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

F. E. LaBar†

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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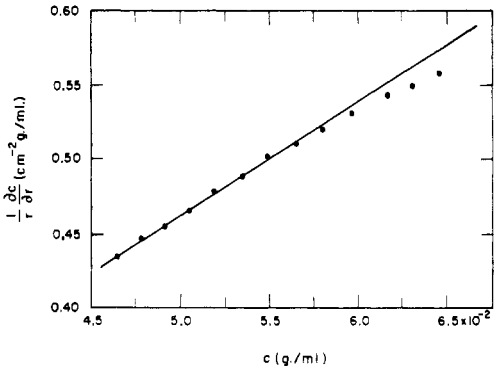

FIGURE 1: Defective schlieren phase plate yields experimental data that do not give the linear graph according to Method II of Van Holde and Baldwin (1958): sucrose solution with c^0 = 54.78 mg/ml.

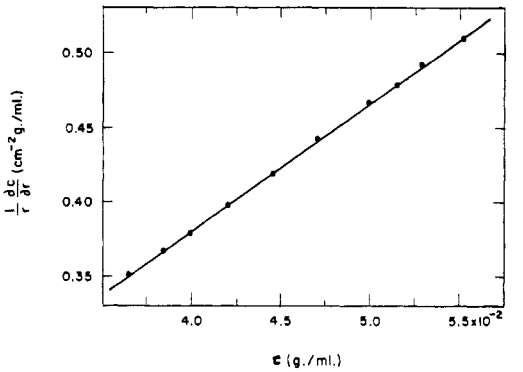

FIGURE 2: Nondefective phase plate gives data that obey the linear relation of Method II of Van Holde and Baldwin (1958): sucrose solution with c^0 = 44.80 mg/ml.

Experimental Section

Materials. Sucrose was obtained from the National Bureau of Standards (sample 17, Lot No. 6004); concentrations of sucrose solutions were calculated from the weights of sucrose and distilled water, corrected for air buoyancy. Chymotrypsinogen A, chromatographically homogeneous, Lot No. CGC 762, was purchased from Worthington Biochemical Corp.; this lot of material is known to be centrifugally homogeneous at sedimentation equilibrium (LaBar, 1965). The supporting buffer for the experiments with chymotrypsinogen A had an ionic strength of 0.2, a pH of 7.2, and concentrations of 17.6 $\times$ 10^{-3} M in KH_2PO_4 and 60.8 $\times$ 10^{-3} M in $Na_2HPO_4 \cdot 7H_2O$; the density of this buffer was calculated from tables of Svedberg and Pedersen (1940). The value of $\bar{v}$ used for chymotrypsinogen A was 0.721, as measured by Schwert (1951). The values of $\bar{v}$, of densities and nonideality contributions of solutions, of sucrose were taken from the data of Gosting and Morris (1949).

This work was done on two different Spinco Model E analytical ultracentrifuges; both instruments were focused according to the procedure described below. The heavy rotor ANJ was used at speeds of 7928 and 9945 rpm, while the conventional AND rotor was used at all higher speeds. Cells with double-sector, Epon-filled centerpieces and Fluorochemical FC-43 oil for the base were used in centrifugation; these items were purchased from Spinco Division, Beckman Instruments.

Methods. FOCUS OF SCHLIEREN OPTICS. Most of the details of the focus of the optical system have already been described (LaBar and Baldwin, 1962; LaBar, 1965) and need not be redescribed here. However, a focusing step extremely important to Archibald measurements with schlieren optics will be taken up here. Trautman (1958) demonstrated the necessity for laterally positioning the source slit on the optic axis in the present type of study. The recent availability from Spinco of a light-source mounting, with vernier and adjusting screw assembly for precision lateral adjustment, made possible readily this positioning of the light source on the axis. The position of focus was determined to be that for which the two flanking fringes at the meniscus, as described by Trautman, were of maximal sharpness and symmetry about the meniscus. The sensitivity of positioning with this method was found to be $\pm$0.03 cm (LaBar, 1963). The magnitude of the error introduced into measurements when the light source is not correctly positioned will be taken up later in the Discussion.

