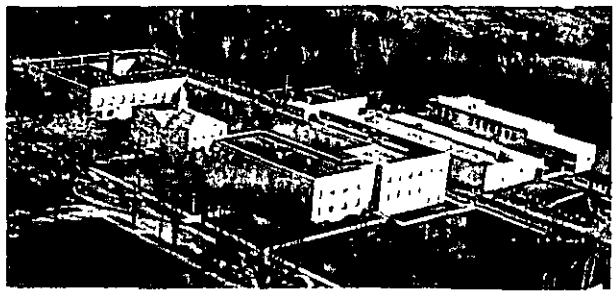

THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

THE MOLECULAR PROPERTIES OF NATURALLY OCCURRING POLYSACCHARIDES

The Effect of Ion Binding on the Molecular Properties of Low Molecular Weight Polysaccharides

Project 2236

Report Seven

Final Report

to

THE PIONEERING RESEARCH COMMITTEE
PIONEERING RESEARCH PROGRAM

January 28, 1963

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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SUMMARY

Diffusion experiments have been conducted in the Spinco Model E electrophoresis-diffusion apparatus using the Rayleigh diffusiometer. The diffusion coefficients of the cellodextrins from cellobiose through cellopentaose in water are reported. These results are combined with measurements taken from other sources to provide a picture of the molecular weight dependence of diffusion coefficients for the polysaccharides from glucose through cellohexaose.

The cellodextrins are examined in the light of the theoretical equations of Kirkwood and Riseman for the dependence of diffusion coefficients on molecular weight for rigid rod and random coil polymers. By fitting the theoretical diffusion equation for the rigid rod to glucose and cellobiose, the diffusion coefficient of cellohexaose may be predicted to within 14%.

The dependence of diffusion coefficients on molecular weight is also described by means of a semiempirical equation suggested by Longsworth. The results of the diffusion of cellobiose in the presence of aqueous sodium chloride are used in conjunction with this empirical relation to estimate the apparent molecular weight of cellobiose in this solution. The apparent molecular weight is then used in combination with a theoretical expression for the sedimentation equilibrium of a solute in the presence of supporting electrolyte. From this analysis the binding coefficient for cellobiose in the presence of a sodium chloride supporting electrolyte has been calculated.

An extensive analysis of Rayleigh fringe patterns has been conducted. From this analysis the concentration dependence of the diffusion coefficient of cellobiose has been determined. The results of fringe analysis on cellotriose and higher members of the cellodextrin series indicate that the samples are polymolecular. This polymolecularity is thought to be the result of progressive bacterial decomposition of the cellodextrins in water. The degree of polymolecularity is low and fortunately does not complicate the conclusions drawn concerning the molecular weight dependence of diffusion coefficients, partial specific volumes, and ion binding effects. Fringe analysis is thought to provide an excellent means of detecting impurities and may rival chromatographic techniques in sensitivity.

The effect of molecular weight on the partial specific volume of cellodextrins in water has been examined. A decrease in partial specific volume is observed as one proceeds from glucose to cellotriose. This has been attributed to the replacement of intermolecular distances with carbon-oxygen bond distances. A simple quantitative theory has been devised to account for this decrease. The partial molar volume of a given member of the series is taken to be the sum of the partial molar volumes of the anhydroglucose units plus an additional partial molar volume associated with the ends of the molecule.

Above cellotriaose the partial specific volume increases with increasing molecular weight. This is attributed to increased volume requirements of the higher homologues resulting from co-operative motion between anhydroglucose units. At high molecular weights partial specific volumes are known to become independent of molecular weight. This fact has been used to estimate the portion of the partial specific volume increase resulting from co-operative motion between molecular segments. This has been treated on a simple theoretical basis and is given quantitative

expression. Using partially methylated cellulose as a basis, the extra partial molar volume associated with the co-operative motion of a polymer segment in the cellulose molecule was estimated.

Finally, diffusion coefficients obtained by three different experimental techniques were compared. Diffusion measurements taken in the Spinco Model E electrophoresis-diffusion instrument employing the Rayleigh diffusiometer are the most accurate. Measurements conducted in the Spinco Model H ultracentrifuge using the transient state analysis of Van Holde and Baldwin are less accurate. Diffusion experiments conducted in the ultracentrifuge using synthetic boundary cells in conjunction with the Rayleigh optical system are at present the least accurate of the techniques.

INTRODUCTION

Since this is the final report on Project 2236 it might be well to review some of the general problems in the physical chemistry of polysaccharides and to place the present work in proper perspective. Organic and biochemical studies allow us to determine the chemical behavior, configuration, and the chemical structure of the sugar units comprising the polysaccharide. A large amount of work of this nature is available on a large number of naturally occurring polysaccharides. We have at present, however, a scarcity of information concerning the physicochemical nature of these substances. Specifically, we lack information on the size, shape, and hydrodynamic properties of the polysaccharides. This is particularly true of the hemicelluloses, and the plant hydrocolloids. The long-range goal of our research work in this area is, therefore, to help fill the scientific void in this area.

The size, shape, and hydrodynamic properties of polysaccharides are most intimately related to the frictional resistance that the polymer offers to the flow of solvent. It is for this reason that the frictional properties of high polymers in solution have received considerable theoretical and experimental attention over the years. The transport properties of diffusion, sedimentation in a gravitational field, and viscosity of polymers in solution are controlled primarily by the friction coefficient of the molecule which, in turn, is dependent on the molecular size and conformation of the polymer chain. It is not surprising, therefore, that studies of polymeric transport have been given considerable attention.

The importance of the friction coefficient is readily demonstrated by considering the well-known Svedberg equation for the determination of molecular

weights through the use of the ultracentrifuge. The sedimentation velocity of a molecule in a gravitational field may be expressed by the sedimentation coefficient given by (1, 2)

$$S_o = \frac{M(1 - \bar{v}\rho)}{N_a f_o} \qquad (1)$$

where

S_o is the sedimentation coefficient at infinite dilution

$\bar{v}$ is the partial specific volume of the polymer molecule

ρ is the density of the solvent

N_a is Avogadro's number

f_o is the friction coefficient of the molecule at infinite dilution.

The diffusional transport of the same polymer molecule may be given by the well-known formula of Einstein (3)

$$D_o = \frac{kT}{f_o} \qquad (2)$$

where k is Boltzmann's constant, and T is the absolute temperature. Upon elimination of the friction coefficient from Equations (1) and (2), the Svedberg equation (4) for the determination of molecular weights is obtained; that is,

$$M = \frac{S_o RT}{D_o(1 - \bar{v}\rho)} \qquad (3).$$

This equation is strictly valid only at infinite dilution and applies only to simple two-component systems consisting of solvent and solute. It is apparent from a comparison of Equations (1), (2), and (3) that the friction coefficient and molecular weight are closely related.

As long as one is restricted to two-component systems, the transport properties of diffusion and sedimentation yield information about the polymer molecule which can be readily interpreted. In many polysaccharide systems, notably the hemicelluloses, one must use solvents that contain supporting electrolytes. Certain hemicelluloses are only soluble in water in the presence of strong caustic or in the presence of complexing agents. In such mixed solvents there is a tendency for the supporting electrolyte (e.g., sodium hydroxide) to associate with the polymer molecule in such a manner that there is some doubt as to whether or not one can obtain a reliable measure of molecular weight of a polymer under these conditions. The nature of this interaction is such that it is present even at infinite dilution so that one can only determine an apparent value for the molecular weight. Any determination of the frictional properties of polysaccharides in the presence of supporting electrolytes will also have to include consideration of these ion-binding effects. Until the binding phenomena and its relation to frictional properties are understood, studies in mixed solvents will always yield information of uncertain value. It was with these facts in mind that the present study on the effects of ion binding on the frictional and molecular properties of polysaccharides was initiated.

In our original proposal for research on the molecular properties of naturally occurring polysaccharides, we planned to study the homologous series of oligosaccharide alditols. These were to have been prepared from the corresponding cellodextrin series. Since the preparation of the alditols would have resulted in the destruction of our entire cellodextrin starting material it was felt that the wise procedure would be to examine the cellodextrin series first and then proceed to the alditol series. It should be pointed out that the preparation of the cellodextrins is a laborious and time-consuming procedure and hence these polysaccharides are extremely valuable.

There are at present no studies on the molecular and frictional properties of the cellodextrins and certainly no knowledge of the behavior of these oligosaccharides in the presence of a supporting electrolyte. For this reason most of our research has been devoted to an examination of the cellodextrins in water and in water in the presence of sodium chloride as a supporting electrolyte.

This report contains the results of partial specific volume measurements and diffusion experiments on the cellodextrins from cellobiose through cellopentaose. These results have been supplemented with measurements on glucose and cellohexaose obtained from other sources. Certain generalizations can be drawn from the results of these measurements which should apply to low molecular weight polysaccharides.

The molecular weight dependence of partial specific volume has certain trends that may make it possible to predict the partial specific volumes of simple linear polysaccharides. A minimum has been observed in the curve of partial specific volume versus degree of polymerization, and a theory has been devised to explain this on a semiquantitative basis.

Diffusion coefficients have been examined as a function of molecular weight and the results interpreted in the light of theoretical equations for diffusion. The results of the molecular weight dependence of diffusion coefficients in water have been used in conjunction with diffusion coefficients obtained in the presence of supporting electrolytes to obtain an estimate of the ion-binding coefficient necessary for the interpretation of ultracentrifuge experiments.

An analysis of skewness of Rayleigh fringes from diffusion experiments has been presented and the results have been used to determine the concentration

dependence of diffusion coefficients for cellobiose and to test the purity of the remaining cellodextrins.

During the last report period we had intended to carry out sedimentation velocity experiments in the ultracentrifuge. Four sedimentation experiments were conducted in double sector synthetic boundary cells using the Rayleigh interference optical system. No accurate sedimentation coefficients could be obtained because of the rapid diffusion of the boundaries compared to the rate of sedimentation. Apparently, future sedimentation experiments will have to be conducted in single sector synthetic boundary cells using the schlieren optical system and maximum allowable gravitational fields. Budget considerations did not allow us to repeat this phase of the research work.

