e^{-\tau})} \tag{24}$$

which may also be written

$$\left(\frac{A}{H}\right)^2 = D\left[\frac{2\pi}{s\omega^2}\, (e^{2\omega^2 st} - 1)\right] \tag{25}$$

The quantity $(A/H)^2$ may be determined from observed sedimentation boundaries as a function of time, while s may be evaluated using the procedure described above from the same source of experimental data. Thus $(A/H)^2$ may be plotted against $(2\pi/s\omega^2)\ (e^{2\omega^2 st} - 1)$ over a range of time in which $2\omega^2 st$ is sufficiently small compared with unity to warrant Faxén's solution, and one obtains a straight line with a slope equal to the required diffusion coefficient. Since Faxén's solution assumes $2\omega^2 st$ to be sufficiently small, equation 25 may be expanded to give approximately

$$(A/H)^2 = 4\pi Dt \tag{26}$$

which indicates a plot of $(A/H)^2$ vs. $4\pi t$ to yield a straight line with a slope D. This method of evaluation of D requires no s value in advance, and appears to be convenient for practical purposes. However, it is recommended that use be made of a more complete method based on equation 25 for accurate evaluation of D.

As noted in the foregoing lines, Faxén's solution assumes the initial condition of the form of equation 12 which may substantially be suited to the synthetic boundary-forming cell. Therefore, strictly speaking, no information can be derived from it about the sedimentation process which obtains in the standard cell. Experiments on many macromolecular systems demonstrate that even in the standard cell a plot of $\ln r_*$ vs. t follows quite accurately a straight line when the peak of the concentration gradient curve separates from the meniscus. However, it is observed in this case that the straight line cuts through the horizontal line defined by $\ln r_* = \ln r_1$ with a finite intercept Δt, as schematically shown in figure 3. Comparison with equation 21 suggests that in the standard cell the position of the meniscus r_1 can no longer be regarded as a free boundary at $t = 0$. Nevertheless, the linearity of $\ln r_*$ vs. t observed for $t > \Delta t$ is generally so remarkably good that it is customary to assume observed sedimentation boundaries in a standard cell as if they were originated from as hypothetical sharp free boundary formed at the meniscus at a time $t = \Delta t$. If this assumption holds, one

may modify equation 21 to give

$$\ln r_* = \ln r_1 + \omega^2 s(t - \Delta t) \qquad (t > \Delta t) \tag{27}$$

Correspondingly, equations 25 and 26 must be modified such that

$$\left(\frac{A}{H}\right)^2 = D\left[\frac{2\pi}{s\omega^2}\left(e^{2\omega^2 s(t-\Delta t)} - 1\right)\right] \tag{28}$$

$$\left(\frac{A}{H}\right)^2 = 4\pi D(t - \Delta t) \tag{29}$$

The value of Δt may be determined by linear extrapolation of the observed $\ln r_*$ vs. t plot, as illustrated in figure 3; it is frequently referred to as the "zero-time correction." Exact evaluation of Δt is immaterial for the determination of s, because only the slope of $\ln r_*$ against t is important. Such is not the case, however, for the evaluation of D by means of equation 28, especially when the maximum position of the sedimentation boundary curve moves rapidly towards the bottom of the cell; in such a case the value of s is necessarily so large that the interval of time in which Faxén's solution may hold is considerably shortened.

It is of considerable interest to investigate theoretically how closely this empirical modification of Faxén's original solution is applicable to data of sedimentation velocity experiments from the standard cell. To this end solution must be obtained to the Lamm equation subject to conditions 2 and 4. If, as in the original analysis of Faxén, interest is confined only to the sedimentation behavior at very early stages of ultracentrifugation, the second condition in equation 4, i.e., the condition at the cell bottom, may be replaced, to a good approximation, by

$$c(r, t) = \text{finite} \qquad (r = r_2 = \infty, t > 0) \tag{30}$$

The set of auxiliary conditions thus obtained may be referred to as the "semi-infinite cell" condition. MacCosham and Fujita (21) worked out a solution to equation 1 under this set of conditions and confirmed that the empirical proce-

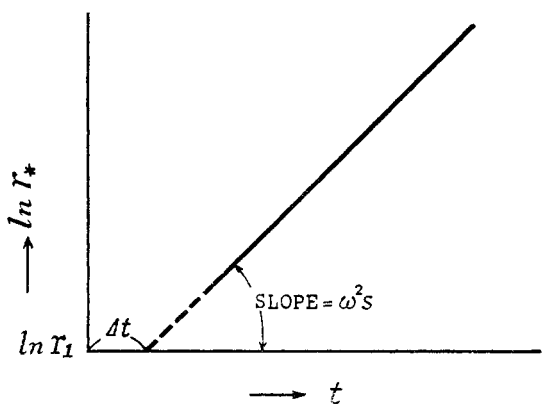

FIG. 3. A $\ln r_*$ vs. t curve with a zero-time correction Δt; r_1 is the radius to the cell meniscus from the center of rotation.

dure mentioned above holds fairly accurately for systems having s large enough or D small enough or both, i.e., $2D/s\omega^2r_1^2$ sufficiently small compared with unity.[3] The theoretical expression for the zero-time correction Δt has been obtained in terms of s, D, and other related parameters. According to their solution, the zero-time correction originates from the situation that diffusion of the dissolved solute in the vicinity of the meniscus differs from that in an unlimited medium (free diffusion). Actually, this is not the only factor for the zero-time correction. One of the possible factors may be that some finite time (5–15 min.) is required in currently used ultracentrifuges before the rotor attains full speed. This factor should become important in systems where solutes sediment very rapidly. No mathematical solution is yet available to the Lamm equation in which this effect is taken into account.

IV. A Solution of the Faxén Type in Which s Depends on c

In Faxén's original solution both sedimentation and diffusion coefficients were assumed to be independent of solute concentration. At present it is a well-recognized experimental fact that sedimentation coefficients of macromolecular systems generally vary with concentration. Thus it has frequently been reported that s for many linear high-molecular-weight substances in dilute solution follows a concentration dependence of the type:

$$s = s_0/(1 + kc) \tag{31}$$

where s_0 is the value of s at infinite dilution, and k is, in almost all cases investigated, a positive quantity characteristic of a given polymer–solvent pair. Wales and Van Holde (27) demonstrated that k for solutions of linear high polymers having flexible chains is closely related to their intrinsic viscosities. To test equation 31 it is customary to plot $1/s$ against c and observe its linearity. The dependence of s on concentration in dilute protein solutions is known to obey better a linear equation

$$s = s_0(1 - kc) \tag{32}$$

rather than equation 31. The form of equation 32 may be considered as a special case of equation 31 for kc sufficiently small compared with unity, but no such restriction on kc is necessary when one treats equation 32 as an empirical relation.

