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A Procedure for the Measurement of Molecular Weights by the Archibald Method. I. Theoretical Analysis*

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ABSTRACT: The Archibald method of measuring molecular weights relies at present on an empirical extrapolation of the gradient curve of $\partial c/\partial r$ *vs.* r to the ends of the solution column. In this first paper an extrapolation procedure is examined by means of computer solutions to the differential equations, and also by study of the Fujita–MacCosham equation [Fujita, H., and MacCosham, V. J. (1959), *J. Chem. Phys. 30*, 291]. A linear extrapolation of $\partial c/\partial r$ *vs.* r gives satisfactory accuracy, provided certain conditions are met. It is also shown that the error can be large when these conditions are not met.

The method introduced by Archibald (1947) is one of the most common methods for measuring molecular weights with the analytical ultracentrifuge. Its popularity stems from the fact that the method permits relatively rapid measurements, as opposed to other centrifugal methods. (For a detailed discussion see Schachman, 1959.) More recently the theory for this method has been extended for use with nonideal, heterogeneous systems (Kegeles *et al.*, 1957). In spite of this and other developments, no thoroughly sound theoretical guide exists for the practical evaluation of data by extrapolation to the ends of the solution column as required by the method. The need for extrapolation arises because optical phenomena cause deterioration of schlieren patterns at both ends of the solution column and thereby prevent direct reading at the ends; nor does the Rayleigh optical system circumvent this difficulty to permit unambiguous measurements at the ends (LaBar and Baldwin, 1962).

Peterson and Mazo (1961) used a digital computer to study the extrapolation to the meniscus, but they were unable to find general conditions for the extrapolation and recommended that the extrapolation be carried out with a computer by successive iteration using constants from the formula of Fujita and MacCosham (1959). The purpose of this study is to establish and to test a procedure for carrying out such extrapolations in routine experimental work.

Fundamental Equations and Methods for Extrapolation. The Archibald condition, based upon the boundary condition that the flow of each component is zero at the ends of the solution column at all times, is given for general application by (Kegeles *et al.*, 1957; Fujita *et al.*, 1962; Kotaka and Inagaki, 1964)

$$(1/rc)(\partial c/\partial r) = M^*(t)\omega^2(1 - \bar{v}\rho)/RT \quad (\text{at } r = r_a \text{ and } r_b) \quad (1)$$

$$M^*(t) = M(t)[1 - M(t)Bc + 0(c^2)] \quad (2)$$

where r, r_a, and r_b are the radial distances to any given point, to the meniscus, and to the base, respectively; t is time; c is the concentration at r and t in grams per unit volume; $\partial c/\partial r$ is the concentration gradient at r and t in grams per volume per distance; $M^*(t)$ and $M(t)$ are the apparent Archibald molecular weight and Archibald molecular weight, respectively, at r and t; B is the

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nonideality coefficient for the solution; $\bar{v}$ is the partial specific volume of the solute (assumed the same for all macromolecular species, if more than one is present); ρ is the density of the solution; ω is the angular velocity; and R and T are the gas constant and absolute temperature. The apparent weight-average molecular weight, M_w^*, for the solution at initial concentration, c^0, is best obtained from the apparent Archibald molecular weight by taking the limit as $\sqrt{t} \to 0$ of $M^*(t)$, as recommended by Yphantis (1959).

Several methods have been used to extrapolate the data to the ends of the solution column in order to obtain experimental values for the left side of eq 1. The first of these was introduced by Archibald (1947): values of $(1/rc)(\partial c/\partial r)$ are plotted *vs.* r and evaluated at the ends by extrapolation. Archibald carried out this with theoretical data; Mommaerts and Aldrich (1958) applied the technique to experimental data obtained with Rayleigh interference optics. The latter workers were forced to use a free-hand extrapolation to get molecular weights, and this procedure introduced an uncertainty of 5% into their results.

Fujita *et al.* (1962), in studying the system *polystyrene in methyl ethyl ketone*, carried out the extrapolation to the ends with a plot of log $[(1/rc)(\partial c/\partial r)]$ *vs.* r.