THEORETICAL BACKGROUND

THERMODYNAMICS OF IRREVERSIBLE PROCESSES

According to the thermodynamics of irreversible processes, (5, 6) diffusion in a multicomponent system can be represented in terms of fluxes $\vec{J}_i$ and generalized forces or affinities $\vec{X}_k$. The $\vec{J}_i$ represent the flow of solute species i passing one square centimeter of a plane perpendicular to the direction of flow. The units of $\vec{J}_i$ are g./sq. cm. sec. The plane to which $\vec{J}_i$ is referred is called the frame of reference. The symbol $\vec{J}'_i$ will be used for the diffusion flux referred to the local center of mass.

For systems not too far from equilibrium, the fluxes are postulated to be linear functions of the generalized forces. This can be expressed in the general form,

$$\vec{J}'_i = \sum_k L_{ik} \vec{X}_k \qquad (4)$$

where the L_{ik} are phenomenological coefficients. The coefficients L_{ii} represent direct effects and the L_{ik} represent coupled effects. If a "proper choice" of fluxes and affinities has been made the phenomenological coefficients will be symmetric, i.e.,

$$L_{ik} = L_{ki} \qquad (5)$$

This fundamental theorem is due to Onsager (7, 8).

The generalized forces are chosen so that,

$$T\theta = \sum_i \vec{J}'_i \cdot \vec{X}_i \qquad (6)$$

where θ is the rate of entropy production and the dot indicates the scalar product of the vectors. For a diffusing species the generalized force is the negative gradient of the chemical potential (4),

$$\vec{X}_i = -\nabla\mu_i. \tag{7}$$

Whenever the diffusion of solute species is considered, one must pay careful attention to the frame of reference. Whenever there is a volume change on mixing there will be bulk flow of the solute species in addition to diffusional transport. For the present we will only consider relations between the reference frame fixed at the local center of mass and the reference frame fixed on the diffusion cell. If $\vec{V}$ is the velocity of the mass fixed reference frame with respect to the cell fixed reference frame, then

$$\vec{J}_i' = \vec{J}_i - C_i\vec{V} \tag{8}$$

where $\vec{J}_i'$ and $\vec{J}_i$ have been defined previously.

In the work reported herein we are interested in the diffusion of a simple sugar in water. For such a simple two-component system, the diffusion flux becomes,

$$\vec{J}_1' = -L_{11}\nabla\mu_1 \tag{9}$$

According to Fick's first law (4)

$$\vec{J}_1 = -D_1\nabla C_1 \tag{10}$$

If there is no volume change on mixing, Equation (8) can be written as (4),

$$\vec{J}_1' = \vec{J}_1/\bar{v}_o\rho \tag{11}$$

where $\bar{v}_o$ is the partial specific volume of the solvent and ρ is the density of the solution. Upon substituting Equation (11) into Equation (10) and comparing the result with Equation (9) we find that,

$$D_1 = L_{11} \left(\partial \mu_1 / \partial C_1\right)_{P,T} \bar{v}_o \rho \tag{12}$$

The chemical potential can be written as,

$$\mu = \mu^o + RT \ln yC \tag{13}$$

where μ^o is the chemical potential in the standard state, μ is the chemical potential and y is the activity coefficient on the concentration scale. Upon taking the partial derivative of the chemical potential with respect to concentration and substituting into Equation (12) one obtains,

$$D = \frac{kT}{f}\left[1 + C\frac{\partial \ln y}{\partial C}\right]_{T,P} \bar{v}_o \rho \tag{14}$$

where the quantity L/C has been replaced with the friction coefficient f. The subscript 1 has been dropped since we are dealing with a single solute species.

Tests of Equation (14) have proven difficult because of the difficulty in determining the concentration dependence of the friction coefficient. A simplifying assumption that can be made is that f is proportional to the viscosity of the solution so that,

$$f/f^o = \eta/\eta_o \tag{15}$$

where η is the viscosity of the solution and η_o is the viscosity of the solvent. When Equation (15) is substituted into Equation (14) one finds that,

$$D = D_o\left[1 + C\,\frac{\partial \ln y}{\partial C}\right]_{T,P} \bar{v}_o \rho \left[\frac{\eta}{\eta_o}\right] \tag{16}$$

where D_o is given by Equation (2) which is Einstein's result,

$$D_o = kT/f_o \qquad (2)$$

Agreement of Equation (16) with experimental results has been very good for aqueous solutions of glycolamide (9), α-alanine (10) and urea (11) to concentrations as high as 0.5 molar. Fair agreement has been observed for aqueous sucrose (12) up to concentrations of 0.2 molar. Poor agreement is found for n-butanol in water (13).

MOLECULAR THEORY OF DIFFUSION

At the present time we have not completed our survey on the molecular theories of diffusion; however, it seems profitable to give a discussion in terms of what we have found thus far. Riseman and Kirkwood (14) have discussed the use of the statistical mechanics of irreversible processes in connection with the Brownian motion and hydrodynamic properties of polymer molecules.

The general theoretical treatment is based on a polymer molecule composed of $2n + 1$ identical structural elements attached to a rigid or flexible framework consisting of reference frames numbered from $-n$ to $+n$. There are $2n$ bond vectors $\vec{b}_\ell$ of magnitude b directed from element $\ell - 1$ to $\ell \geq 1$ and from elements ℓ to $\ell + 1$ for $\ell \leq -1$. A friction coefficient ζ is associated with each structural element and will depend on the nature of the fluid and the structure of the element.

The theory results in the following expression for the translational diffusion coefficient of a rigid rod;

$$D = (kT/\zeta z)[1 + 2\lambda \ln (z-1)] \qquad (17)$$

where $\underline{z} = 2\underline{n}$ and $\lambda = \zeta/(6\pi\eta_o\underline{b})$,

with,

ζ = the friction coefficient of the structural unit

η_o = the viscosity of the solvent

$\underline{b}$ = the magnitude of the bond vector

$\underline{n}$ = an integer as discussed above

The corresponding case of a random coil polymer has also been solved and the translational diffusional coefficient is given by,

$$D = \frac{kT}{z\zeta}\left(1 + \frac{8}{3}\lambda_o z^{1/2}\right) \qquad (18)$$

where $\lambda_o = \zeta/[(6\pi^3)^{1/2}\eta_o\underline{b}]$.

Our use of the results of Kirkwood and co-workers (14) will be limited to a test of translational diffusion equations; however, it should be pointed out that theoretical expressions are available for the rotary diffusion coefficients of rodlike and random coil polymers. In addition, theoretical expressions are available for the intrinsic viscosity, real and imaginary components of the complex shear modulus and complex intrinsic viscosity of solutions containing rigid rod and random coil molecules. All of these quantities are related through the friction coefficient of the structural element, ζ.

EXPERIMENTAL MATERIALS AND PROCEDURES

MATERIALS

The cellobiose used in diffusion experiments was obtained from Eastman Organic Chemicals. The rest of the cellodextrins used in the present work were made available to us through the courtesy of Dr. N. S. Thompson and Mr. W. Bliesner.

The cellodextrins prepared by Mr. Bliesner were obtained by chromatographic separation. The separation was accomplished on a column containing an adsorbant composed of a 50-50 mixture of charcoal (Darco G-60) and Cellite (Cellite 545). The adsorbant was pretreated with stearic acid-ethanol solutions. The adsorbant and the use of a stearic acid-pretreatment is similar to that reported elsewhere (16, 17).

Whatman cellulose powder was hydrolyzed with fuming hydrochloric acid according to the method of Jermyn (15). The solution resulting from the acid hydrolysis was neutralized with sodium bicarbonate and filtered to remove salts and undissolved materials. The resulting filtrate containing the cellodextrins was added to the column and washed with distilled salts and glucose. The cellodextrins were then eluted with a solution consisting of 40% ethanol by volume. The individual members of the cellodextrin series were then purified by the procedure described by Wolfram and Dacrons (18).

At one point in the preparation, the samples were inadvertently subjected to bacterial attack. The solutions were heated to destroy the bacteria and since all sugars were recrystallized to constant melting or decomposition points they were still considered to be suitable for the present work.

Several sources of water were used during the course of this work. Partial specific volume measurements were conducted in distilled water obtained from an ordinary Barnstead laboratory still. The water used in diffusion experiments on cellobiose was distilled water obtained from the distillation of deionized water containing alkaline potassium permanganate.

In diffusion measurements on cellotriaose and all higher members of the cellodextrin series the water was prepared as described by Bauer (19) for density measurements with 6 place accuracy.

The water of highest purity was stored in polyethylene bottles which had been leached for a total of six weeks in several changes of water distilled from alkaline permanganate and including at least three changes of distilled water of the highest purity (prepared as described by Bauer).

Solutions in all density and diffusion experiments were prepared in calibrated volumetric flasks and were used directly from these flasks. The amount of solute and the amount of solvent added to each flask were determined by weight.

In studying the effect of salt on the diffusion of cellobiose in water, Baker analyzed reagent-grade sodium chloride was used.

PARTIAL SPECIFIC VOLUME

The determination of partial specific volume must be done with considerable care. This is particularly important in the ultracentrifugal determination of molecular weights where the accuracy of the partial specific volume measurement determines the accuracy of the molecular weight. For example, an error in the determination of partial specific volume will cause an error three times as large in the molecular weight.

Care must be exercised in the choice of the pycnometer used in the specific volume determination. In general, pycnometers of the Gay-Lussac type are unsuitable for accurate density work. Those of the Sprengel-Ostwald type are preferable. A Sprengel bottle (20) was used exclusively in the present experiments. The bottle has a volume of 20.5 ml. and an additional 5 ml. are required for proper filling.

The procedures required for accurate density determination have been described by Bauer and Lewin (20) and by Kraemer (21). The reader is referred to these authors for a detailed discussion. Only a brief discussion will be given here.

The Sprengel bottle was filled by attaching 7/27 female ground-glass joints to the stems and then drawing solution into the bottle. The bottle was then immersed in a constant temperature bath at 30.000 $\pm$ 0.005°C. The height of the liquid in the capillaries was adjusted by passing braided nylon fishline down one capillary to the meniscus and thereby removing a portion of the liquid. The capillary was subsequently dried in the same manner. The volume of the dilatometer was determined by using distilled water and a value of 0.995646 g./cc. for the density of water at 30.00°C. A typical calibration curve is shown in Fig. 1 where the volume of the dilatometer is plotted as a function of the sum of the liquid levels in the two capillaries.