From the kinetic theoretical point of view, when s depends on c, the diffusion coefficient D should in general depend on c owing to the contribution of the frictional coefficient to these quantities. Roughly speaking, there is a general trend for the variation of D with c in dilute solutions that D increases monotonically with c for substances of sufficiently high molecular weight and decreases monotonically for substances of relatively small molecular size.

[3] The dimensionless parameter $2D/s\omega^2r_1^2$ is an equivalent to

$$\epsilon = 2D/s\omega^2r_0^2$$

which plays a fundamental role in the analysis of sedimentation data from the synthetic boundary-forming cell. Otherwise stated, the same symbol ϵ will be used for both $2D/s\omega^2r_1^2$ and $2D/s\omega^2r_0^2$ in the discussion which follows.

Since both s and D generally vary with c, particularly in systems of proteins and of other high-molecular-weight substances, it is not sufficient to analyze sedimentation velocity data for such systems in terms of the classical Faxén solution which neglects these effects. However, one finds readily that it is formidably difficult to solve the complete Lamm equation by taking explicitly into account a concentration dependence of either s or D or both, because of the nonlinearity of the equation which manifests itself in such cases. Quite recently, a first solution to this problem has been obtained for the case in which s is allowed to vary linearly with c in accordance with equation 32, while D is left independent of c (11). The two basic assumptions as well as the infinite cell condition as had originally been introduced in Faxén's analysis were also adopted in the solution, so that it is only useful for practical purposes under such restrictions that the classical Faxén solution may also apply. The general solution obtained is so complicated in form that only its essential features which tell the effects of a concentration dependence of s on sedimentation behavior are outlined below.

Figure 4 shows graphically the concentration gradient curves for different values of kc_0, where c_0 is the initial concentration of the solution. In this figure, the curve corresponding to $kc_0 = 0$ represents the boundary curve for s independent of c, i.e., that derived from the classical solution described fully in a

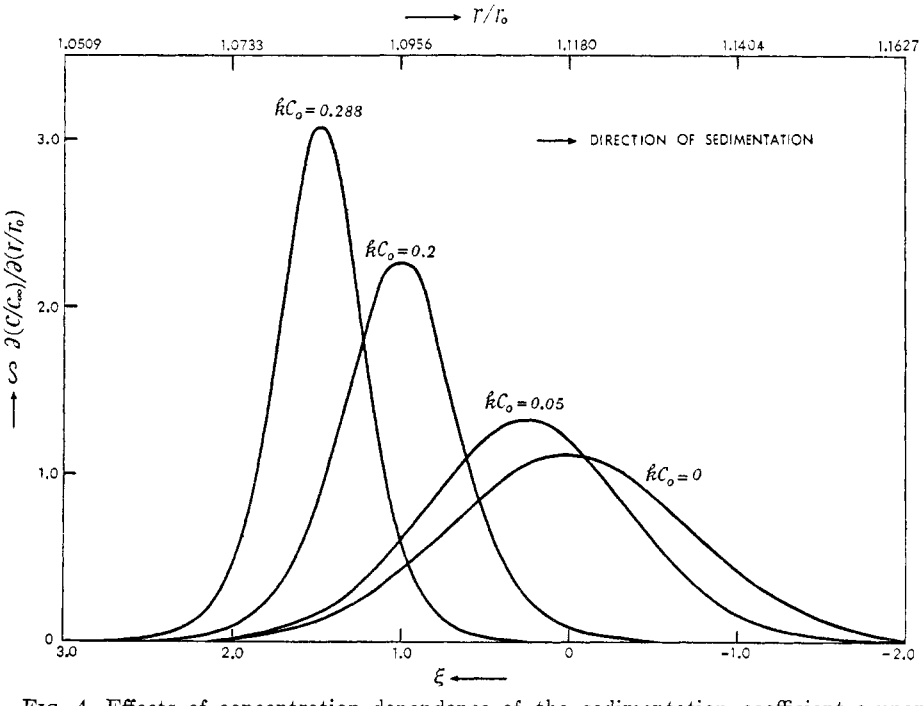

FIG. 4. Effects of concentration dependence of the sedimentation coefficient s upon the concentration gradient curve. s varies with c in accordance with the equation: $s = s_0(1 - kc)$. c_0 is the initial concentration of the solution, and c_∞ is the concentration far in front of the boundary between solvent and solution phases.

previous paragraph. It is found that, with increasing dependence of s on concentration (*1*) the shape of the boundary curve is markedly sharpened, (*2*) the position of the maximum gradient is noticeably shifted towards the positive side of ξ, i.e., the solvent side, but (*3*) symmetry of the curve is practically maintained except at the edges. These facts are in harmony with what one may observe in actual systems involving a concentration dependence of s; thus it appears that the result (*1*) is no more than the behavior which is usually called a "sharpening effect."

The position r_* of the maximum gradient of a sedimentation boundary curve is shown in this case to be displaced with time t in accordance with

$$\ln r_* = \ln r_0 + s_0(1 - kc_0)\omega^2 t + (1 - kc_0)kc_0(s_0\omega^2 t)^2 + O[(s_0\omega^2 t)^3] \quad (33)$$

$s_0\omega^2 t$ having been assumed to be sufficiently small in comparison with unity, the third and higher terms in this expansion may be dropped to obtain:

$$\ln r_* = \ln r_0 + s_0(1 - kc_0)\omega^2 t \quad (34)$$

This is exactly of the same form as equation 21, except for the difference that s in equation 21 is here replaced by $s_0(1 - kc_0)$; this is actually the value of the sedimentation coefficient for the given initial concentration c_0. Thus one may evaluate s for a given concentration from the displacement of the maximum gradient in exactly the same manner as described previously for the case in which s is independent of concentration. By plotting s thus obtained against the corresponding concentration and extrapolating back to infinite dilution, the value of s_0 may be determined, together with the slope constant k, if the given system obeys equation 32. This is the procedure which has customarily been employed for the determination of s as a function of concentration. It is apparent that further theoretical investigation must be attempted to confirm whether this usual procedure is reliably used for other types of concentration dependence of s, particularly for the type given by equation 31, which has a much wider applicability than equation 32.