Still another method consists of plotting $\partial c/\partial r$ *vs.* r, evaluating $\partial c/\partial r$ at the ends, and then calculating $(1/rc)\cdot(\partial c/\partial r)$ to obtain molecular weights (Ginsburg *et al.*, 1956; Richards and Schachman, 1959). Peterson and Mazo (1961) with the use of a computer found that $\partial c/\partial r$ *vs.* r was linear over 10% of the cell near the meniscus, but they were unable to present general conditions that would ensure a linear gradient. More recently Weston and Billmeyer (1963) reported the existence of a very short linear region at both ends of the column.

An entirely different approach from those above, that attempts to avoid the extrapolation directly, was introduced by Ehrenberg (1957). This method requires that the experiment be run at high speed and assumes that the curve of $\partial c/\partial r$ *vs.* r becomes horizontal (and constant with r) near the meniscus at short times; however, these conditions, even if obtained, do not permit measurements of molecular weights at the base of the column.

Theory

The dependence of $\partial c/\partial r$ on r is given by $\partial^2 c/\partial r^2$, the derivative of the gradient curve with respect to r. A theoretical study of this dependence should yield conditions under which a linear extrapolation of $\partial c/\partial r$ *vs.* r is valid. Toward this end we will enploy the equation of Fujita and MacCosham (1959), which is a solution of the Lamm differential equation for ultracentrifugation in a semiinfinite cell. This equation may be used to describe sedimentation at the meniscus so long as a plateau region $(\partial c/\partial r) = 0$ exists in the cell (Yphantis, 1959; LaBar and Baldwin, 1963.)

$$\frac{c}{c^0} = \frac{\exp(-\epsilon T)}{2}\left\{\left[1 - \Phi\left(\frac{T - Z}{2T^{1/2}}\right)\right] - \frac{2}{\sqrt{\pi}} T^{1/2} \exp\left[-\frac{(T - Z)^2}{4T}\right] + [1 + Z + T]\left[1 - \Phi\left(\frac{T + Z}{2T^{1/2}}\right)\right]\exp(Z)\right\} \quad (3)$$

$$\Phi(x) = (2/\sqrt{\pi}) \int_0^x \exp(-q^2)dq \quad (4)$$

$$\epsilon = 2D/r_a{}^2\omega^2 s,\ T = 4Dt/\epsilon^2 r_a{}^2,\ Z = \frac{2}{\epsilon} \ln (r/r_a) \quad (5)$$

where r is the radial distance from the center of rotation, r_a is the position of the meniscus, D is the diffusion coefficient, s is the sedimentation coefficient, and ω and t are, as before, angular velocity and time.

The first derivative of c with respect to r near the meniscus is

$$\frac{\partial c}{\partial r} = \frac{c^0 \exp(-\epsilon T)}{\epsilon r}\left\{[2 + Z + T]\left[1 - \Phi\left(\frac{Z + T}{2T^{1/2}}\right)\right]\cdot \exp Z - \frac{2T^{1/2}}{\sqrt{\pi}} \exp\left[-\frac{(T - Z)^2}{4T}\right]\right\} \quad (6)$$

and the second derivative is

$$\frac{\partial^2 c}{\partial r^2} = \frac{c^0 \exp(-\epsilon T)}{\epsilon^2 r^2}\left\{[(6 - 2\epsilon) + (2 - \epsilon)T + (2 - \epsilon)Z]\left[1 - \Phi\left(\frac{Z + T}{2T^{1/2}}\right)\right]\exp Z - \frac{2}{\sqrt{\pi}}\left[\frac{2 + (2 - \epsilon)T}{T^{1/2}}\right]\exp\left[\frac{-(T - Z)^2}{4T}\right]\right\} \quad (7)$$