The partial specific volume of the polymer is related to the corresponding apparent property by the relation,

$$\bar{v} = \phi_v + C \frac{\partial \phi_v}{\partial C} \qquad (19)$$

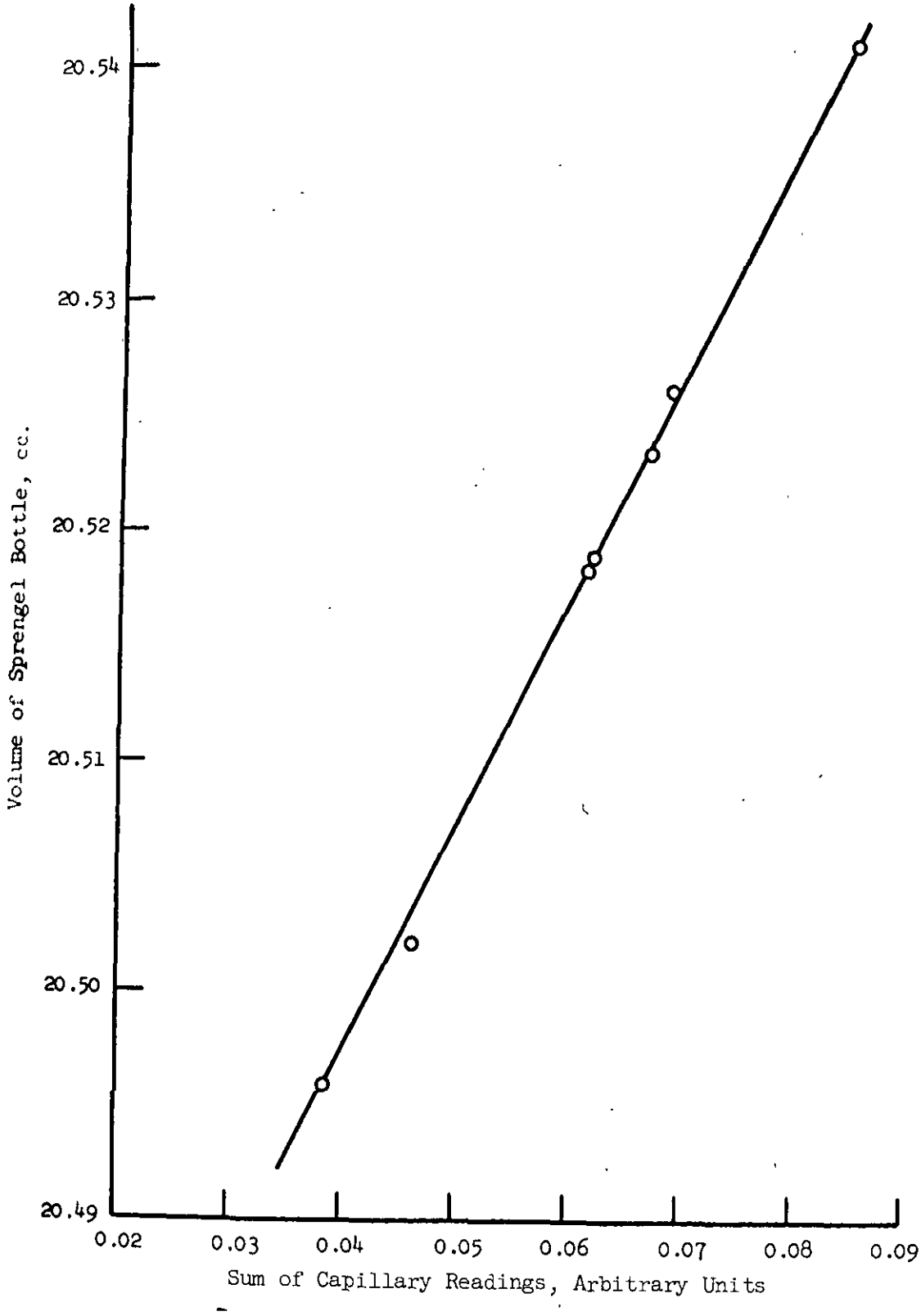

Figure 1. Calibration Curve for the Sprengel Bottle

where

$\bar{v}$ is the partial specific volume of the polymer in ml./g.

ϕ_v is the apparent specific volume in ml./g.

C is the polymer concentration in g./ml.

The apparent specific volume is the quantity computed directly from experimental measurements and is given by

$$\phi_v = \frac{1}{\rho_o} - \frac{1}{C}\frac{\rho - \rho_o}{\rho_o} \tag{20}$$

or

$$\phi_v = v\left[\frac{1}{m_o} - \frac{100}{P}\left(\frac{1}{m_o} - \frac{1}{m}\right)\right] \tag{21}$$

where

ρ_o is the density of the solvent in g./ml.

ρ is the density of the solution in g./ml.

m_o is the mass of solvent in the pycnometer in g.

m is the mass of solution in the pycnometer in g.

P is the per cent of polymer by weight

v is the volume of the pycnometer in ml.

In the present work it is assumed that the last term in Equation (19) is negligible and, therefore, the partial specific volume and apparent specific volume are identical. In actual practice the apparent specific volume is only slightly concentration-dependent at concentrations less than 1 g./100 ml. For very accurate work the measurements should be carried out at several concentrations. Problems of accuracy and the labor involved prevented determinations at more than a single concentration.

In accurate dilatometric work it is necessary to correct for buoyancy effects in weighing. The ratio of the true weight to the apparent weight is given by,

$$1 + \rho_a (v_i - v_w) = 1 + R_i \qquad (22)$$

where

ρ_a is the density of air, in g./ml.

$\underline{v_i}$ is the specific volume of the substance being weighed in ml./g.

$\underline{v_w}$ is the specific volume of the weights in ml./g.

For the present work, the difference in $\underline{R}$ values for the weights $\underline{m}$ and $\underline{m_o}$ did not exceed the permissible error in $\underline{v}$ and the correction was not applied. All weighings were carried out to 0.1 mg., however, and the same side of the balance arm was used in all weighings. The weights were uncalibrated but the same weights were used in all determinations.

THEORY OF FREE DIFFUSION

Free diffusion techniques were employed in the present experimental program. A brief description of these will be given here. For a more complete discussion the reader is referred to the excellent review article of Gosting (4).

The diffusion experiments were conducted in cells of the Tiselius type. In free diffusion the experiments are conducted in a vertical rectangular cell. A side view of such a cell is shown in Fig. 2. The bottom of the cell is filled with a solution at a solute concentration of $\underline{C_2}$. The top of the cell is filled with a less concentrated solution $\underline{C_1}$ or as in the case of experiments in the present work with pure solvent. The density of Solution 2 is always greater than Solution 1 so as to avoid convective mixing of the solutions.

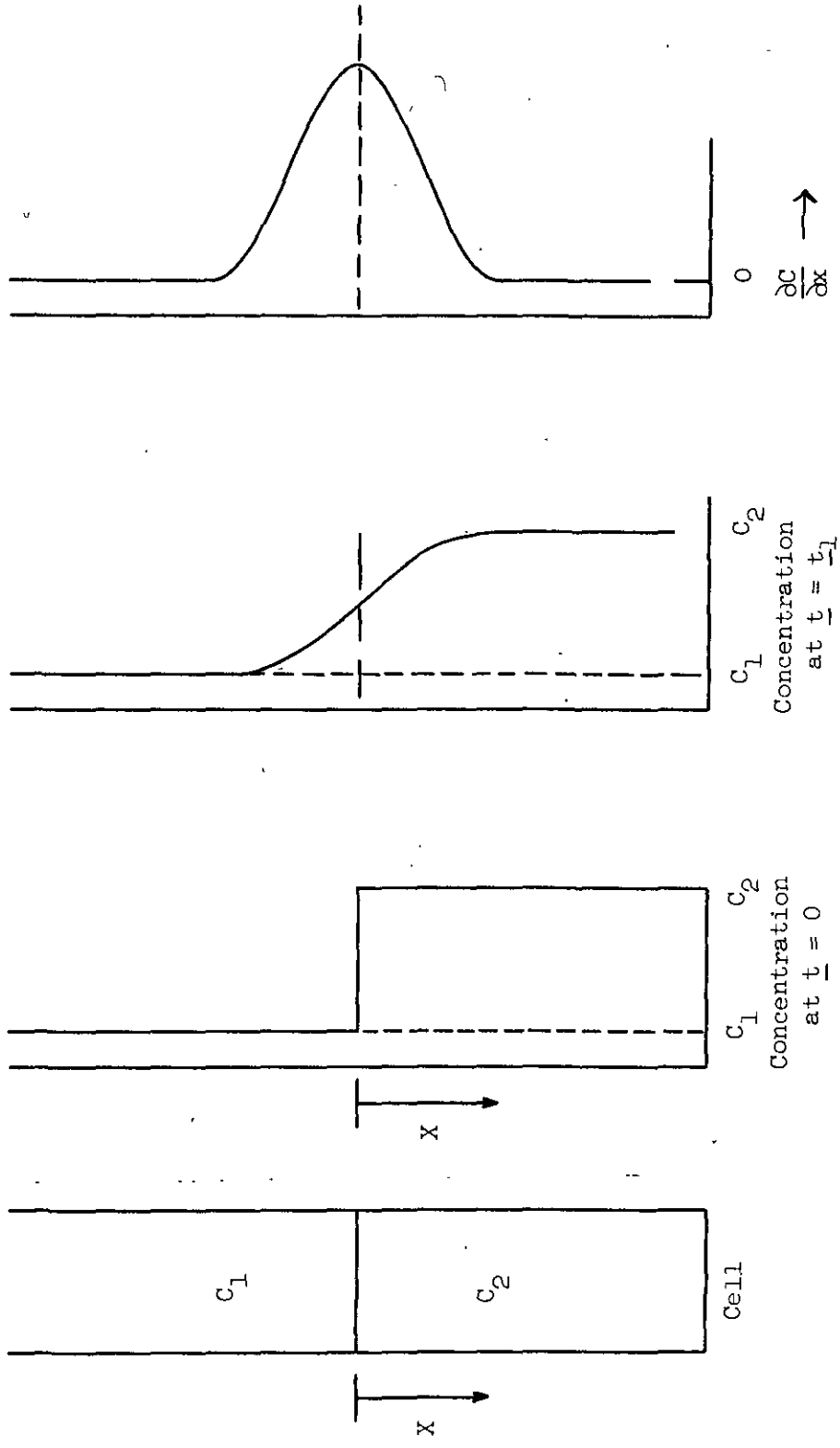

Figure 2. The Free Diffusion Experiment

At the start of the experiment a sharp boundary is formed at the interface between the solutions. This is the condition existing at the start of the experiment when $\underline{t} = 0$. At some later time when $\underline{t} = \underline{t}_1$ the concentration within the cell will be represented by the sigmoidal curve in Fig. 2. The concentration gradient curve is also shown. In free diffusion the concentrations at the ends of the cell are not permitted to vary.