It is shown that the ratio of height to area, H/A, for the system obeying equation 32 can be represented with a sufficient degree of accuracy by

$$\frac{H}{A} = \frac{1}{2r_0}\left[\frac{e^{-2\omega^2 s_0 t}}{\pi\epsilon(1 - e^{-2\omega^2 s_0 t})}\right]^{1/2}\left[\frac{\Phi'(x)}{1 + \Phi(x)} + 2x\right] \quad (35)$$

where

$$\epsilon = 2D/s_0\omega^2 r_0^2 \quad (36)$$

$$x = \frac{kc_0}{\sqrt{\epsilon}}(1 - e^{-2\omega^2 s_0 t}) \quad (37)$$

and $\Phi'(x)$ is the derivative of the error integral with respect to its argument. By combining this equation with the value of s_0 obtained, one may evaluate the diffusion coefficient D from data of H/A as a function of t; a detailed account of

the procedure can be found in a recent article by Baldwin (7), where a practical application of it to data on bovine plasma albumin in dilute solutions is also described. His analysis discloses that D values which are evaluated from sedimentation velocity measurements in terms of data on height–area ratio are extremely sensitive to a slight dependence of s upon concentration. Thus, when his data on bovine albumin are analyzed in terms of the classical Faxén solution neglecting the concentration dependence of s, one obtains a peculiar value of D which changes appreciably with the duration of ultracentrifugation, while, when use is made of the more appropriate equation 35 for the analysis, one finds a D value which is not only free of such an anomaly but which agrees quite well with the value previously reported by Gosting (15) from precision free-diffusion experiments on the same sample.

In the solution for a concentration-dependent sedimentation described above, the dependence of D on concentration was ignored although, as noted before, both s and D may concomitantly depend on concentration. This neglect, however, is justified so far as the Lamm equation, subject to the conditions of the Faxén type, is concerned. In fact, any solutions of the Faxén type assume that

$$\epsilon = 2D/s_0\omega^2 r_0^2 \ll 1$$

so that concentration dependence in D, if any, should play a far less important role in the determination of sedimentation pattern than does that in s.

V. The Exact Solution of Archibald

The first exact solution to the Lamm equation free of the basic assumptions employed in solutions of the Faxén type was obtained by Archibald (2), assuming both s and D independent of concentration, under the initial and boundary conditions obtained in the standard cell, i.e., equations 2 and 4. The concentration distribution in the cell is represented by an infinite series as

$$c(r, t)/c_0 = e^z(z_2 - z_1)/(e^{z_2} - e^{z_1}) + \sum_{n=1}^{\infty} M(\alpha_n, 1, z)e^{(\alpha_n - 1)\tau} \int_{z_1}^{z_2} e^{-z}M(\alpha_n, 1, z)\,dz \Big/ \int_{z_1}^{z_2} e^{-z}[M(\alpha_n, 1, z)]^2\,dz \quad (38)$$

where

$$\tau = 2\,\omega^2 st, \qquad z = \omega^2 sr^2/2D, \qquad z_i = \omega^2 sr_i^2/2D \qquad (i = 1, 2) \quad (39)^4$$

The function $M(\alpha_n, 1, z)$ is an eigenfunction associated with a confluent hypergeometric equation as

$$\frac{d^2M}{dz^2} + \left(\frac{1}{z} - 1\right)\frac{dM}{dz} - \frac{\alpha_n}{z}M = 0 \quad (40)$$

and α_n is the associated eigenvalue determined from the relation:

$$\frac{d}{dz}M(\alpha_n, 1, z) = M(\alpha_n, 1, z) \qquad (z = z_1 \text{ and } z_2) \quad (41)$$

[4] It should be noted that z_1 is the reciprocal of ϵ appearing in solutions of the Faxén type.

The evaluation of α_n involves a really laborious computational effort, even if interest is confined only to the first several of them. Thus, Archibald (2) had to satisfy himself with determining only the value for $n = 1$. Values for $n = 1$, 2, and 3 have quite recently been computed by Waugh and Yphantis (28) using a differential analyzer at the Massachusetts Institute of Technology. It should be noted, however, that these eigenvalues depend on the choice of z_1 and z_2. Accordingly, the values obtained by these authors for particular sets of the values of z_1 and z_2 (hence, of s, D, ω, r_1, and r_2) may not be utilized for general purposes. An approximate analytic expression of α_n in terms of the parameters related was derived later by Archibald (3), together with an approximate equation for the concentration distribution which is more tractable for practical computations. This will be discussed in the next paragraph in connection with more recent developments in the approximate treatment of the sedimentation differential equation.

A close examination of the solution of the Archibald type reveals that the series in equation 38 converges rapidly under the condition that

$$\tau = 2\,\omega^2 st \gtrsim O(1) \tag{42}$$

and

$$\epsilon = 2D/s\omega^2 r_1^2 \gtrsim O(1) \tag{43}$$[5]

These are characteristically contrasted to inequalities 10 and 11 upon which Faxén's solution was based. Thus one finds that Archibald's solution may be well suited to the description of sedimentation processes of relatively small molecules at later stages of ultracentrifugation. Of course, this is largely a qualitative statement of the basic character of the solution, and the actual estimate of the ranges of τ and ϵ in which the series in equation 38 is practically useful depends on a particular combination of the parameters s, D, ω, and r_1.

Although the above solution is of considerable interest from the mathematical point of view in that it represents an exact solution to the Lamm equation, it is too complicated to be useful for the practical analysis of experimental data. Archibald (4) has demonstrated methods for the evaluation of s and D from appropriate experiments in terms of this exact solution. Unfortunately, they require the greatest precision in the data that are most difficult to obtain (29). In general, the difficulty of finding means to determine s and D for substances of small molecular size from measurements of sedimentation velocity lies in the fact that in such systems, either the maximum of the concentration gradient curve remains sufficiently close to the meniscus or, even when the boundary is separated therefrom, it is not sufficiently sharp to be accessible to an accurate determination. Some theoretical sedimentation gradient curves illustrative of this situation are shown graphically in figure 5, where the numerical values of the parameters used in the calculation are given in the legend. These curves have been computed using not the exact Archibald solution but an approximate solution which will be described below. It is observed from the figure that at the relatively initial stages of ultracentrifugation there appears a maximum, though quite

[5] The inequality means that neither quantity is very small in comparison to unity.