TEST OF PHASE PLATE. A step important to this study is the necessity for checking the quality of the phase plate supplied with the instrument. Need for this check is demonstrated by the fact that in the course of the present study some phase plates were found to be faulty. These phase plates were replaced with nondefective ones by the manufacturer.

A measure of the error introduced from a faulty

TABLE 1: Errors Introduced by a Defective Phase Plate into Measurements of the Molecular Weight of Sucrose at Sedimentation Equilibrium.[a]

Type of Phase Plate	c^0 (mg/ml)[b]	Error in M_w (%)	Error in M_z (%)
Defective	54.78	−2.8	−9.9
Nondefective	44.80	−0.5	−1.8

[a] The molecular weight of sucrose is 342.3. Corrections for nonideality were made from Gosting and Morris (1949), and the specific refractive increment used for conversion to concentration was taken from this same reference to be 1.431 $\times$ 10^{-1} ml/g at 25°. Measurements were made at 25° and 42,040 rpm. [b] c^0 is the initial concentration of the solution. Except for the difference in solutions and the change of phase-plates, the focus of the optics and other experimental variables are identical.

phase plate into measurements of molecular weight at sedimentation equilibrium is presented in Table I (this result, along with all subsequent results of studies on sucrose, are taken from LaBar, 1963). M_w and M_z are calculated from Method I, equation 31, and Method II, equation 36, respectively, of Van Holde and Baldwin (1958); the graphs in Figures 1 and 2 are according to Method II, and their advantage for detecting a defective phase plate lies in the fact that the slope of a graph need not be evaluated and that the parameters may even be left in units of optical displacements, rather than converted to units of concentration as shown here. The departure from linearity of the data of Figure 1 is conclusive proof that this phase plate is defective; adherence of the data to a straight line in Figure 2 shows that the phase plate, used as a replacement, is of good optical quality and may be relied upon to yield correct data.

MEASUREMENT OF c^0, INITIAL CONCENTRATION. In order to obtain highly accurate values for molecular weights, the initial concentration of the solution must be measured carefully. Synthetic boundary forming experiments, using capillary centerpieces, may introduce appreciable errors unless employed cautiously. The data of Table II show that satisfactory results may be obtained when the level of the measured solution is well below the level of the capillary; but when the level of the solution rises toward the level of the capillary, errors in the measurement make the results unsatisfactory for use in determining molecular weights. This type of boundary-forming experiment was used only to measure the initial concentration of solutions of sucrose.

TABLE II: Variation as a Function of Sample Volume of Measurements of Initial Concentration of Sucrose with a Synthetic-Boundary, Capillary Centerpiece.[a]

	Init Concn (mg/ml)		
Vol. (ml)	Actual[b]	Measured	Error (%)
0.13	9.728	9.693	−0.36
0.16	9.728	9.588	−1.44
0.19	9.728	9.539	−1.94
0.19	9.734	9.574	−1.64

[a] These data were obtained with sucrose using Rayleigh interference optics for greater accuracy (LaBar, 1963). The volume of liquid needed to reach the level of this capillary is 0.21 ml. [b] Computed from the weights of sucrose and water at 25°, corrected for air buoyancy.

In the case of chymotrypsinogen A, the molecular weight is great enough to allow movement of the boundary away from the meniscus at high speeds. This technique was used in these studies and offers the same advantages for work with schlieren optics as with Rayleigh optics (Yphantis, 1964; LaBar, 1965). Prior to centrifugation at 20°, the solutions of chymotrypsinogen A were dialyzed *vs.* buffer at 4° for 24 hr. The superiority of the moving boundary method over the synthetic boundary forming method is shown in Table III. Even though broad peaks for the data of Table III were observed, due to the relatively high diffusion coefficient of chymotrypsinogen A, correction for radial dilution using the position of maximum height gave values in excellent agreement with each other for two very different values of r_p, whereas the synthetic boundary forming experiment gave a value that was low by 2.1%. This is in agreement with the observations of Table II because a volume of 0.18 ml was required for the higher refractive indices employed in schlieren work (with a volume of 0.13 ml, the plateau ahead of the boundary would have been lost before the pattern would be resolved sufficiently to permit photography). Values for c^0 of chymotrypsinogen A, used in calculations of Archibald molecular weights, were obtained from moving boundary experiments at 50,740 and 52,640 rpm with corrections for radial dilution by $(r_p/r_a)^2$.