If the diffusion coefficient is independent of concentration the course of diffusion may be described by Fick's second law,

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \tag{23}$$

This second-order partial differential equation is readily solved for the case of free diffusion. Integration of Equation (23) results in the following equation for concentration;

$$C = \bar{C} + \frac{\Delta C}{2}(2/\pi^{1/2}) \int_0^{x/[2(Dt)^{1/2}]} [\exp(-\beta^2)]\, d\beta \tag{24}$$

and for the concentration gradient,

$$\frac{\partial C}{\partial x} = [\Delta C/2(\pi Dt)^{1/2}] \exp[-x^2/4Dt] \tag{25}$$

where $\underline{\bar{C}}$ is the mean solute concentration, $\underline{\bar{C}} = (\underline{C}_1 + \underline{C}_2)/2$ and $\Delta\underline{C}$ is the initial concentration difference across the boundary given by $\Delta\underline{C} = \underline{C}_2 - \underline{C}_1$.

In our measurement of diffusion coefficients we employed the Rayleigh diffusiometer of the Spinco Model H electrophoresis-diffusion apparatus. A simplified diagram of a typical Rayleigh diffusiometer is shown in Fig. 3. The diagram represents a top view of the instrument. The viewer is looking down into the diffusion cell.

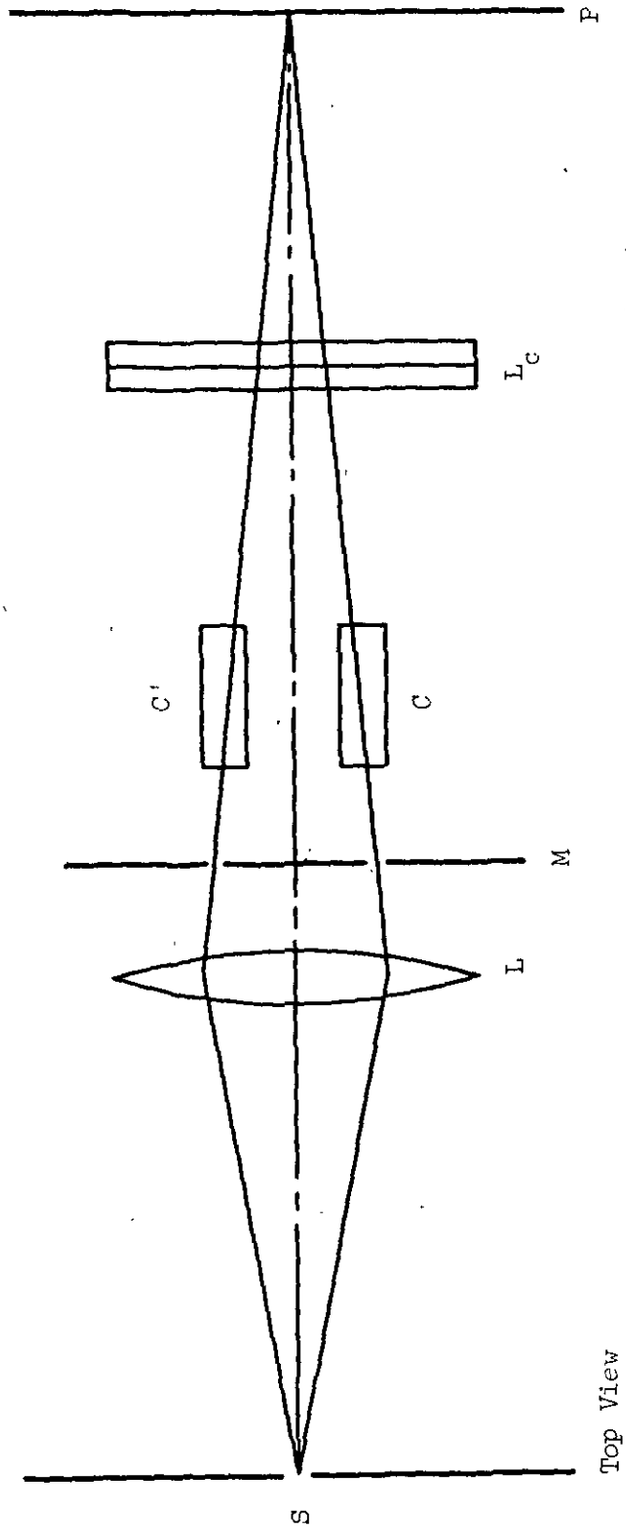

Figure 3. Diagram of the Rayleigh Diffusiometer

Monochromatic light from a point source S is focused by a long focal length lens L onto a photographic plate P. Two vertical slots M allow one band of light to pass through diffusion cell C and the other to pass through an identical reference cell C' filled with solvent. In the interferometer used in this laboratory the reference cell C' is not present as a separate entity. The windows of cell C have been extended beyond the edge of the cell. The extended windows and the water in the thermostat form the reference path. The cylinder lens L_c focuses each level of the cell to a corresponding level at the photographic plate P so that rays deflected by the concentration gradient are brought back into the proper level to interfere with rays from the reference path.

The resulting fringe patterns are shown in Fig. 4. The patterns are in a horizontal position as they would be observed at the photographic plate in our instrument. The left-hand side of the fringe pattern represents the top of the cell and the right the bottom. The corresponding concentration curve is shown below the fringe pattern.

The total number of fringes crossing the horizontal field is given by,

$$J = \frac{a\ \Delta n}{\lambda} \qquad (26)$$

where J is the total number of fringes, a is the cell thickness along the light path, Δn is the refractive index difference across the initial boundary and λ is the wavelength of the monochromatic light. If the refractive index is a linear function of concentration, each fringe represents a change in concentration given by $(C_2 - C_1)/J$. A linear dependence of refractive index on concentration is often assumed although corrections for nonlinearity are not difficult to make.

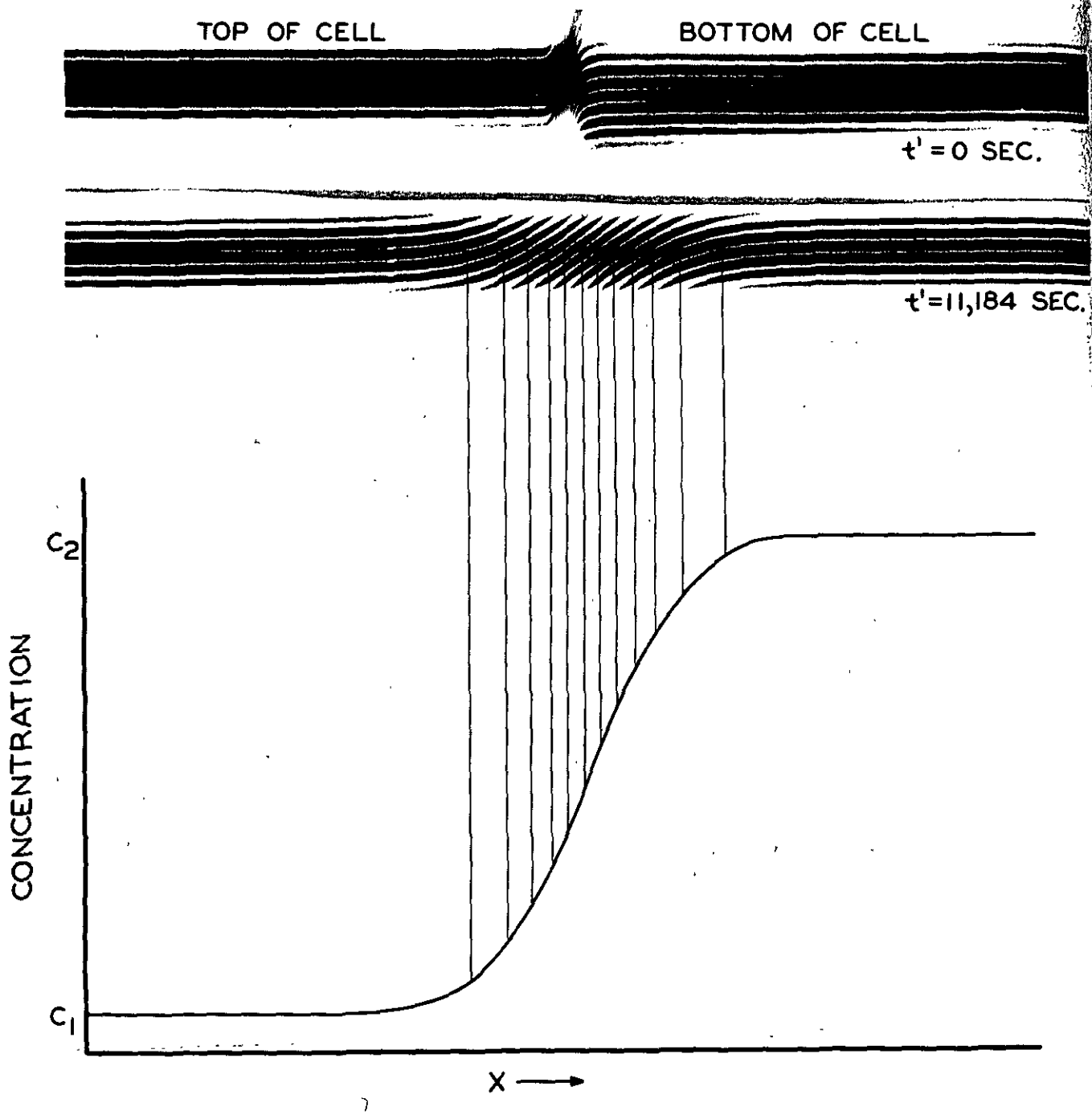

Figure 4. Typical Rayleigh Fringe Patterns
DB-20; Photos No. 1-10; $J = 12.52$; $\Delta t = 213.9$ sec.