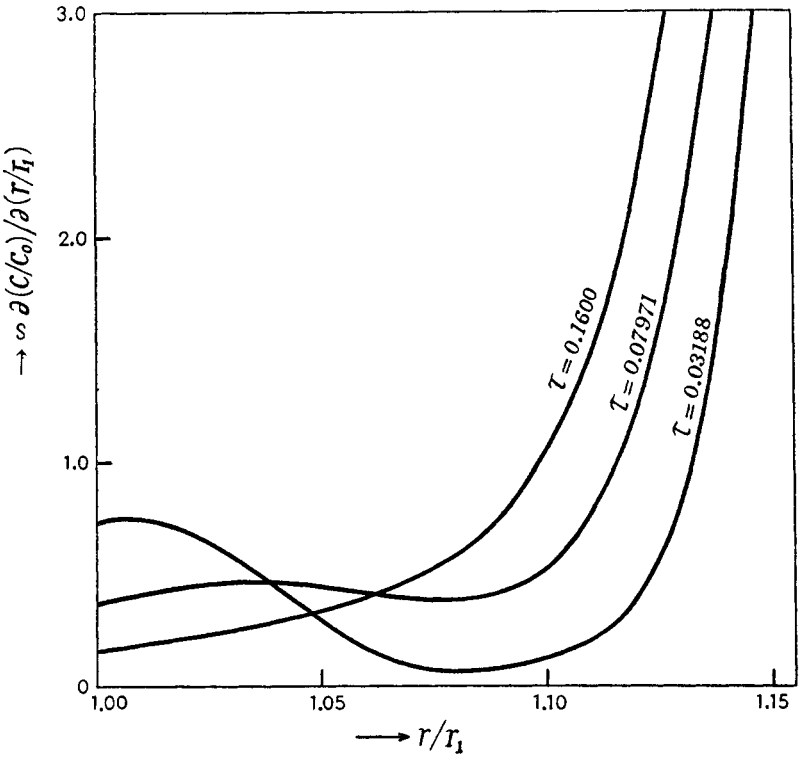

FIG. 5. Calculated concentration gradient curves in a cell with $r_1 = 6.067$ cm. and $r_2 = 7.003$ cm. The curves correspond to different values of reduced time $\tau = 2\omega^2 st$. The following values are used for the calculation: $\omega^2 = 3.919 \times 10^7$ (rad./sec.)^2, $s = 0.565 \times 10^{-13}$ sec., $D = 2.372 \times 10^{-6}$ cm.^{2}/sec.

diffuse, close to the meniscus, but with increasing time the boundary becomes appreciably flattened and eventually disappears completely. It is also important to note that the gradient curves indicated do not return back to the baseline ahead of their maxima. It is shown that for a system which has a much higher ϵ than chosen for this computation, the calculated boundary curves exhibit eventually neither a maximum nor a zero gradient over the entire cell. These sedimentation patterns make a marked contrast to those for systems belonging to the category of Faxén's solution; in the latter, particularly when the initial boundary between solution and solvent is formed away from the meniscus, there is observed a sedimentation boundary which has a definite "bell" shape and approaches zero-gradient at both of its edges, as illustrated in figure 2. The marked upturn of the gradient curves in figure 5 as observed in the centrifugal side of the cell is accounted for by restricted diffusion of solutes affected by the cell bottom.[6] Calculations in terms of an approximate solution to the Lamm

[6] In addition to the upturn of the gradient in the vicinity of the cell base, a sharp upturn of gradient may be observed in the region adjacent to the meniscus at very early stages of ultracentrifugation. This may be ascribed to a similar restricted diffusion in the region, as understood from the calculation of MacCosham and Fujita (21).

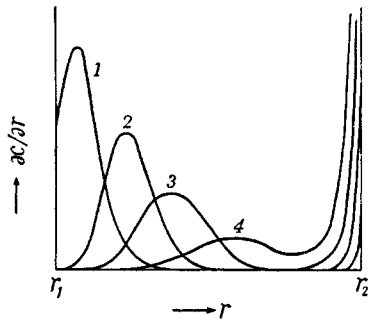

FIG. 6

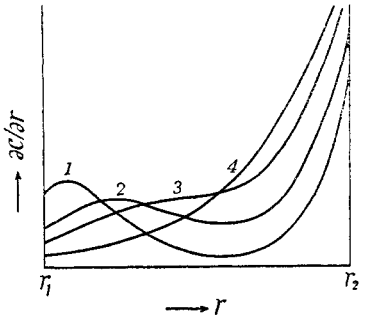

FIG. 7

FIG. 6. Schematic diagram of the change in concentration gradient distribution with the duration of ultracentrifugation for a system with a sufficiently small value of ϵ; the numbers 1, 2, 3, and 4 are in order of the time elapsed from the start of centrifugation.

FIG. 7. Schematic diagram of the change in concentration gradient distribution with the duration of ultracentrifugation for a system with a relatively large value of ϵ; the numbers 1, 2, 3, and 4 are in order of the time elapsed from the start of centrifugation.

equation which will be described below show that for systems having small enough values of ϵ this upturn is confined in a sufficiently narrow layer adjacent to the cell base over a considerably long interval of time from the start of ultracentrifugation, while for systems having relatively large ϵ the corresponding region covers a considerably extended area in the cell, except for very early stages of sedimentation. Summarizing the results from these and related considerations, one can describe for the progressive change of sedimentation boundary pattern with the duration of ultracentrifugation two such schematic diagrams as shown in figures 6 and 7; figure 6 presents the patterns for a system belonging to "Faxén category," and figure 7 shows those for a system belonging to "Archibald category." These two patterns represent, rather ideally, two extremes of the type of solutions to the Lamm equation as classified in terms of the basic parameter of the problem: $\epsilon = 2D/s\omega^2 r_1^2$. There may be a variety of substances which belong to the range of ϵ intermediate between these two extremes.