On substituting into eq 7 the series expansions

$$\Phi(x) = (2/\sqrt{\pi})[x + 0(x^2)] \quad (8a)$$

$$\exp(-x) = 1 - x + 0(x^2) \quad (8b)$$

and neglecting terms, which for most experimental conditions contribute <2%, we obtain

$$\frac{\partial^2 c}{\partial r^2} \doteq \frac{c^0 \exp(-\epsilon T)}{\epsilon^2 r^2}\left\{(6 - 2\epsilon) - \frac{2T^{-1/2}}{\sqrt{\pi}}[2 + (3 - \epsilon)Z]\right\} \quad (9)$$

The substitutions

$$\delta = r - r_a,\ r^2 \doteq r_a{}^2/(1 - 2\delta/r_a),\ Z \doteq \frac{2\delta}{\epsilon r_a} \quad (10)$$

transform eq 9 into

$$\frac{\partial^2 c}{\partial r^2} \doteq \frac{c^0(1 - 2\delta/r_a)\exp(-\epsilon T)}{\epsilon^2 r_a^2}\left\{(6 - 2\epsilon) - \frac{2\epsilon r_a}{\sqrt{\pi Dt}}\left[1 + \frac{(3 - \epsilon)\delta}{\epsilon r_a}\right]\right\} \quad (11)$$

The term containing $(Dt)^{-1/2}$ always exceeds the constant term $(6 - 2\epsilon)$ as long as the plateau exists. Thus, eq 11 states that the gradient curve, $\partial c/\partial r$ *vs.* r, will have a negative slope at the meniscus and the steepness of this slope will decrease with time. This situation is observed routinely in experiment.

In experiment, the direct reading of data can be made reliably to within 0.015 cm of the ends of the column; the extrapolation to the ends is usually carried out on data taken from 0.015 to 0.050–0.100 cm of the ends. Accordingly, for $0 < \delta < 0.075$ cm, $r_a = 6.00$ cm, if the term $[1 + (3 - \epsilon)\delta/\epsilon r_a]$ is constrained to vary no greater than 5% (this reflects a rather minor dependence of eq 11 on r), then the gradient curve will be linear in r near the ends provided $\epsilon > 0.5$. An estimation for the time required to achieve this linearity for practical work may be obtained by imposing the further condition that the gradient at $\delta = 0.075$ cm be about one-half its value at the meniscus (this restriction will cause the gradient curve to have a moderate, and not too steep, slope: a desirable property for extrapolation). Since the term containing the error function of eq 6 normally contributes 94% or more of the value of $\partial c/\partial r$, the condition may be expressed as

$$\left(\frac{\partial c}{\partial r}\right)_{r'} \Big/ \left(\frac{\partial c}{\partial r}\right)_{r_a} \doteq \frac{\left[1 - \Phi\left(\frac{Z' + T}{2T^{1/2}}\right)\right]}{\left[1 - \Phi\left(\frac{T^{1/2}}{2}\right)\right]} = \frac{1}{2} \quad (12)$$

where r', Z', and δ' are evaluated with $\delta = 0.075$ cm. Using expansion 8a and rearranging, we have

$$\sqrt{\pi}T^{1/2} - T = 2Z' \quad (13)$$

Since T is relatively small, it may be neglected to give

$$T^{1/2} = (2/\sqrt{\pi})Z' \quad (14)$$

or from eq 5 and 10

$$(Dt)^{1/2} = (2/\sqrt{\pi})\delta' \quad (14a)$$

$$Dt \doteq \delta'^2 \quad (14b)$$

Thus $Dt = 0.56 \times 10^{-2}$ cm^2 for $\delta' = 0.075$ cm; this analysis predicts that $\partial c/\partial r$ will be linear and have a moderate slope at r near the meniscus for $Dt = 10^{-2}$ cm^2.