The two basic measurements needed for analysis of Rayleigh fringes are the total number of fringes J and the horizontal location x_j at which each fringe j crosses the horizontal line of sight. A set of reduced cell co-ordinates, Z^*, are defined by,

$$\frac{2j - J}{J} = \Phi(Z^*) \tag{27}$$

where $\Phi(Z^*)$ is the Gaussian error function given by

$$\Phi(Z^*) = \frac{2}{(\pi)^{1/2}} \int_0^{Z^*} [\exp(-\beta^2)]\, d\beta \tag{28}$$

Extensive tables of this function are available (22). For a two-component system in which the refractive index is a linear function of concentration and in which D is independent of concentration, Z^* reduces to $x/2(Dt)^{1/2}$. The diffusion coefficient can then be computed from the quantity,

$$\frac{x_{J-j} - x_j}{2MZ^*} = 2(Dt)^{1/2} \tag{29}$$

where M is the magnification factor for the Rayleigh diffusiometer. The diffusion coefficient is then determined by averaging among a number of different fringe pairs.

PROCEDURE FOR DIFFUSION EXPERIMENTS

Diffusion experiments were performed in a Spinco Model H Electrophoresis-Diffusion instrument. The actual diffusion was allowed to take place in Tiselius cells. Two sizes of cells were used; a 2-ml. micro cell and a 11-ml. standard cell. Since the diffusing boundary must be sharpened at the start of the experiment by withdrawing solvent and solution from the diffusion boundary, the total amount of

solution required for the operation of either cell is the same; approximately 25 ml. in either case. The main difference between the cells lies in the cell thickness along the optical path. The micro cell has the shorter cell thickness and will according to Equation (26) have a fewer number of total Rayleigh fringes, other factors being equal.

The cells were filled using a layering technique as described by Gosting (4). The bottom half of the U-shaped Tiselius cell is filled with solution and the center section of the cell is isolated. Solvent is layered on top of the solution in one limb of the cell. The other limb is filled with solution. The cell channels are then brought into alignment and a long stainless steel needle is lowered into the system to the point at which the boundary is desired. Fluid is withdrawn from the system by means of an automatic sampling system and as a result of the density difference between solution and solvent a sharp boundary will be formed just below the tip of the needle.

When a sharp boundary is formed the sharpening procedure is stopped and the needle is withdrawn. The time is noted and three photographs were taken as soon as possible. Across any diffusing boundary there will be an integral number of fringes plus a certain fraction of a fringe. These early photographs were used to obtain fractional fringe data. It was generally not possible to see individual fringes crossing the boundary on these early photographs.

All fringe patterns were recorded on Eastman Type M photographic plates using 16-sec. exposures. The plates were developed in Eastman D-11 developer for 5 minutes.

The first photograph on which individual fringes could be noted was generally obtained after about 5 minutes of free diffusion, at which time the

fourth picture was taken. Three subsequent photographs were taken 15 minutes apart and thereafter photographs were taken at progressively increasing time intervals.

FRINGE MEASUREMENT AND CALCULATIONS

The determination of fringe fractions and fringe positions were, for the most part, carried out on a Gaertner traveling microscope although, in a few early experiments with cellobiose, the Wilder microprojector, Model C was used. The position of each fringe was determined several times and an average of at least two readings were used to determine the fringe position.

There are several methods of fringe pairing that may be employed in the computation of diffusion coefficients from Equation (29). In the present work we have employed the method of Longsworth (23, 24). The first fringe is paired with the first fringe past the center of the pattern, the second with the second past the center, etc. In this way diffusion coefficients are compared linearly across the boundary. By examining the time and concentration dependence of diffusion coefficients calculated in this manner we can obtain information on the concentration dependence of diffusion coefficients and also obtain information on the presence of impurities in the sample. This will be discussed in some detail in a later section of this report.

The computations for diffusion coefficients were carried out on the IBM 1620 computer. Two programs were used in the computations and these Fortran programs are given in Appendix I.

The first program calculates the average diffusion coefficient $\underline{D}_{\underline{A}}$ based on the reduced height-area ratio (4). This is computed from a knowledge of the experimentally observed times $\underline{t}'$, the fringe number $\underline{j}$, the total fringe number $\underline{J}$,

and the fringe locations x_{J-j} and x_j. The second computer program is used to correct for the finite thickness of the boundary at the time the needle is withdrawn. This correction is made on the following basis.

If it is assumed that the boundary was essentially Gaussian at the time sharpening was stopped, then one can show (25) that the following relation applies:

$$D'_A = D_A(1 + \Delta t/t') \qquad (30)$$

where t is the true diffusion time, t' is the experimentally observed time, and Δt is a small time increment that must be added to t' to obtain the true time t. The diffusion coefficient, D'_A, is the slightly erroneous diffusion coefficient based on the times t' and D_A is the proper corrected diffusion coefficient. A plot of D'_A versus $1/t'$ will extrapolate linearly to the observed D_A.

The second computer program is designed therefore to carry out this extrapolation by the method of least squares. The diffusion coefficient D'_A is represented as a function of t' according to Equation (30) and the resultant slope is used to calculate the time correction. This is then used in the first program and the computations are repeated. The entire procedure is repeated until the time correction is less than one second.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the present experimental investigation will be given in five sections. First, the results of our measurements on the partial specific volumes of the cellodextrins will be given and interpreted in the light of a theory which is developed. This will be followed by a discussion of the results of diffusion experiments. Following this, several methods of determining diffusion coefficients will be compared. Next, the usefulness of an analysis of fringe skewness will be discussed. Finally, the results of our experiments on cellobiose will be used to obtain the binding coefficient for cellobiose in aqueous sodium chloride solutions.

RESULTS OF PARTIAL SPECIFIC VOLUME MEASUREMENTS

The results of partial specific volume measurements on the cellodextrins, sucrose, raffinose, cycloheptaamylose, and methyl cellulose in water are given in Table I. The cellodextrins from cellobiose through cellopentaose were determined at 30.00°C. and are the results of the present research work. The sources of the results on the other compounds are indicated in the appropriate footnotes.

Some rather interesting observations can be made when the partial specific volumes are plotted as a function of the degree of polymerization, D.P. Such a plot is shown in Fig. 5. The partial specific volume is seen to drop from a value of 0.621 ml./g. for glucose to a minimum value of 0.6109 ml./g. at a D.P. of 3, for cellotriose. Above a D.P. of 3, the partial specific volume begins to rise to a value of 0.6279 ml./g. for cellopentaose at a D.P. of 5.

This rather strange behavior is readily explained on the following basis. The initial decrease in partial specific volume in going from glucose through the

TABLE I

PARTIAL SPECIFIC VOLUMES OF THE CELLODEXTRINS, SUCROSE, RAFFINOSE, AND CYCLOHEPTAAMYLOSE IN WATER

Compound	Molecular Weight	Concentration, moles/liter	Partial Specific Volume, $\bar{V}_s$, ml./g.	Partial Molar Volume, $\bar{V}_m$, ml./mole
Glucose[a]	180.16		0.621	111.9
Cellobiose[b]	342.30	0.03100	0.6148	210.4
Cellotriose[b]	504.45	0.01910	0.6109	308.2
Cellotriose[b]		0.00960_3	0.6110	308.2
Cellotetraose[b]	666.59	0.01497	0.6132	408.5
Cellopentaose[b]	828.73	0.00621_3	0.6279	520.3
Sucrose[c]	342.30		0.613	209.9
Raffinose[c]	594.52		0.6078	306.6
Cycloheptaamylose[d]			0.627	
Methyl cellulose[e] (D.P. about 355)			0.729	

[a]Reference (24), temperature of 25°C.

[b]Data from the present work, temperature of 30.00°C.

[c]Reference (12).

[d]Reference (26).

[e]Reference (27).

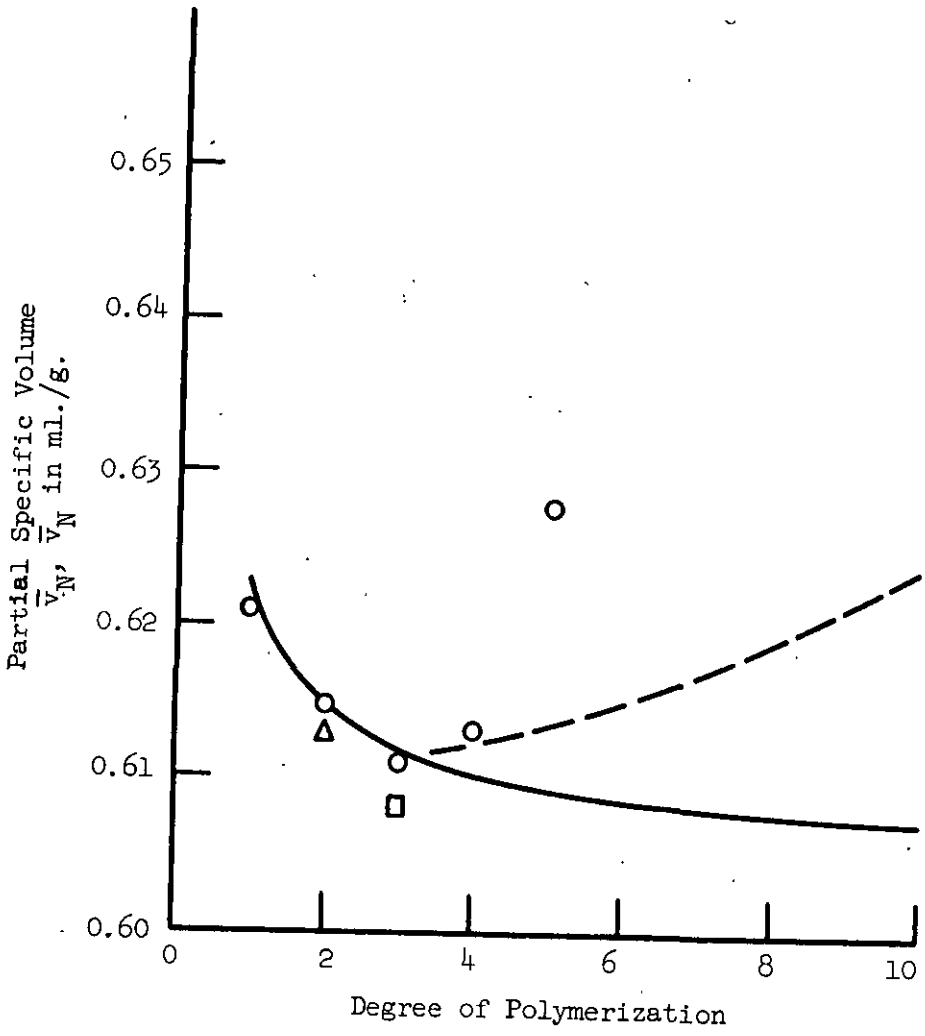

Figure 5. Partial Specific Volumes as a Function of Degree of Polymerization. Open Circles Represent the Cellodextrins; Triangle, Sucrose; and Square, Raffinose

disaccharides of sucrose and cellobiose to the trisaccharides of raffinose and cellotriose is due primarily to the replacement of intermolecular distances with the shorter carbon-oxygen atomic bond distances. The increase in partial specific volume above a D.P. of 3 is due to the increased space requirements of the higher members of the series. This increased space requirement has its origin in the co-operative motion (28) of the structural unit of the molecule.