As observed from figure 6, the sedimentation pattern of the Faxén type is characterized not only by the appearance of a distinct maximum gradient but also by the existence of a zero-gradient region ahead of the maximum. This latter condition means that there is a region centrifugal to the maximum gradient where concentration is independent of position. This region is often called the "plateau region" (in the concentration distribution diagram). On the basis of a consideration of the conservation of mass, Baldwin (5) has shown that under this condition there obtains the relation:

$$-2s\omega^2 t = \ln\left(1 - \left[\int_{r_1}^{r_t} (r^2 - r_1^2)\frac{\partial c}{\partial r}\,dr\right] \Big/ c_0 r_1^2\right) \tag{44}$$

where r_t denotes a position chosen arbitrarily in the plateau region, i.e., in the zero-gradient region in the concentration gradient diagram. This equation can be used directly and simply for the calculation of s, when the concentration gradient curve between the meniscus and the point r_t is measured as a function

of time. This method has an advantage over the classical one which resorts to the movement of the maximum gradient, in that it may apply for the case when a distinct maximum does not separate from the meniscus within the interval of time accessible to experiment. From the considerations given above, it is expected that the boundary remains sufficiently close to the meniscus over a period of ultracentrifugation when a substance has a sufficiently small s so as not to satisfy the condition $2D/s\omega^2 r_1^2 \ll 1$, one of the basic assumptions for the solutions of the Faxén type. As a matter of fact, this prediction can be confirmed from the calculation of MacCosham and Fujita (21) cited previously. However, there is some doubt whether the method of Baldwin can be applied reliably for substances having extremely small s or large enough D. In such a case, the parameter $2D/s\omega^2 r_1^2$ may necessarily be so large that the sedimentation pattern would be of the type predicted by a solution of the Archibald type and, therefore, no such well-defined zero-gradient region is observed away from the meniscus as required in the derivation of equation 44 (see figures 5 and 7). Thus it appears essential in a practical application of equation 44 to ascertain in advance if the chosen position r_t lies in a true plateau region. This can be done by use of a double cell in which the solvent is placed on one side and the solution on the other; the two schlieren curves coincide in the zero-gradient or plateau region. Klainer and Kegeles (19) have recently combined this method with one of Archibald's previous procedures (4) to determine simultaneously the diffusion coefficient D and the molecular weight of solute M, and have applied it to some practical systems with favorable results.

The method of Baldwin is essentially equivalent to that of Gutfreund and Ogston (16), who expressed the conservation of mass on the basis of concentration distribution in the form:

$$\frac{1}{2} c_0(r_t^2 - r_1^2) = \int_{r_1}^{r_t} cr \, dr + \frac{1}{2} c_0 r_t^2(1 - e^{-2s\omega^2 t}) \tag{45}$$

If, in accordance with Yphantis and Waugh (32), one introduces $Q(t)$, the average fractional supernatant concentration at time t, by

$$Q(t) = \int_{r_1}^{r_t} cr \, dr \Big/ \int_{r_1}^{r_t} c_0 r \, dr \tag{46}$$

equation 45 can be rewritten

$$-2\omega^2 st = \ln\left(1 - \frac{(r_t^2 - r_1^2)}{r_t^2}[1 - Q(t)]\right) \tag{47}$$

This equation can also be used to determine s when the concentration distribution between the meniscus and the plateau region is measured as a function of time. It is, however, inconvenient for use with the optical systems now generally employed—for example, the Lamm scale method, the Philpot schlieren system, and their variants—because sedimentation patterns measured in these systems are not the concentration distribution but the concentration (refractive index) gradient distribution. It would be well suited to the light-absorption or the

integral-fringe interferometric systems, where boundary curves are given in the form of concentration against cell distance.

As noted above, either Baldwin's method or that of Gutfreund and Ogston assumes the existence of a plateau region ahead of the boundary or the meniscus, so that both are essentially best suited to high-molecular-weight solutes which are generally characterized by sufficiently small values of ϵ. For solutes of low enough molecular weight the plateau region may not always be obtained definitely, and another method must be sought. An interesting contribution to this problem has recently been given by Yphantis and Waugh (32), who based their treatment on the exact solution of Lamm's equation developed by Archibald.

Instead of defining r_t as a position chosen in the plateau region, they choose it rather arbitrarily somewhere in the central region of the cell, and consider the average fractional concentration, $Q(t)$, in the phase centripetal to this point. They calculated $Q(t)$ as a function of reduced time $\tau = 2\omega^2 st$ for several values of a new parameter σ defined by $\sigma = \omega^2 s/D$, using the exact solution of Archibald described above.[7] The results they obtained are shown in figure 8. It should be noted that this graph depends moreover on the particular values of r_1, r_2, and r_t chosen (in their case, these are 6.067, 7.003, and 6.600 cm., respectively). Beyond the initial, coincident phase of the plots shown in figure 8, each Q is related to an infinite number of paired values of τ and σ. If two (or more) values of Q are obtained for different times, such that at least one is beyond the initial stage mentioned, then it should be possible to determine what particular values of s and D give the observed values of Q. A useful procedure for effecting this determination has been developed by Yphantis and Waugh themselves (32). They (33) applied it to some biologically important substances with results which compared very favorably with the values from the classical displacements of the boundary gradient curves. During the initial stage of centrifugation ($\tau \ll 1$)$Q(t)$ is essentially independent of σ, hence of D, except for extremely low values of σ. Therefore, an experimental Q determined in this region leads directly to a value of s. It is a simple matter to show that the curve of $1 - Q$ for $\sigma = \infty$, i.e., $D = 0$, can be represented by equation 47. Accordingly, the initial, coincident phase of Q vs. τ plots is the time interval of ultracentrifugation where the method of Gutfreund and Ogston (and hence the method of Baldwin) can be applied safely to evaluate s, irrespective of the value of D.[8] For instance, it follows from figure 8 that for a system with σ corresponding to curve 1 the range of applicability of the method of Gutfreund and Ogston is confined to τ smaller than about 0.03.

In the general method of Yphantis and Waugh described, the position of r_t can be chosen arbitrarily somewhere in the central part of the cell. In order to make

[7] σ is related with ϵ by a relation, $\sigma = 2/\epsilon r_1^2$. Thus, larger σ corresponds to smaller ϵ for a fixed value of r_1. It should be noted that σ is not a dimensionless quantity but has a dimension of (length)$^{-2}$.