An analysis for the base, carried out in a manner analogous to the Fujita–MacCosham analysis at the meniscus, yields an expression for $\partial c/\partial r$ like eq 6, but in which the signs before the error function and $T^{1/2}$ terms are positive rather than negative. This expression for sedimentation at the base is likewise valid only so long as a plateau is present in the cell. The second derivative is identical with eq 9, except that the sign before the $T^{1/2}$ term is positive; this difference means that the slope of the gradient curve will always be positive at the base, but the steepness of the slope will decrease with time, as it does at the meniscus. However, the change in sign before the error function at the base gives

$$\sqrt{\pi}T^{1/2} + T = -2Z' \quad (15)$$

$$T^{1/2} = (-2/\sqrt{\pi})Z' \quad (16)$$

$$(Dt)^{1/2} = (-2/\sqrt{\pi})\delta' \quad (16a)$$

$$Dt \doteq \delta'^2 \quad (16b)$$

Hence, for the same δ, the gradient curve at the base should be linear at the same time as that required for linearity at the meniscus.

These theoretical results indicate that the gradient curve will be linear with radial distance, and have a moderate slope, over a useful range at both ends of the column for $Dt \sim 10^{-2}$ cm^2 and $\epsilon > 0.5$. In order to examine in detail the behavior of the gradient curve for an ideal, homogeneous solution under conditions employed in experiment, we turn to the analysis of theoretical digital computer data. Such a solution is shown to be homogeneous by measurement of the same value for $M(t)$ at both ends of the column (Archibald, 1947; Trautman, 1956).

Analysis of Theoretical Computer Data. The availability of numerical solutions to the equation of Mason and Weaver (1924) (D. A. Yphantis, private communication 1960) provided data for a test of the basic theory described above by analysis of $\partial c/\partial r$ *vs.* r curves corresponding to widely different conditions. However, for clarity it must be pointed out that these numerical solutions are obtained from the summation equation of Mason and Weaver. This summation equation gives values at early times, when the plateau is present, that converge to those given by the more exact integral equation of Mason and Weaver (1924). Numerical calculations have demonstrated that this convergence is adhered to closely. The Mason–Weaver integral equation resembles the equation of Fujita and MacCosham, eq 3; both describe sedimentation in a semiinfinite cell, but the former in a rectangular cell under a constant field, and the latter in a sector cell under a centrifugal field. These differences are minimal at the short times under investigation here, and the integral equation of Mason and Weaver has analytic form at the meniscus identical with eq 3 with these differences: (1) it lacks the exponential multiplier in time, $\exp(-\epsilon T)$, (2) it contains a linear dependence on r

in place of the parameter Z, (3) it contains a parameter in time which differs from the parameter T by a constant. That the Mason–Weaver solutions are good approximations for this study is demonstrated by the fact that only dependence (2) contains a different radial dependence and this is minimized at the ends of the column where extrapolations of $\partial c/\partial r$ *vs.* r are made.

The numerical solutions presented here are consistent with theoretical experiments having a meniscus position at 6.00 cm and a base position at 7.00 cm. Since the parameters of both the Mason–Weaver and Fujita–MacCosham equations are in reduced form, changes in these parameters cannot be interpreted uniquely in terms of specific changes in experimental variables. In general, however, a decrease in ϵ reflects an increase in speed or molecular weight. It will be convenient for this analysis to think of changes in ϵ as reflecting changes in speed with the molecular weight held constant.

Figure 1 depicts the behavior of the gradient curve at an early time for a theoretical experiment at moderately low effective centrifugal field. The linear extrapolation at the meniscus yields an excellent result; while the extrapolation at the base gives a low value, this value is still within the 2% accuracy of the schlieren optical system. The per cent difference between the actual values at the ends and the extrapolated values were calculated for the data of Figure 1 and for data of differing conditions; the results are shown in Table I.

TABLE I: Error in Gradient at Ends of Column as a Result of Linear Extrapolation of Computer Data (in Per Cent).