With this qualitative reasoning in mind it is interesting to attempt a theoretical interpretation of the experimental results. Assume that the partial molar volume is made up of two additive parts. The first consists of the volume associated with the anhydroglucose units and the second is the volume associated with the ends of the molecule. The partial molar volume $\bar{V}_N$ for the cellodextrins can be represented by,

$$\bar{V}_N = N\bar{V}_a + 2\bar{V}_e \qquad (31)$$

where N is the number of anhydroglucose units in the molecule, $\bar{V}_a$ is the partial molar volume associated with an anhydroglucose unit and $\bar{V}_e$ is the partial molar volume associated with the end of the molecule. The terms $\bar{V}_a$ and $\bar{V}_e$ can be evaluated from the first three members of the cellodextrin series. The partial molar volumes of glucose and cellobiose are known and one has two equations in two unknowns from which one observes that $\bar{V}_a$ = 98.5 ml./mole and $\bar{V}_e$ = 6.7 ml./mole. From the cellobiose and cellotriose sugars one finds $\bar{V}_a$ = 97.8 ml./mole and $\bar{V}_e$ = 7.4 ml./mole. The agreement between the values obtained from the three sugars is extremely good and one is justified in using average values of $\bar{V}_a$ = 98.15 ml./mole and $\bar{V}_e$ = 7.05 ml./mole.

The partial specific volume is given by,

$$\bar{v}_N = \frac{N\bar{V}_a + 2\bar{V}_e}{NM_a + M_H + M_{OH}} \qquad (32)$$

where M_a is the molecular weight of an anhydroglucose unit, M_H is the atomic weight of hydrogen and M_{OH} is the molecular weight of the OH-group. The partial specific volumes of the cellodextrins are, therefore, given by

$$\bar{v}_N = \frac{N(98.15) + 2(7.05)}{N(162.14) + 18.02} \qquad (33)$$

A test of Equation (33) is shown in Fig. 5 where the results of this analysis are represented by the solid line for the first ten members of the cellodextrin series. The equation represents the first three members of the series quantitatively as expected and then fails on the higher members. It should, however, be pointed out that the partial specific volume calculated for cellopentaose by means of this formula is still within 3% of the experimental value.

It is apparent that an additional term must be added to the total molar and partial specific volumes if these quantities are to be treated on a quantitative basis. This added volume is believed to have its origin in the added volume required for the co-operative motion of the anhydroglucose units. For example, for a given motion of the end of the polymer molecule to take place, the remaining units must move in a restricted manner. This will cause the members to sweep out larger volumes than if they were free to move independently. If this additional volume is swept out faster than it is replaced by solvent molecules the partial molar and molar volumes will increase.

For the purpose of general discussion we will designate this additional partial molar volume by the term $\bar{V}_c$ where the subscript c refers to co-operative

motion of the anhydroglucose units. The partial specific volume can then be expressed in the general form,

$$\bar{v}_N = \frac{N\bar{V}_a + 2\bar{V}_e + \bar{V}_c}{NM_a + M_H + M_{OH}} \qquad (34)$$

where, in general, the volume $\bar{V}_c$ will be a function of the degree of polymerization.

The authors have attempted to estimate $\bar{V}_c$ in several ways. One very simple way is to assume that the molecules are rigid rods that sweep out a conical volume which cannot be refilled as fast as it is emptied. The rigid rod molecule is assumed to sweep out a volume equal to twice the volume of a cone given by,

$$2x(\text{volume of cone}) = 2[(\pi/3)(\frac{L}{2}\sin\theta)^2(\frac{L}{2}\cos\theta)] \qquad (35)$$

where L is the length of the molecule given by $L = NL_o$ where L_o is the length of a monomer unit which may be taken as 5.15×10^{-8} cm. The angle θ is one half the angle of the apex of the cone and N_a is Avagadro's number. The total volume per mole is given by

$$\bar{V}_c = \bar{V}_\theta = (\pi x 10^{-24}/12)\, N_a L_o^3 N^3 \sin^2\theta \cos\theta \qquad (36)$$

where $\bar{V}_\theta$ has the units of ml./mole.

For the purpose of estimation we assume that $\bar{V}_\theta$ is equal to the difference between the experimentally determined partial molar volume and that calculated from Equation (33). For cellotetraose $\bar{V}_\theta$ is 1.8 ml./mole and θ is 1°49'. If one assumes that θ is a constant for the remaining members of the series, $\bar{V}_\theta$ can be calculated. The partial specific volume is then calculated by substituting the results from Equation (36) into Equation (34).

The partial specific volumes calculated on the basis of Equation (36) are given by the dashed line in Fig. 5 for cellotetraose and higher sugars. For the glucose, cellobiose, and cellotriose polymers the dashed and solid lines are identical.

It is apparent that the increase in partial specific volume is predicted only qualitatively. There are a number of ways in which improved estimates of $\bar{V}_\theta$ can be obtained. The methods are, however, quite involved and will not be worth carrying out until the partial specific volumes of cellohexaose and celloheptaose are known. Rather than consider these methods in detail it seems more profitable to discuss the general features that a theory must have in order to describe the partial specific volume dependence on molecular weight.

These general features can be predicted from what is known about the partial specific volumes of high molecular weight polymers. First of all, the partial specific volumes of high molecular weight polymers are known to be independent of molecular weight. The reason for this lies in the manner which a high molecular weight polymer moves in solution. This movement is thought to take place by means of segmental jumps (28) involving short segments of the polymer chain. In order for a segment to move in solution there must be sufficient energy and space associated with the segment and its neighbors. This must involve a certain amount of co-operative motion between segments.

If the molecule is large enough one can assign an average size to the jumping segment. As the result of a jump the segment will sweep out a volume $\bar{V}_s$ per mole of segments which cannot be refilled with solvent as it is emptied. If N_s represents the number of segments in a molecule and N_m the number of anhydroglucose units in the segment, then

$$N_s = N/N_m \tag{37}$$

where N is the degree of polymerization.

In the limit as the molecular weight becomes large the extra molar volume resulting from co-operative motion becomes,

$$\bar{V}_c = (N/N_m)\bar{V}_s \tag{38}$$

and upon substituting into Equation (34) one finds

$$\bar{v}_N = \frac{N\bar{V}_a + 2\bar{V}_e + (N/N_m)\,\bar{V}_s}{NM_a + M_H + M_{OH}} \tag{39}$$

If the molecule is large enough M_H, M_{OH}, and $2\bar{V}_e$ will be negligible compared to the other terms in the expression and one has,

$$\bar{v}_N = \frac{\bar{V}_a + (\bar{V}_s/N_m)}{M_a} \tag{40}$$

Since $\bar{V}_a$ and M_a are known and $\bar{v}_N$ is in principle available from experiment one can determine the ratio $\bar{V}_s/N_m$. The partial specific volume, the volume of an anhydroglucose unit and the molecular weight of an anhydroglucose unit are constant and therefore the ratio $\bar{V}_s/N_m$ is a constant.

The partial specific volume of the cellulose molecule in water is unknown at high molecular weights because of the insolubility of the molecule. The partial specific volume of partially methylated cellulose is available (21) for a molecule with a D.P. of 355, a degree of substitution of 1.26, and a partial specific volume of 0.729 ml./g. For lack of anything better it is assumed that for cellulose of high molecular weight $\bar{v}_N$ = 0.729 ml./g., M_a = 162.14 and $\bar{V}_a$ = 98.15. Substituting these values into Equation (40) one finds that $\bar{V}_s/N_m$ = 8.45 ml./mole for cellulose.

The results of the discussion thus far are illustrated in Fig. 6, where the experimental partial specific volumes and those computed by Equations (32) and (39) are indicated.

As the degree of polymerization decreases $\underline{N}_s$ will decrease and the co-operative motion between segments necessary for a given segment to move will decrease. The jump frequency (29) of a segment at the end of the molecule will increase as will the volume swept out by the segment. In other words, $\underline{\bar{V}}_s$ is expected to increase when the total number of segments in the molecule decreases far enough. This increase in $\underline{\bar{V}}_s$ should be followed by a decrease as the number of co-operating segments approaches zero. As the length of the molecule becomes much smaller than the length of a segment the partial specific volume should approach the values reported for the cellodextrins.

In summary, $\underline{\bar{V}}_c$ should be negligible from a D.P. of 1 to 3. It should increase as the co-operative motion between anhydroglucose units increases and then pass through a maximum and approach an asymptotic limit of $(\underline{N}/\underline{N}_m)\underline{\bar{V}}_s$ as the molecular weight becomes large.

RESULTS OF DIFFUSION EXPERIMENTS

The results of the diffusion experiments on cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose in water are given in Table II. Where possible, the diffusion coefficients at several concentrations are reported. Diffusion coefficients of cellopentaose and cellohexaose are given at only one concentration since there was insufficient time to study the effect of concentration on these samples. Time also did not permit experimental work to be conducted on celloheptaose.