TABLE III: Comparison of Methods for Measuring the Initial Concentration of a Solution of Chymotrypsinogen A.

Method	Speed (rpm)	Time (min)[a]	c^0 (in units of area/cm²)[b]
Synthetic boundary	15,220	...	0.3351
Moving boundary[c]	50,740	167.4	0.3422
Moving boundary[d]	50,740	191.7	0.3422

[a] Corrections for each acceleration were made by adding one-third of the time required for that acceleration. [b] Schlieren bar angle set at 80.0°; areas from photographs were corrected to zero time by multiplying by radial dilution factor $(r_p/r_a)^2$, where r_p is radial position of maximal height and r_a is position of meniscus. [c] These data were obtained by centrifugation at 50,740 rpm for 18.5 min after acceleration from 39,460 rpm. [d] These data were obtained by centrifugation at 50,740 rpm for 113.5 min after acceleration from 27,690 rpm.

DETERMINATION OF CONCENTRATIONS AT ENDS OF COLUMN. Since measurements of molecular weights by the Archibald method in this study were made while a plateau ($\partial c/\partial r = 0$) existed in the cell, it was justifiable to determine the concentrations at the ends simply from

$$c_a = c^0 \exp(-2s\omega^2 t) - \int_{r_a}^{r_{p'}} (\partial c/\partial r)dr \qquad (1a)$$

and

$$c_b = c^0 \exp(-2s\omega^2 t) + \int_{r_{p'}}^{r_b} (\partial c/\partial r)dr \qquad (1b)$$

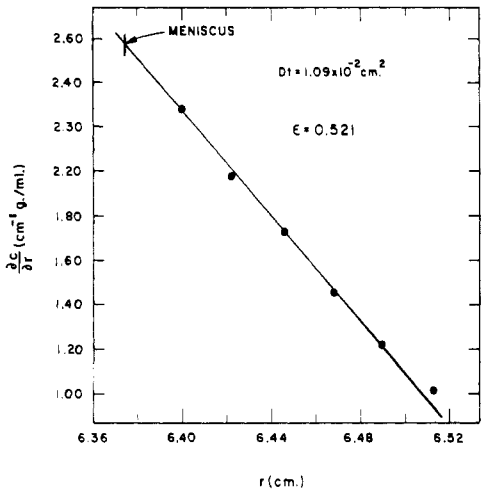

FIGURE 3: Linear extrapolation at the meniscus of concentration gradient *vs.* r for experimental data of a sucrose solution having c^0 = 44.80 mg/ml and centrifuged at 42,040 rpm for 37.29 min.

where c_a and c_b are the concentrations at the meniscus and base, respectively; c^0 is the initial concentration; $\partial c/\partial r$ is the concentration gradient at r, the radial coordinate; r_a, r_b, and r_p' are the radial positions of the meniscus, base, and appropriately the beginning or end of the plateau region, respectively; s is the sedimentation coefficient; ω is the angular velocity; and t is time. The sedimentation coefficient of sucrose is taken from LaBar and Baldwin (1963), that of chymotrypsinogen A from Schwert (1951).

DETERMINATION OF MOLECULAR WEIGHTS. Molecular weights according to Archibald (1947) are determined at the ends from

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

$$M^*(t) = M(r)[1 - M(t)Bc + 0(c^2)] \quad (3)$$

where, in addition to the parameters defined in eq 1, $M^*(t)$ and $M(t)$ are the apparent Archibald molecular weights respectively at c_a or c_b and t, B is a nonideality coefficient, $\bar{v}$ is the partial specific volume of the solute, ρ is the density of the solution, and R and T are the gas constant and absolute temperature.