[8] For large σ (hence small D) Q vs. τ curves do not differ significantly from that for $\sigma = \infty$ ($D = 0$) over a considerable interval of time from the start of centrifugation. This causes difficulty in determining D by Yphantis and Waugh's method, because their method is practically equivalent under this condition to the method of Gutfreund and Ogston, which tells nothing of D.

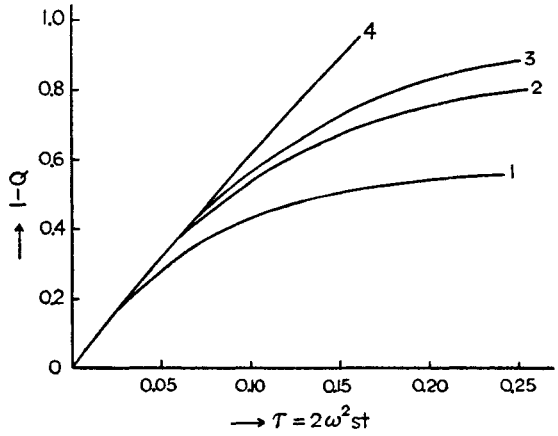

FIG. 8. The average fractional supernatant concentration Q vs. reduced time τ ($= 2\omega^2 st$) curves for various values of parameter σ ($= \omega^2 s/D$), calculated from exact solutions. For curve 1, $\sigma = 0.467$; for curve 2, $\sigma = 0.933$; for curve 3, $\sigma = 1.399$; and for curve 4, $\sigma = \infty$ ($D = 0$).

possible measurements of $Q(t)$ after the rotor has been brought to rest, they devised a separation cell, as they termed it, by slight modifications of the commercially available standard ultracentrifuge centerpiece. As for the details of construction and manipulation of such a cell, reference should be made to their original article (33). One of the characteristic features of this new method would be that optical measurements which are almost exclusively involved in the classical sedimentation velocity experiments are no longer necessary for obtaining the experimental data required in this case. Assays of $Q(t)$ are the only measurements required on the solutions obtained from the ultracentrifuge. Such assays may be performed by chemical, physical, or biological methods in accordance with the relevant character of the solutes concerned.

VI. Approximate Treatments of the Lamm Equation

The method of Yphantis and Waugh (32) requires constructions of Q vs. τ plots for a number of σ values under given cell parameters (r_1, r_2, and r_t). The Q vs. τ graph prepared by them (figure 8), as noted before, refers to a specific separation cell having $r_1 = 6.067$ cm., $r_2 = 7.003$ cm., and $r_t = 6.600$ cm. It is tedious to construct cells with exactly these cell parameters; moreover, Q vs. τ curves for σ values other than those chosen are required in general. These problems could be solved without difficulty if Archibald's exact solution to the Lamm equation were tractable for numerical computations of solute distributions in a given cell for any paired values of τ and σ (or ϵ). As noted previously, however, this is not actually the case, particularly for small enough values of τ and ϵ for which the infinite series in equation 38 converges so slowly that the solution is practically of no value for the purpose concerned. For these reasons, suitable approximate solutions which readily yield solute distributions with sufficient accuracy have been investigated on the basis of a practically identical idea.

Archibald (3) has developed approximations to the solute distribution in the cell where $z_2 - z_1 \approx 1$. Waugh and Yphantis (28) showed that Archibald's approximations are applicable even for larger differences of z_2 and z_1 ranging from 3 to 9. Considerable computation, however, is required in obtaining the results demanded even with this simplified solution.

A better and simpler approximate solution to the Lamm equation has recently been worked out by Yphantis and Waugh (32) and independently by Fujita (12). The calculation of solute distributions in the sector-shaped cell is complicated because of a radially varying centrifugal field. In the ultracentrifuge cells currently used, the opening of their radial edges is as small as 4° and, in addition, the distance between their meniscus and bottom is sufficiently small compared with that between the meniscus and the axis of the rotor, i.e., $(r_2 - r_1)/r_1 \ll 1$. These cell conditions allow one to assume that the physical situation in the actual sector-shaped cell be, to a first approximation, the same as that in a rectangular cell with a uniform field of force. Solutions for such a rectangular system were worked out by Mason and Weaver (22) more than thirty years ago in connection with their treatment of the settling of spherical particles under gravity. Yphantis and Waugh, and independently Fujita, have modified slightly such classical solutions of Mason and Weaver by taking into account a first-order effect of the sectoral, radially varying field and obtained practically identical approximate solutions to the Lamm equation. Yphantis and Waugh's solution reads

$$c(y, \tau)/c_0 = e^{y/\alpha}/\alpha(e^{1/\alpha} - 1) + e^{y/2\alpha} \sum_{m=1}^{\infty} E_m(\tau)(\sin m\pi y + 2\pi m\alpha \cos m\pi y) \quad (48)$$

where $E_m(\tau)$ is given by

$$E_m(\tau) = \frac{16\alpha^2\pi \exp\left[- \left(\alpha m^2\pi^2 + \frac{1}{4\alpha}\right)\gamma\tau \right] m[1 - (-1)^m e^{1/2\alpha}]}{(1 + 4\alpha^2 m^2\pi^2)^2} \quad (49)$$

In equation 48 the symbols y, α, and γ are, respectively, defined by

$$y = (r - r_1)/(r_2 - r_1) \quad (50)$$

$$\alpha = 1/\sigma\bar{r}(r_2 - r_1) = D/\omega^2 s\bar{r}(r_2 - r_1) \quad (51)^9$$

and

$$\gamma = \bar{r}/2(r_2 - r_1) \quad (52)$$

The quantity $\bar{r}$ is left undetermined in their treatment, but in practice it may be chosen as the radius to a point taken somewhere in the central region of the cell,

[9] α can be expressed in terms of

$$\epsilon = 2D/s\omega^2 r_1^2$$

as

$$\alpha = \frac{\epsilon}{2}\left(\frac{r_1}{\bar{r}}\right)\left(\frac{r_1}{r_2 - r_1}\right)$$

without serious loss of accuracy of the results.[10] In general, if the ratio of $r_2 - r_1$ to $\bar{r}$ is made smaller, hence γ is made larger, the physical situation is closer to the rectangular system and therefore the approximation should become more accurate. In Fujita's solution, which is slightly different from the above in its details, the quantity equivalent to $\bar{r}$ is expressed in terms of other related physical parameters, and thus the final solution is uniquely determined.