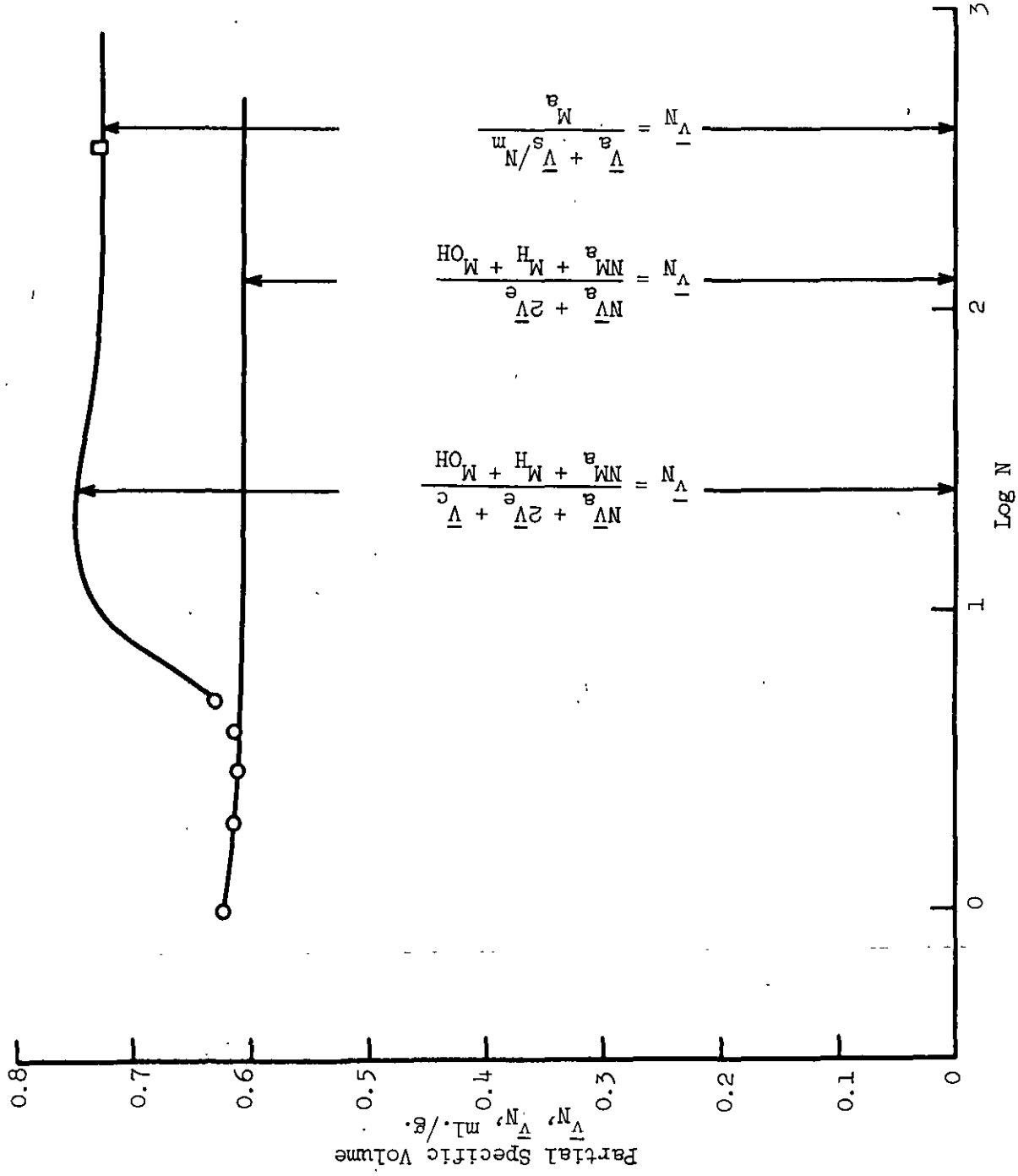

Figure 6. Partial Specific Volume of Cellodextrins as a Function of Degree of Polymerization

TABLE II

DIFFUSION COEFFICIENTS FOR THE CELLODEXTRINS IN WATER
AT 30.00 ± 0.02°C.

Expt. Code	Compound	ΔC, moles/l.	$\bar{C}$, moles/l.	J	Δt, sec.	$D_A \times 10^6$, sq.cm./sec.	Std. Dev. x 10^6, sq.cm./sec.	Cell Size, ml.
DB-5	Cellobiose	0.02824	0.01412	32.68	56.8	5.706	0.018	2
[a]	Cellobiose	0.01823	0.00912		42	5.713	0.016	Ult. cent.
DB-12	Cellobiose	0.008648	0.004324	39.42	65.2	5.739	0.012	11
DB-11	Cellobiose	0.002684	0.001342	12.24	50.2	5.944	0.060	11
DB-19	Cellotriose	0.013522	0.006761	21.98	50.2	4.868	0.017	2
DB-16	Cellotriose	0.009656	0.004828	63.91	43.5	4.864	0.009	11
DB-17	Cellotriose	0.006126	0.003063	41.41	82.1	4.844	0.011	11
DB-18	Cellotriose	0.001878	0.000939	13.44	73.1	4.908	0.022	11
DB-21	Cellotetraose	0.010016	0.005008	87.06	117.2	4.202	0.009	11
DB-20	Cellotetraose	0.001536	0.000768	12.42	213.9	4.006	0.054	11
DB-22	Cellopentaose	0.002074	0.001037	21.18	39.1	3.817	0.022	11
[a]	Cellohexaose		0.00103			3.179	0.084	

[a] Obtained by James Tostevin in the ultracentrifuge using the Rayleigh Optical system.

The diffusion coefficients are reported in terms of $\underline{D}_A$, the diffusion coefficient based on the reduced height-area ratio, along with the standard deviation. The total number of fringes $\underline{J}$ and the zero time corrections $\Delta\underline{t}$ are reported for all experiments conducted in the electrophoresis-diffusion instrument. The zero-time corrections are three to ten times larger than those reported by Gosting and Morris (12). This is due to the fact that the tip of our sharpening needle was blunt. Gosting and Morris used a specially shaped needle in their work on sucrose.

The refractive index difference across the initial boundary, $\Delta\underline{n}$, may be calculated from the total number of fringes $\underline{J}$, the cell thickness along the optical path $\underline{a}$, and the wavelength of the monochromatic light λ, through the use of Equation (26). The cell thickness was $\underline{a}$ = 0.6457 cm. for the 2-ml. micro cell and $\underline{a}$ = 2.596 cm. for the 11-ml. standard cell. The wavelength of the monochromatic light is $\lambda = 5462.2 \times 10^{-8}$ cm. The refractive index difference across the initial boundary was calculated from Equation (26) and may be expressed as a function of concentration difference $\Delta\underline{C}$. The resulting expressions are given as follows,

Cellobiose	$\Delta n = 21.05\ \Delta C - 434\ (\Delta C)^2$	(41)
Cellotriose	$\Delta n = 12.46\ \Delta C + 2114\ (\Delta C)^2$	(42)
Cellotetraose	$\Delta n = 11.62\ \Delta C + 802\ (\Delta C)^2$	(43)
Cellopentaose	$\Delta n = 9.304\ \Delta C$	(44)

It is apparent from Table II that the diffusion coefficients of the cellodextrins decrease with increasing molecular weight. In order to examine this feature of the results more closely one can consider that the diffusing molecules are spheres. Stoke's law may then be used to evaluate the friction coefficient in Einstein's equation, Equation (2), with the result that the diffusion coefficient

for a sphere, D_s, is given by (4)

$$D_s = RT[6\pi\eta\ (3M\bar{V}/4\pi N_a)]^{-1/3} \qquad (45)$$

where η is the viscosity of the solution, M is the molecular weight of the solute and N_a is Avagadro's number. In the past, when this equation has been tested for small molecules it has not yielded accurate results. The failure of the equation is due in part to the failure of Stoke's law when the size of the molecule approaches that of the solvent and in part to the neglect of hydration and asymmetry of the molecules. For large molecules that are not too asymmetric, the product $(M\bar{V})^{1/3}$ is approximately constant and for small molecules decreases with increasing molecular weight.

By assuming $\bar{V}$ to be approximately the same for a large number of substances, Polson (30) simplified Equation (45) to yield,

$$D = KM^{-1/3} \qquad (46)$$

where K is an empirical constant. This was tested on a variety of compounds with a molecular weight range from 20 to 294,000 and was found to fail for molecular weights below 1000. Polson and van der Reyden (31) carried out an empirical extension of this equation to give a better fit of these data. This resulted in the relation,

$$D = K_1M^{-1/3} + K_2M^{-2/3} + K_3M^{-1} \qquad (47)$$

where K_1, K_2, and K_3 are empirical constants. Longsworth (23) has pointed out that this relation can be represented by the simpler expression,

$$D = A/(M^{1/3} - B) \qquad (48)$$

in which A and B are empirical constants. This has the advantage that a plot of $DM^{1/3}$ versus D is linear. This expression can also be written in terms of molecular volumes to yield,

$$D = A'/(\bar{V}^{1/3} - B') \qquad (49)$$

where A' and B' are empirical constants. It should be pointed out that in spite of the fact that Equations (46), (47), (48), and (49) are empirical they still have a useful relation to Equation (45) which has a molecular basis.

The relations of Longsworth have been tested for a variety of substances and it is, therefore, interesting to examine our experimental results in this connection. Equation (48) may be tested by making a plot of $D\bar{V}^{1/3}$ versus D. Such a plot is shown in Fig. 7 for glucose through cellopentaose. If we choose to eliminate glucose from consideration, Equation (49) can be made to represent the cellobiose through cellopentaose sugars with an average deviation of 2%, by the relation

$$D = 2.385 \times 10^{-5}/(\bar{V}^{1/3} - 1.792) \qquad (50)$$

A single relation of this form has been found to represent a number of aliphatic amino acids, peptides, sugars, aromatic and heterocyclic amino acids at 1°C. with an average deviation of 1.4% (23).

An analogous test can be made with Equation (48) by simply plotting $DM^{1/3}$ versus D. Again a straight line should be obtained if the equation is to represent the experimental results. The results of a test of this equation are shown in Fig. 8. It is apparent from the marked curvature of the plot that Equation (48) fails to represent the dependence of diffusion coefficient upon molecular weight.