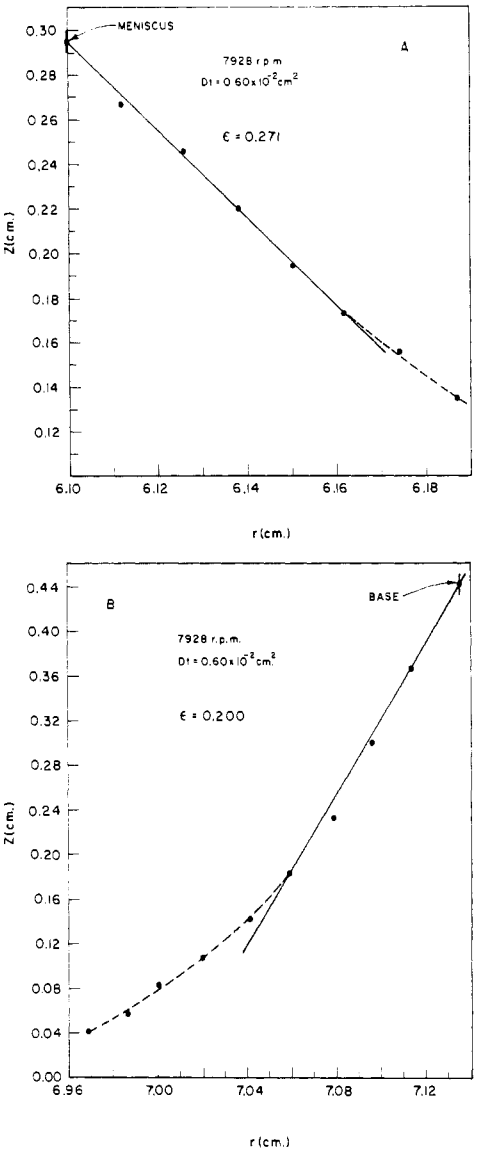

FIGURE 4: Linear extrapolations at both ends of column on a solution of chymotrypsinogen A, c^0 = 15 mg/ml, after 104.5 min with schlieren bar angle of 70°. The gradient is left in terms of vertical displacements (Z) on the photographic plate. The solid line used for extrapolation is the best line through data near the end of the column; the dashed line reflects the behavior of the data over the interval examined. Points with vertical slashes at the ends are the extrapolated values, as opposed to the other data points.

Results

A preliminary experiment performed on the well-studied system of *sucrose in water* under conditions favorable for a linear gradient at the meniscus established: first, that a linear gradient is observed experimentally in accordance with theoretical predictions, and second, that under such conditions, values for molecular weight by the Archibald method can be obtained within the estimated 2% error inherent to schlieren optics. Proof of the first objective is given in Figure 3, that of the second in Table IV. The gradient curve in Figure 3 is linear at the meniscus over more than 0.10 cm. This situation was obtained by using the relatively long column height of 0.6796 cm.

An extensive study of the behavior of the gradient curve at both ends of the column over a wide range of centrifugal fields was carried out with the homogeneous