The solution thus obtained, equation 48, is much simpler than Archibald's exact solution involving a series of confluent hypergeometric functions or even than his own approximation. In fact, with this solution one can calculate solute distributions in a given cell in a quite straightforward manner up to the numerical accuracy as desired. Furthermore, the expression for a distribution may be readily integrated to yield an analytic form of the average fractional supernatant concentration $Q(t)$ as

$$Q(t) = \frac{1}{y_t}\left(\frac{e^{y_t/\alpha_1}}{e^{1/\alpha} - 1}\right) + \frac{2\alpha}{y_t}\sum_{m=1}^{\infty} E_m(\tau) \sin m\pi y_t \qquad \left(y_t = \frac{r_t - r_1}{r_2 - r_1}\right) \quad (53)$$

From this one may compute easily Q vs. τ plots for various values of σ as required in the application of the method of Yphantis and Waugh described previously. The degree of approximation of equation 48 to the exact solution is satisfactory so far as numerically investigated; a typical example of the comparison is shown in figure 9, where the solid line has been obtained from solutions by a differential analyzer. One may also readily obtain an analytic expression for the concentration gradient distribution by simply differentiating equation 48 with respect to r, and the resulting equation is again tractable for straightforward numerical computations. Thus the concentration gradient curves shown in figure 5 have been computed from such an approximate solution, though actually the equation derived by Fujita was used instead of that of Yphantis and Waugh cited above.

On examination, it is found that all the approximate solutions described still involve a similar inherent disadvantage as does the original exact solution of Archibald, in that all the infinite series involved in them converge slowly for small values of τ and ϵ; hence they are of no practical use for obtaining theoretical information about the sedimentation of large molecules at early stages of ultracentrifugation. For sufficiently small values of τ and ϵ one may turn back to the classical solution due to Faxén or its variants. However, there must be an intermediate region in which both τ and ϵ are neither so small as to warrant Faxén's approximations nor so large as to make rapid convergence of the series in Archibald's solution or its variants. The mathematical theory of sedimentation behavior useful for such an intermediate region is certainly desired,[11] because there is now much interest in the determinations of molecular size by the ultracentrifugation of substances which have intermediate molecular weights available neither by the peak movement nor by the mass transport analysis.

[10] In their numerical computation Yphantis and Waugh (32) chose $\bar{r} = r_t = 6.600$ cm

[11] The solution considered by MacCosham and Fujita (21) is applicable for any ϵ, provided τ is sufficiently small. It is therefore somewhat significant in relation to this problem.

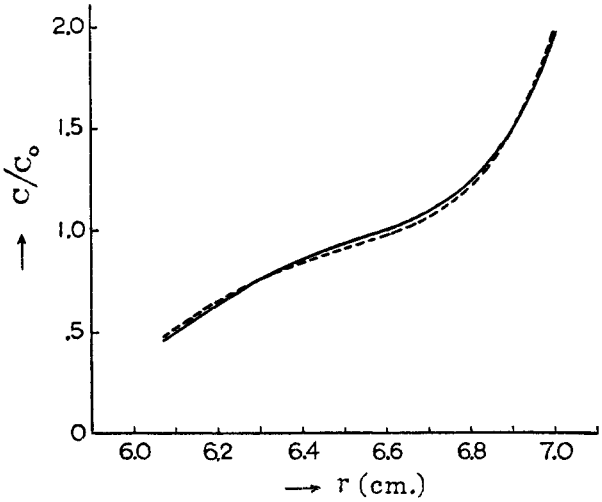

FIG. 9. Comparison of the exact solution and Yphantis and Waugh's approximate solution to the Lamm equation. The ordinate gives the ratio of concentration at a time 4 hr. from the start of centrifugation to the initial concentration. The abscissa gives the radial position in centimeters. The following values are used for the calculation: $s = 0.356 \times 10^{-13}$ sec., $D = 2.989 \times 10^{-6}$ cm.^{2}/sec., $r_1 = 6.067$ cm., $r_t = 6.600$ cm., $r_2 = 7.003$ cm.

In solutions of the Archibald type considered in this and the previous paragraphs, the dependence of s or D or both on concentration is ignored. This is partly justified, because, as noted before, those solutions are primarily suited to solutes of low molecular weight. For such solutes the dependence of s or D on concentration is usually much less pronounced than that for solutes of high molecular weight.

Either the exact or the approximate solution of the Archibald type discussed above is valid only for sedimentation in the standard cell, because both assume a uniform initial solute distribution, i.e., equation 2. For the analysis of sedimentation velocity data from synthetic boundary-forming cells in which a sharp initial boundary is formed between solution and solvent (or solutions of different concentrations) in the central part of the cell, solution of the Lamm equation must be obtained with the initial condition of the form of equation 3. Such a solution should reduce to Faxén's, as discussed in Section III, for sufficiently small values of τ and ϵ, but for the purpose of obtaining a theoretical basis for the sedimentation analysis of low-molecular-weight solutes from synthetic boundary-cell data, its behavior for more general values of these parameters need be explored mathematically and in detail. No attempt to the solution of this problem has as yet been reported, however.

Since in synthetic boundary-forming cells the disturbing effect arising from restricted diffusion in the vicinity of the meniscus is practically absent, distinct, bell-shaped sedimentation boundaries could be obtained with much smaller and more highly diffusible molecules than those which may be studied in the standard cell by conventional peak-displacement measurements. This situation implies a remarkable advantage of the synthetic boundary-forming cell over the conven-

tional standard cell in the sense that the peak-movement method is the most reliable and accurate now available for the determination of the sedimentation coefficient. However, it should be noted that in experiments with a sharp initial boundary formed away from the cell meniscus the region of uniform concentration ahead of the boundary is disturbed earlier than in experiments with a uniform initial concentration distribution, by diffusion resulting from the concentration gradient near the cell bottom. This apparently limits the applicability of the synthetic boundary-forming cell to molecules of not too small size. In order to emphasize the availability of this new type of cell, particularly for the sedimentation analysis of low-molecular-weight solutes, definite theoretical information should be obtained from the Lamm equation concerning the effect of diffusion on sedimentation patterns to be observed in a cell of this type.