$Dt \times 10^2$ (cm^2)	Meniscus		Base	
	ϵ	% Error	ϵ	% Error
0.20	0.0185	+3.1	...[a]	...
0.15	0.0555	+1.3	0.0408	...[b]
0.20	0.0555	+2.3	0.0408	...[b]
0.40	0.0555	+4.6	0.0408	−9.2
0.60	0.0555	+6.8	0.0408	−5.2
0.40	0.111	+0.7	0.0816	−7.7
0.80	0.111	+1.9	0.0816	−3.0
1.20	0.111	+1.9	0.0816	−3.5
0.17	0.185	−1.3	0.136	−15.9
0.67	0.185	+0.1	0.136	−2.2
2.00	0.185	+1.3	0.136	−3.3
0.50	0.555	0.0	0.408	−1.6
2.00	0.555	+0.1	0.408	−0.3
0.20	1.111	−0.1	0.816	−0.4
4.00	1.111	0.0	0.816	0.0

[a] Ehrenberg condition: data at the base are not used
[b] Theoretical data at the base are too highly curved to permit a linear extrapolation; this phenomenon would readily be apparent in experiment as well.

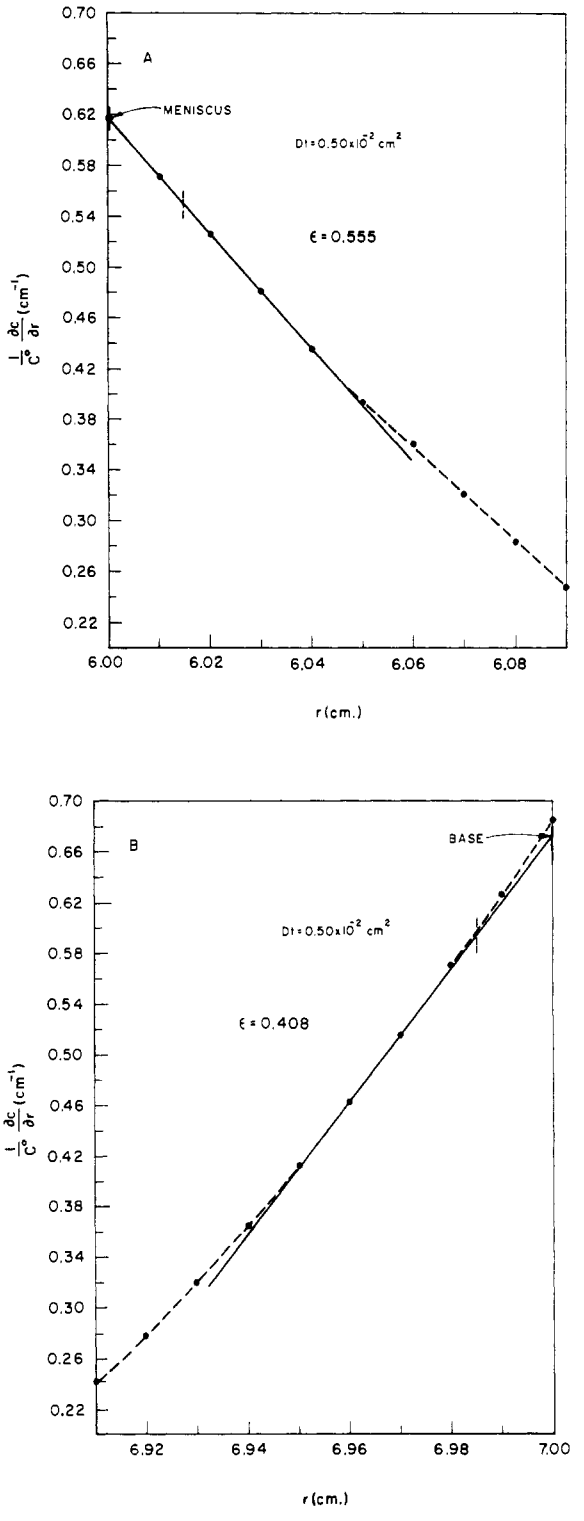

FIGURE 1: Linear extrapolation of computer data at both ends of the column. $(1/c^0)(\partial c/\partial r)$ is the gradient curve normalized by the initial concentration, and r is the radial distance. The solid line is the best straight line for extrapolation through the points near the ends, and the dashed curve shows actual behavior of the data when the points depart from a line. In experiment data within 0.015 cm cannot be obtained accurately; the vertical dash marks define this interval.