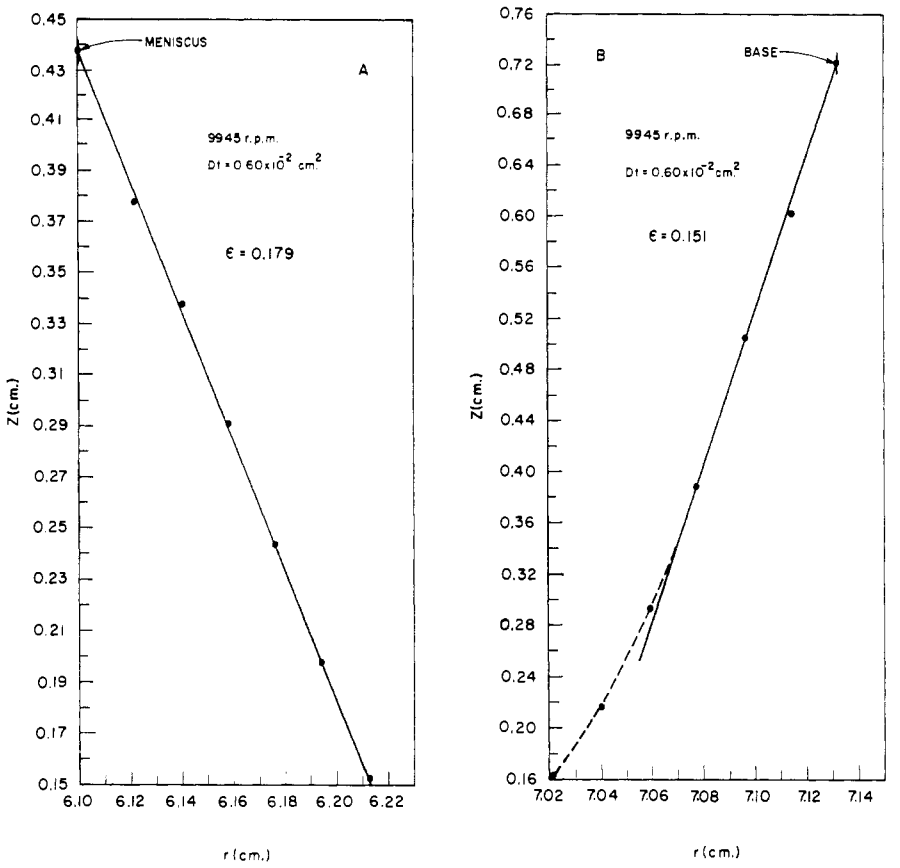

FIGURE 5: Linear extrapolations on a solution of chymotrypsinogen A, c^0 = 15 mg/ml, after 104.9 min with schlieren bar angle at 70°. Notation is the same as that of Figure 4.

TABLE IV: Molecular Weight of Sucrose Measured by Linear Extrapolation at the Meniscus.[a]

Time (min)	$Dt \times 10^2$ (cm²)	$M(t)$	Error in $M(t)$ (%)
13.29	0.390	347.6	+1.6
21.29	0.624	344.7	+0.7
37.29	1.093	348.4	+1.8
53.29	1.562	343.8	+0.4

[a] Values obtained from a sucrose–water solution with c^0 = 44.80 mg/ml, column height, H = 0.6796 cm, and speed = 42,040 rpm. Values for the nonideality and diffusion coefficients were taken from Gosting and Morris (1949); the molecular weight of sucrose is 342.3. This same experiment run on to sedimentation equilibrium gave a molecular weight of 340.7 with schlieren optics, an error of −0.5%; ϵ = 0.521.

system *chymotrypsinogen A in phosphate buffer*. The graphs in Figure 4 at 7928 rpm show that the curves are linear at the two ends over different intervals. Figure 5 illustrates that the gradients at the two ends remain linear for this same time at the speed 9945 rpm. However, the region of linearity is greater at the meniscus for ϵ = 0.179 than it is at the base for ϵ = 0.200. This difference indicates that the degree of linearity is not merely a function of ϵ and Dt but is also a function of which end of the column is being examined. Thus considerable care had to be taken in reading the data for calculations of molecular weight at the base, not only because of this shorter interval, but because this interval, itself, decreases as ϵ decreases. The errors in apparent molecular weight at different speeds and times are given in Table V. All of these values were obtained by linear extrapolations, except in two cases: these include the two at the meniscus at 17,250 rpm in which the gradient pattern begins to reflect the Ehrenberg condition that becomes dominant at higher speeds. Figure 6A shows the gradient pattern at the meniscus for the earlier of the two nonlinear extrapolations; Figure 6B shows the corresponding pattern at the base where the linear extrapolation line is taken over a very short interval. The results in Table V show that the linear extrapolation is not suitable for the base under these conditions; a similar conclusion may be drawn in general for very early times at all speeds.

The molecular weight of chymotrypsinogen A used in Table V is calculated from the data of amino acid

sequence by Hartley (1964) to be 25,767; sedimentation equilibrium studies with interference optics (LaBar, 1965), using the value of Schwert (1951) of 0.721 for $\bar{v}$, gave values in agreement with this calculated value within from −0.7 to +0.3% at a concentration one-tenth of those employed here. In calculating the errors in Table V, the nonideality coefficient B was assumed to be 0. While the errors of the table on the positive side at the meniscus at low speeds may indicate that this assumption is incorrect, the fluctuation in errors at a given speed prevent an extrapolation to zero time to permit an estimate of B to prove this. All of the experiments reported in Table V were performed on the same aliquot of chymotrypsinogen A at c^0 = 15 mg/ml; except for positioning in the two different rotors, the ultracentrifuge cell was left untouched, except for shaking, between runs. When the speed was increased to 27,690 rpm, the gradient at this concentration at the meniscus became so steep that some of the light passing through the gradient was deflected so strongly that it did not reach the plane of the photographic plate. This difficulty was circumvented by using a less concentrated solution with c^0 = 10 mg/ml; this permitted an investigation of the Ehrenberg modification.