The synthetic boundary-forming cell provides another interesting technique that cannot be obtained with a standard cell That is, it makes it possible to determine a differential sedimentation velocity between two concentrations of the same molecule. Schachman and Harrington (18c) have shown experimentally that for s decreasing with increasing concentration the differential sedimentation boundary has a much lower s than either of the s values corresponding to the concentrations of the given two solutions. Hersh and Schachman (17) have shown that this behavior of a differential boundary can be accounted for by simple mass considerations.

VII. Pressure-dependent Sedimentation

At speeds of rotation usually employed in measurements of sedimentation velocity, a large pressure difference, which may amount to several hundred atmospheres, is produced between the liquid–air meniscus and the bottom of the cell. Since the viscosity and density of the solvent and the specific volume of the solute may vary with pressure, it is expected that sedimentation processes in such a field of high-pressure gradient should differ more or less from those in a field of uniform pressure. In order to attain a high precision in the evaluation of the intrinsic sedimentation coefficient of a given substance, a correction must be applied to sedimentation data with respect to this pressure effect, along with, among other things, the elimination of the concentration dependence effect by means of extrapolation to infinite dilution.

This problem was early considered by Mosimann and Signer (23) and was recently worked out more specifically by Oth and Desreux (24) and Cheng and Schachman (8). Singer (25) has discussed the effect of pressure upon the configuration and the orientation of flexible macromolecules sedimenting in a field of high pressure gradient. He showed that the pressure effect upon the frictional coefficient of such a macromolecule is practically negligible within the range of pressure obtained in ordinary ultracentrifuge cells. This implies that values of the diffusion coefficient D obtained from free diffusion experiments may be combined with s from ultracentrifugal measurements to calculate the molecular weight M by means of the Svedberg relation. On the basis of the Lamm equation without the diffusion term, i.e., equation 5, Fujita (10) has made a mathematical refinement of the problem as dealt with by Oth and Desreux who

used a rather empirical procedure and, moreover, derived solutions of equation 5 with s which not only depends upon pressure but also varies with concentration in the fashion represented by equation 31. It was shown that the square dilution law no longer holds in cases where s varies with pressure, while it does for s dependent on concentration alone irrespective of the form of its dependence. The effect of hydrostatic pressure on s should become significant for solutes in organic solvents which usually have much larger compressibilities than water. Experimental information about the dependence of the viscosity of solvents on pressure is particularly needed for further development of the theory of pressure-dependent sedimentation processes. Both theoretical and experimental investigations in this field are apparently in a very premature stage, and need for further work is quite apparent.

VIII. References

(1) Alberty, R. A.: J. Am. Chem. Soc. **76,** 3733 (1954).
(2) Archibald, W. J.: Ann. N. Y. Acad. Sci. **43,** 211 (1942).
(3) Archibald, W. J.: J. Appl. Phys. **18,** 362 (1947).
(4) Archibald, W. J.: J. Phys. & Colloid Chem. **51,** 1204 (1947).
(5) Baldwin, R. L.: Biochem. J. **55,** 644 (1953).
(6) Baldwin, R. L.: J. Phys. Chem. **58,** 1081 (1954); J. Am. Chem. Soc. **76,** 402 (1954).
(7) Baldwin, R. L.: Biochem. J. **65,** 490 (1957).
(8) Cheng, P. Y., and Schachman, H. K.: J. Am. Chem. Soc. **77,** 1498 (1955).
(9) Faxén, H.: Arkiv Mat. Astron. Fysik **21B,** No. 3 (1929).
(10) Fujita, H.: J. Am. Chem. Soc. **78,** 3598 (1956).
(11) Fujita, H.: J. Chem. Phys. **24,** 1084 (1956).
(12) Fujita, H.: In manuscript.
(13) Goldberg, R. J.: J. Phys. Chem. **57,** 194 (1953).
(14) Gosting, L. J.: J. Am. Chem. Soc. **74,** 1548 (1952).
(15) Gosting, L. J.: Measurements quoted in an article by R. L. Baldwin, L. J. Gosting, J. W. Williams, and R. A. Alberty, Discussions Faraday Soc. **20,** 13 (1955).
(16) Gutfreund, H., and Ogston, A. G.: Biochem. J. **44,** 163 (1949).
(17) Hersh, R., and Schachman, H. K.: J. Am. Chem. Soc. **77,** 5228 (1955).
(18) (a) Kegeles, G.: J. Am. Chem. Soc. **74,** 5532 (1952).
(b) Pickels, E. G., Harrington, W. F., and Schachman, H. K.: Proc. Natl. Acad. Sci. U. S. **38,** 943 (1952).
(c) Schachman, H. K., and Harrington, W. F.: J. Polymer Sci. **12,** 379 (1954).
(19) Klainer, S. M., and Kegeles, G.: J. Phys. Chem. **59,** 952 (1955).
(20) Lamm, O.: Arkiv Mat. Astron. Fysik **21B,** No. 2 (1929).
(21) MacCosham, V. J., and Fujita, H.: J. Chem. Phys., in press.
(22) Mason, M., and Weaver, W.: Phys. Rev. **23,** 412 (1924).
(23) Mosimann, H., and Signer, R.: Helv. Chim. Acta **27,** 1123 (1944).
(24) Oth, J., and Desreux, V.: Bull. soc. chim. Belges **63,** 133 (1954).
(25) Singer, S.: J. Polymer Sci. **2,** 290 (1947).
(26) Trautman, R., and Schumaker, V.: J. Chem. Phys. **22,** 551 (1954).
(27) Wales, M., and Van Holde, K. E.: J. Polymer Sci. **14,** 81 (1954).
(28) Waugh, D. F., and Yphantis, D. A.: J. Phys. Chem. **57,** 312 (1953).
(29) Williams, J. W.: J. Polymer Sci. **12,** 351 (1954).
(30) Williams, J. W., Baldwin, R. L., Saunders, W. M., and Squire, P. G.: J. Am. Chem. Soc. **74,** 1542 (1952).
(31) Williams, J. W., and Saunders, W. M.: J. Phys. Chem. **58,** 854 (1954).
(32) Yphantis, D. A., and Waugh, D. F.: J. Phys. Chem. **60,** 623 (1956).
(33) Yphantis, D. A., and Waugh, D. F.: J. Phys. Chem. **60,** 630 (1956).