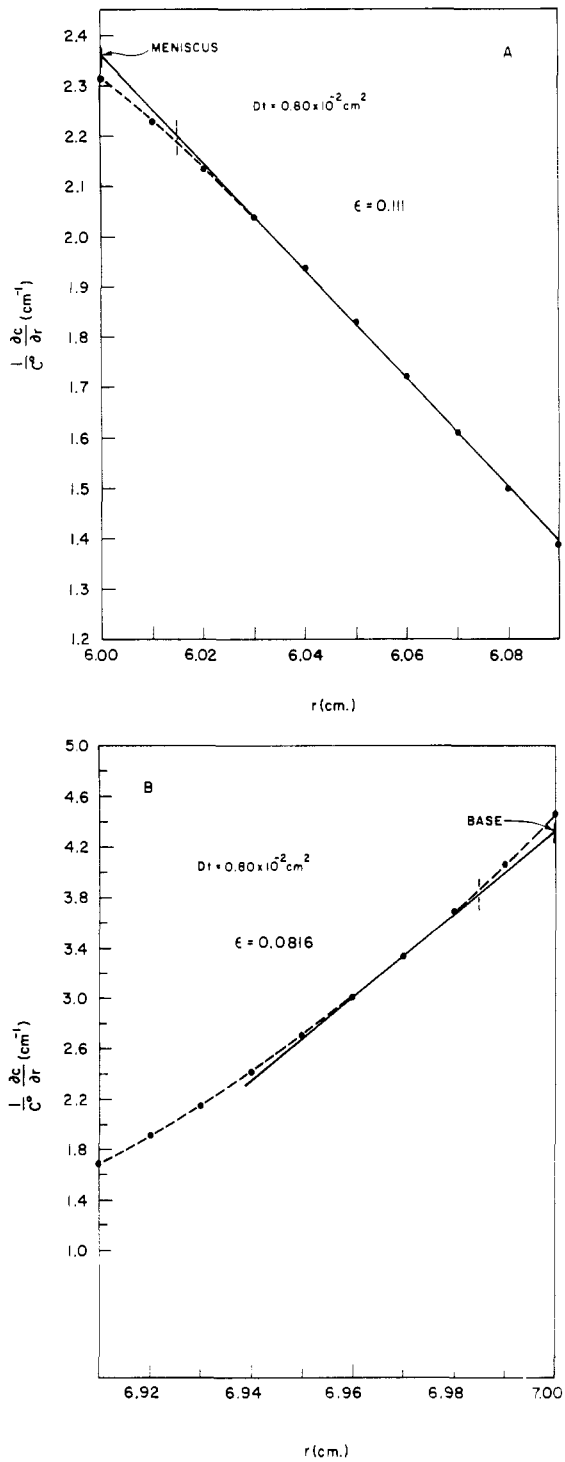

FIGURE 2: Linear extrapolation of computer data at both ends for a theoretical experiment at a fivefold higher effective field than that in Figure 1. The departure from linearity at the ends increases appreciably at this field.

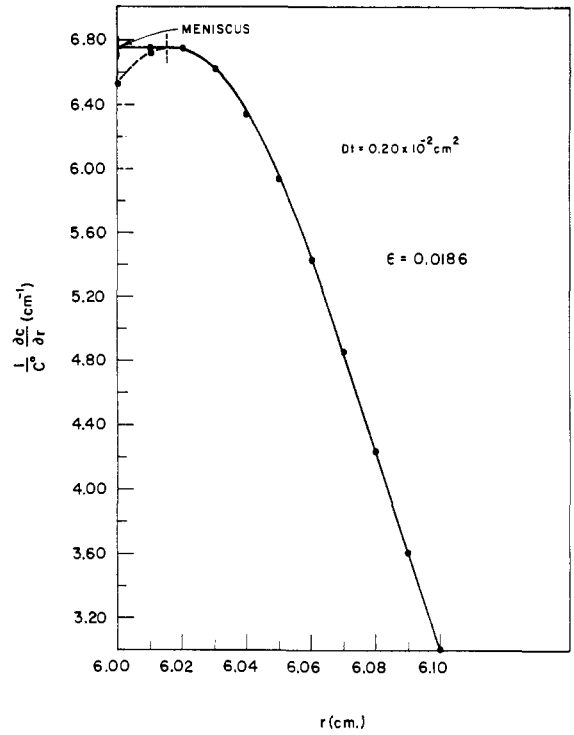

FIGURE 3: Data for a theoretical experiment according to the method of Ehrenberg. The horizontal straight line depicts the expected behavior at the meniscus; the dashed curve shows the actual behavior of the data. Points farther away from the meniscus are linear with distance, in contrast to the behavior of the data of Figure 1.