The results of measurements using the technique of Ehrenberg are presented in Table VI; they were calculated in the same way as those of Table V. Table VI shows that the error in measured molecular weight increases appreciably over an interval of 20 minutes.

TABLE V: Differences between Apparent Molecular Weight from Linear Extrapolation and Molecular Weight at Infinite Dilution on Chymotrypsinogen A.[a]

			Meniscus		Base	
Speed (rpm)	Time (min)	$Dt \times 10^2$ (cm²)	ϵ	Diff in $M^*(t)$ (%)	ϵ	Diff in $M^*(t)$ (%)
7,928	40.5	0.23	0.271	−0.1	0.200	−4.2
7,928	72.5	0.41	0.271	+2.4	0.200	+3.7
7,928	104.5	0.60	0.271	+0.7	0.200	+3.5
7,928	120.5	0.69	0.271	+0.8	0.200	+3.9
7,928	184.5	1.05	0.271	+1.1	0.200	+0.6
9,945	40.9	0.23	0.179	−3.9	0.151	+2.9
9,945	104.9	0.60	0.179	+1.4	0.151	+1.1
9,945	200.9	1.15	0.179	+0.9	0.151	+2.9
15,220	36.3	0.21	0.078	−1.9	0.056	−5.8
15,220	68.3	0.39	0.078	+2.6	0.056	−4.5
17,250	34.2	0.19	0.057	−0.2	0.042	−13.3
17,250	66.2	0.38	0.057	−0.2	0.042	−12.2
17,250	98.2	0.56	0.057	+2.9[b]	0.042	−17.3
				−4.6[c]		
17,250	130.2	0.74	0.057	−1.2[c]	0.042	−16.3

[a] For a solution with c^0 = 15 mg/ml at 20°. Apparent molecular weights were calculated using $\bar{v}$ = 0.721 of Schwert (1951); the molecular weight at infinite dilution of 25,767 was calculated from data of Hartley (1964) on amino acid sequence. Values for D of 9.5 × 10^{-7} cm²/sec and s of 2.54 × 10^{-13} sec for chymotrypsinogen A were also taken from the data of Schwert (1951); r_a was 6.10 cm and r_b was 7.13 cm. [b] Line. [c] Curve.