At longer times for the highest ϵ values shown in the table, the extrapolations at the base yield better values, while values at the meniscus continue to remain good. It is shown in the table for the next lower ϵ (a higher speed with $\epsilon = 0.408$ at the base) that the linear extrapolation at the base also gives good values even at short times. The difference in ϵ values between the meniscus and base at the same speed and molecular weight stems from the differences in radial position used for the base in equations comparable to eq 5: at the base, ϵ is calculated using r_b, the radial position of the base, instead of r_a.

The effect of increasing the field fivefold over that of Figure 1 is illustrated in Figure 2; the error in determining the gradient at both ends of the column by linear extrapolation increases in accord with predictions from eq 11 and its counterpart for the base. An experiment under these conditions at an earlier time possesses considerably more upward curvature at the base; this is reflected by the increase in error given in the table. Other experiments in the table show that this upward curvature at the base increases with increasing speed (decreasing ϵ) and becomes worse at short times for a given value of ϵ.

The graph of Figure 2A points out a behavior at the meniscus which was not predicted from the analysis of eq 11–14b; the gradient tends to curve downward very near the meniscus as the speed increases. This effect will

yield molecular weights which will be too high, but this error remains relatively small as compared with the low values at the base. The errors increase as the speed is raised twofold above that of Figure 2; in fact, the gradient curve at the base becomes so highly curved that it is unworkable at early times. These errors at the meniscus become appreciably larger as the speed increases and approaches the Ehrenberg condition at the meniscus, where the gradient curve is expected to be horizontal (Ehrenberg, 1957).

A theoretical experiment depicting the Ehrenberg condition, in which the boundary is about to move away from the meniscus, is shown in Figure 3. Two significant features are revealed in this figure. First, the curve is not truly horizontal at the meniscus as predicted for this method; but the error introduced here is only +3.1% for $\epsilon = 0.0185$ and in itself will not give rise to highly serious errors in measurements of molecular weight. The second aspect, with bearing on the theoretical result of eq 11, is that the gradient curve exhibits a linear region from 6.06 to 6.10 cm. This unusual property, however, is present also at $\epsilon = 0.0555$ (a lower speed), and as opposed to the first aspect, does affect the extrapolation to the meniscus to give a large error of +6.8% with $Dt = 0.60 \times 10^{-2}$ cm² because under these conditions, while the gradient is most linear from 6.04 to 6.10 cm, it does take on a gradual downward curvature as it approaches the meniscus. This second aspect, at high effective fields, is most deceiving and can introduce relatively large errors into measurements of molecular weight at the meniscus.

Discussion

The results of the theoretical experiments presented in Table I confirm the predictions from the Fujita–MacCosham equations that a workable linear gradient is present at the meniscus and base for $\epsilon > 0.50$ and $Dt \sim 0.50 \times 10^{-2}$ cm². Homogeneity of the sample is demonstrated under these conditions by the fact that the combined errors for the base and meniscus are within the accuracy of the schlieren optical system. The theoretical data demonstrate a phenomenon that was not predicted by this analysis of the equations, namely, that the gradient at the base shows appreciable upward curvature, especially at early times. This will lead to erroneously low values for molecular weights. The computer data establish furthermore that measurements using the linear extrapolation can be made at the meniscus without serious error for ϵ down to 0.10. However, errors at both ends of the column increase as ϵ values decrease, with the meniscus giving high values and the base low values. This rather unusual phenomenon was observed experimentally in studies on myosin by Mommaerts and Aldrich (1958); this finding will be discussed further in the following paper (LaBar, 1966).

Study of the Ehrenberg method with computer data shows that the gradient curve is not horizontal at the meniscus over an interval of 0.020 cm, the first 0.015 cm of which are unreadable under experimental conditions. The length of the linear horizontal interval in Figure 3 is estimated to be 0.005 cm, but a straight line was extended to the meniscus to measure the error from such a linear extrapolation. This theoretical experiment establishes that the linear horizontal interval is too short to allow linear extrapolation to yield accurate values for molecular weight according to the method of Ehrenberg. If deterioration of the data near the meniscus did not prohibit readings to within 0.005 cm of the meniscus, then linear extrapolations could be made and reliable values for molecular weights could be obtained. Until this obstacle is surmounted it is unlikely that the Ehrenberg method will yield results as accurate as those obtained by Archibald measurements at low speeds. Studies at other times in addition to those shown in Figure 3 agree with this finding. The errors introduced by this effect were acceptable for the theoretical case examined, but the following paper (LaBar, 1966) with experimental data shows that this effect tends to give rise to even larger errors.

Calculations to establish the consequence of an erroneous value for $\partial c/\partial r$ at the end show that the error in $\partial c/\partial r$ is carried over directly into the error in molecular weight; this comes about because a given error in the gradient curve introduces a much smaller error through numerical integration into the value found for concentration. This error in concentration is relatively negligible in the evaluation of molecular weight from eq 11, as compared with the error introduced by $\partial c/\partial r$ itself.

Although the present analysis of the Fujita–MacCosham equation at the meniscus and a comparable equation at the base and of computer data for the Mason–Weaver equation has established conditions for linear extrapolations of the gradient curves to the ends of the column, it must be borne in mind that (in these equations) no allowance is made for the dependence on concentration of the sedimentation and diffusion coefficients. The conditions outlined should apply well to experiment but the behavior of the gradient curve may be altered somewhat by concentration dependence of real substances. The following paper will show that this dependence does not affect the applicability of the procedure for linear extrapolation described here.

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A Procedure for the Measurement of Molecular Weights by the Archibald Method. II. Experimental Studies with Sucrose and Chymotrypsinogen A*

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ABSTRACT: In the preceding theoretical paper [LaBar, F. E. (1966), *Biochemistry 5*, 2362 (this issue, preceding paper)] conditions were established where a linear extrapolation of the gradient curve gives accurate molecular weights with the Archibald method. The necessity for using such a linear extrapolation now is shown and experimental tests with model systems are given for the theoretical conclusions of the first paper. Also, certain experimental problems are discussed, including the importance of having the optical system in correct focus and the need to test the schlieren phase plate.

In the preceding communication (LaBar, 1966), a procedure for measuring Archibald molecular weights using a linear extrapolation of the gradient curve was proposed. The procedure is based on an analysis of theoretical equations and was tested by calculations with theoretical computer data. Conditions were found which give relatively small errors when strictly linear extrapolations are employed. These conditions constrain the parameters ϵ to >0.50 and $Dt \sim 0.50 \times 10^{-2}$ cm^2, where $\epsilon = 2D/r_a^2\omega^2 s$, D is the diffusion coefficient, r_a is the radial position of the meniscus, ω is the angular velocity, s is the sedimentation coefficient, and t is time. The theoretical data showed that for the meniscus only the range of ϵ could be extended to >0.10. The purpose of the present study is to show in practice how accurately the molecule weight of a known substance can be determined under these conditions and also to establish whether concentration dependence and actual experimental conditions alter the gradient curve from that predicted from theory and thereby affect measurements of molecular weight.

The substances employed for this study are sucrose and chymotrypsinogen A. The ultracentrifugal behavior of both materials has been examined by sedimentation equilibrium methods (LaBar and Baldwin, 1962; LaBar, 1965). The use of chymotrypsinogen A permits an examination of the modification of the Archibald method by Ehrenberg (1957); this examination points out a practical difficulty in the modification which leads to errors larger than those predicted by the theoretical data.

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