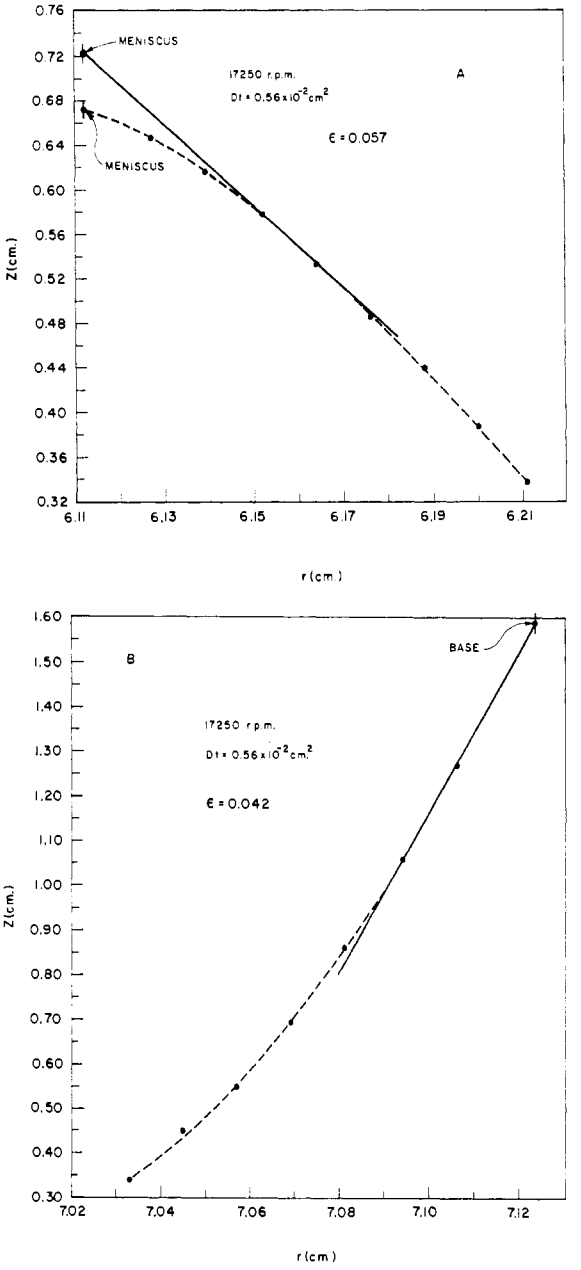

FIGURE 6: Behavior of a solution of chymotrypsinogen A, c^0 = 15 mg/ml, at relatively high field after 98.2 min with schlieren bar angle of 75°. Points away, but not immediately near, the meniscus fall on a straight line.

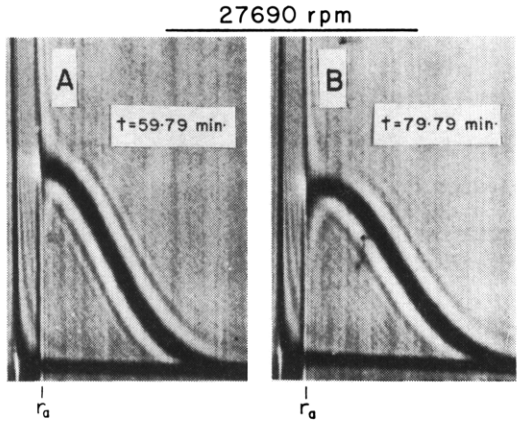

FIGURE 7: Photographs of the gradient at the meniscus at different times for $\epsilon = 0.0222$ with a schlieren bar angle of 75° in an experiment measuring molecular weight according to Ehrenberg (1957); c^0 of solution of chymotrypsinogen A is 10 mg/ml.

TABLE VI: Differences between Apparent Molecular Weight from Ehrenberg Modification and Molecular Weight of Chymotrypsinogen A at Infinite Dilution.[a]

Time (min)	$Dt \times 10^2$ (cm^2)	Diff in $M^*(t)$ (%)
59.8	0.34	+2.6
63.8	0.36	+2.5
67.8	0.39	+4.0
71.8	0.41	+4.5
79.8	0.45	+8.6

[a] For a solution with $c^0 = 10$ mg/ml at 20°. Values were computed as in Table V; ϵ of meniscus = 0.022 with speed = 27,690 rpm.

While this change may be reflected in the difference between the gradient patterns of Figure 7, the present investigator's presumption, before the data were evaluated, was that gradients for the interval 72–80 rather than 60–64 min more properly reflected a horizontal gradient near the meniscus. Obviously, this presumption was incorrect as demonstrated by the results in the table. It is true that this effect was predicted by the theoretical data of the preceding communication, but it was not anticipated that loss of clarity in the pattern near the meniscus would lead to errors as large as 8%. The explanation for this appears to be that the gradient curve is symmetrical and falls considerably after reaching its maximum position, rather than plateauing horizontally toward the meniscus.

Discussion

The results show that linear gradients are found for the predicted conditions. The most favorable conditions for measuring molecular weights according to the Archibald method are low speeds ($\epsilon = 0.175$–0.300 for chymotrypsinogen A speed = 9000–7000 rpm) and moderate times ($Dt \geq 0.50 \times 10^{-2}$ cm^2 for chymotrypsinogen A $t \geq 85$ min). Due to the fact that the gradient is too small for routine work at $\epsilon = 0.271$ (7928 rpm) at the meniscus, values for ϵ should be restricted further so that more precise data may be obtained from well-resolved patterns at high schlieren bar angles; this limits ϵ to values near 0.175. Higher speeds, lower ϵ values, may be employed if one is willing to abandon measurements at the base.

Measurements at different speeds as described by Mueller (1964) will have to be carried out with great care, and interpretations of differences in molecular weight between the meniscus and base as reflecting heterogeneity of the sample should be done with equal caution. It is likely that differences of 5% are not significant; in fact, the data at high speeds with homogeneous chymotrypsinogen A show that this difference can grow to >15%. This effect at low ϵ explains why lower values for molecular weight may be observed at the base than at the meniscus. It probably is the explanation for results of this nature in the measurements of molecular weight of myosin by Mommaerts and Aldrich (1958) where, despite low speeds, ϵ values remained low enough to prevent an accurate extrapolation at the base. In the case of synthetic polymers, on the other hand, differences of 15% and greater are known to be caused by nonideality alone (Kegeles *et al.*, 1957).

The study of Mommaerts and Aldrich was done with interference optics, and it points up an advantage of interference optics over schlieren optics. The lateral position of the source slit, with respect to the optic axis, is not critical with interference optics, whereas it is critical when schlieren optics are used. The measurements reported here were made after careful positioning of the source on the axis. However, some measurements were made inadvertently with the slit 0.25 in. from the position of focus. Values from these data with gradients at low speeds agree well with those of Table V, but at speeds >9945 rpm, results for comparable experiments at the meniscus were in error 5–10% above errors in the table. This finding emphasizes that focusing of the slit on the axis as described by Trautman (1958) may not be neglected with schlieren optics. Since schlieren optics permit simple, direct analysis of experimental data, it may be more advantageous to expend effort in focusing schlieren optics than to differentiate interference data. This view is supported by the finding that measurements with the Archibald method by interference optics give results no more accurate, on the average, than schlieren optics (F. E. LaBar, unpublished data on sucrose; see also Richards and Schachman, 1959).

Finally, the extension of the method of linear extrapolation of $\partial c/\partial r$ *vs.* r to heterogeneous systems is apparent since the addition of one linear function to another will yield a linear function. Such linearity was observed with solutions of ribonuclease, known to contain dimer in addition to monomer; no change in linearity was observed when the dimer was removed

(F. E. LaBar, unpublished data). Thus, good results with heterogeneous systems are expected provided the speed is sufficiently low for the heaviest component. Additional studies are needed to demonstrate whether further limitations over those already set forth here for homogeneous systems must be imposed when this procedure is applied to heterogeneous systems.

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Chemical and Biological Properties of Reduced and Alkylated Staphylococcal Enterotoxin B*

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ABSTRACT: The single disulfide bridge in staphylococcal enterotoxin B has been found to be nonessential for the biological activity and conformation of the protein. Reduction of the disulfide bridge and alkylation of the resulting SH groups with both iodoacetamide and iodoacetate produced derivatives that had the same emetic activity and immunological properties as the native enterotoxin. Reduction and reoxidation of the disulfide bridge again did not produce any immunological or emetic changes. The physical properties of the alkylated enterotoxins and the reduced and oxidized enterotoxin, as measured by viscosity and sedimentation, remained essentially the same as that of the native enterotoxin. Unfolding in 6 M guanidine hydrochloride gave equivalent viscosities for the native and reduced carboxamidomethylated enterotoxins, and the derivative, after removal of the guanidine, regained full biological activity.

Staphylococcal enterotoxins are the substances elaborated by *Staphylococcus aureus* and responsible for emesis and diarrhea in food poisoning. Staphylococcal enterotoxin B has been prepared in a highly purified state by Schantz *et al.* (1965). It has a molecular weight of 35,300 and contains 1.04% sulfur, equivalent to 12 sulfur-containing amino acid residues per molecule. No free sulfhydryl was found either as *S*-carboxymethylcysteine or with titrations using *p*-mercuribenzoate. All the sulfur in the molecule is accounted for by ten methionine residues and two half-cystine residues forming one disulfide bridge per molecule (Spero *et al.*, 1965).

The present communication is concerned with the reduction of the disulfide bridge and the alkylation of the resulting sulfhydryl groups with iodoacetamide and iodoacetate. It also includes studies on the reoxidation of the sulfhydryl groups of reduced enterotoxin. The physical properties and immunological and emetic

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