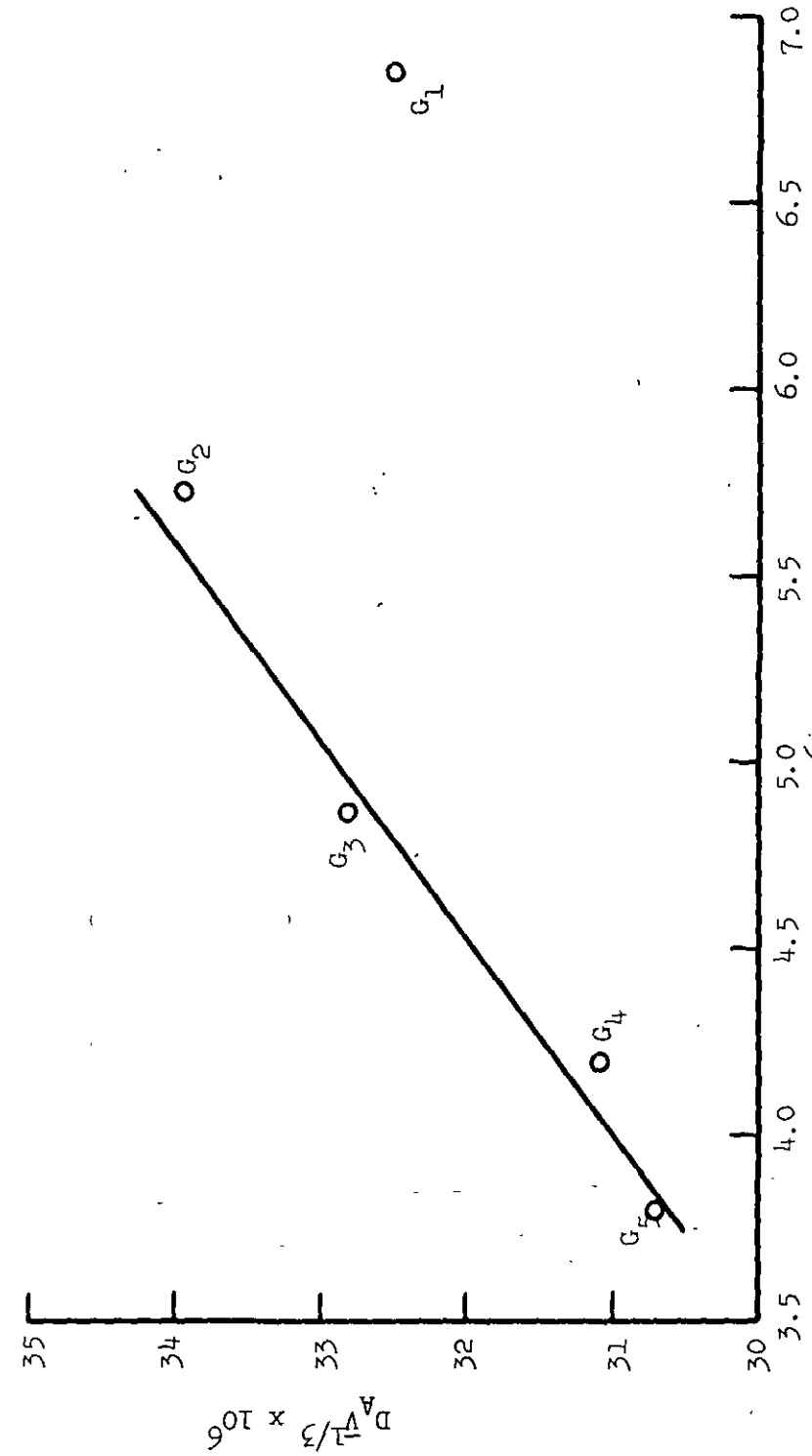

Figure 7. Diffusion Coefficient and Partial Molar Volume Relation for the Cellodextrins. Solid Line Represents Values Calculated from Equation (50). Glucose, G_1; Cellobiose, G_2; Cellotriose, G_3; Cellotetraose, G_4; and Cellopentaose, G_5

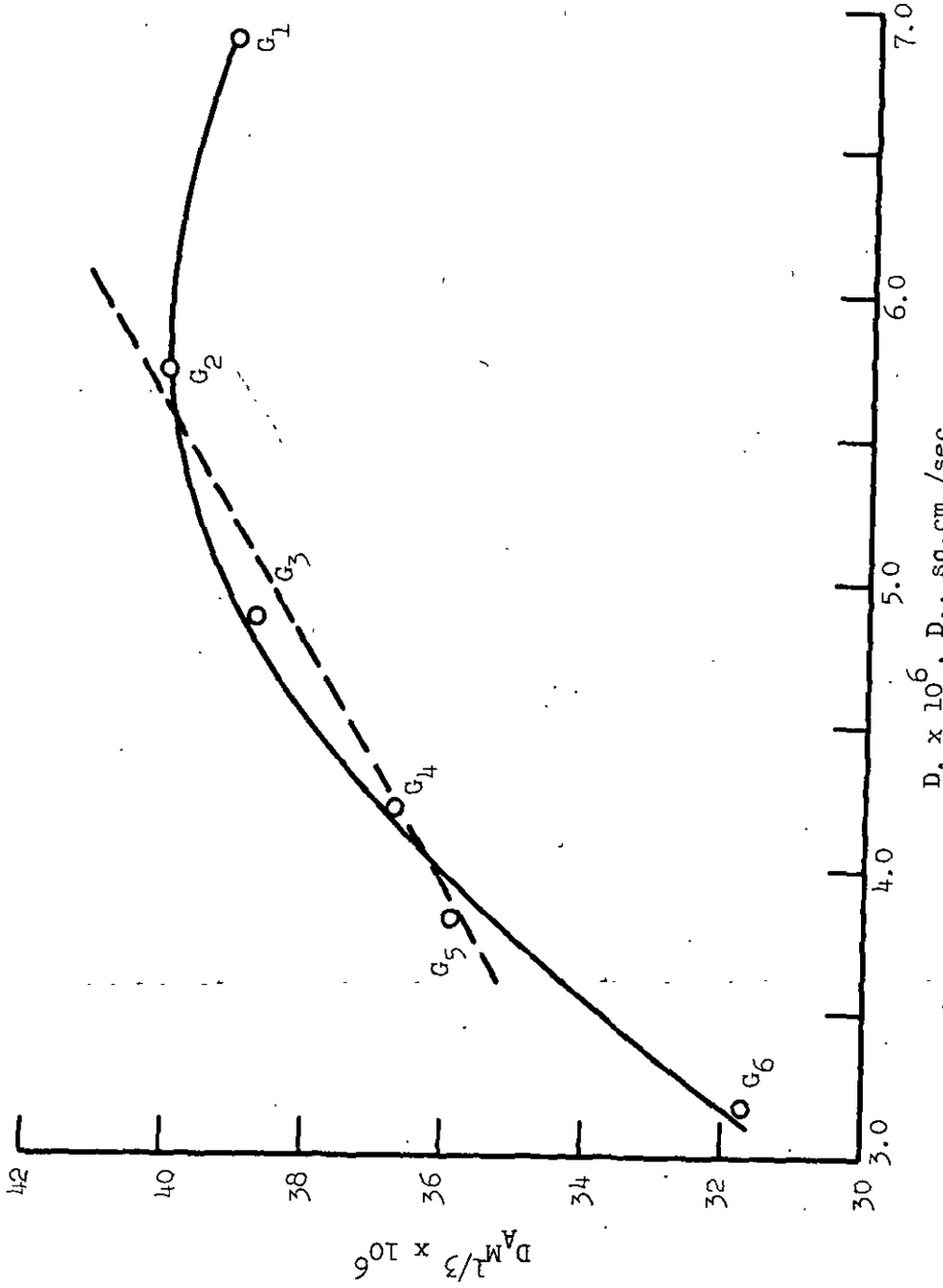

Figure 8. Diffusion Coefficient and Molecular Weight Relation for the Cellodextrins. Dashed Line Represents Calculated Values From Equation (51). Glucose, G_1; Cellobiose, G_2; Cellotriose, G_3; Cellotetraose, G_4; Cellopentaose, G_5; and Cellohexaose, G_6

If we choose to omit glucose and cellohexaose from consideration, Equation (48) can be made to represent the cellobiose through cellopentaose data. Over this restricted range, the relation,

$$D = 2.658 \times 10^{-5}/(M^{1/3} - 2.402) \qquad (51)$$

represents the data with an average deviation of less than 2%. The elimination of glucose and cellohexaose from consideration is of course an arbitrary procedure. Our primary reason for doing this is to obtain a simple expression which may be used in our discussion of ion binding.

It is interesting to examine the molecular weight dependence of the translational diffusion coefficient in the light of the molecular theory of Riseman and Kirkwood (14). It is desirable therefore to examine the experimental results in terms of Equations (17) and (18). There is, however, some question as to what to use for the value of n in these equations since the cellodextrins do not strictly fit the polyethylene-type chain structure on which these equations are based.

For simplicity we shall assume that n is equal to N, the degree of polymerization. When $n = 1$ we have the glucose molecule. On substituting the value of the diffusion coefficient for glucose into Equation (17) one finds,

$$D = \frac{kT}{2\zeta_o} = 6.86 \times 10^{-6} \text{ sq. cm./sec.} \qquad (52)$$

in which the diffusion coefficient of glucose has been corrected to 30.00°C. For cellobiose we can take $D = 5.72 \times 10^{-6}$ sq. cm./sec. and using the above value for kT/ζ_o find that, $2(2.303)\ \lambda_o = 1.41$ so that one has

$$D = \frac{6.86 \times 10^{-6}}{N} [1 + 1.41 \log(2N-1)] \qquad (53)$$

for the entire cellodextrin series. A similar treatment leads to the following expression for the random coil molecule;

$$D = \frac{6.86 \times 10^{-6}}{N}[1 + 0.480\ N^2] \qquad (54)$$

The experimental results for the translational diffusion coefficients are compared in Fig. 9 with the theoretical results for the diffusion of rigid rods and random coil molecules. As expected, the experimental results are closer to the rigid rod model than the random coil. For the rigid rod model the agreement between theory and experiment appears to be within 14% for cellohexaose.

COMPARISON OF DIFFUSION METHODS

In the course of the present experimental program we have obtained diffusion coefficients through the use of three different methods. The results obtained by the three methods are given in Table III. Method I is the Rayleigh interference method applied to free diffusion in the Tiselius cell as described in this report. Method II is the ultracentrifuge transient state method described by Van Holde and Baldwin (32) and is discussed in detail in Quarterly Report Three (33).

Diffusion coefficients obtained by Method III are the result of ultracentrifuge experiments using the Rayleigh optical system in conjunction with the double sector synthetic boundary cell. Free diffusion is made to take place in the sector-shaped cell at low gravitational fields under conditions where sedimentation is presumed negligible. The boundary is formed by layering solvent on top of solution in the gravitational field. The results for this method were obtained by Mr. J. Tostevin (34) on the Spinco Model E ultracentrifuge.

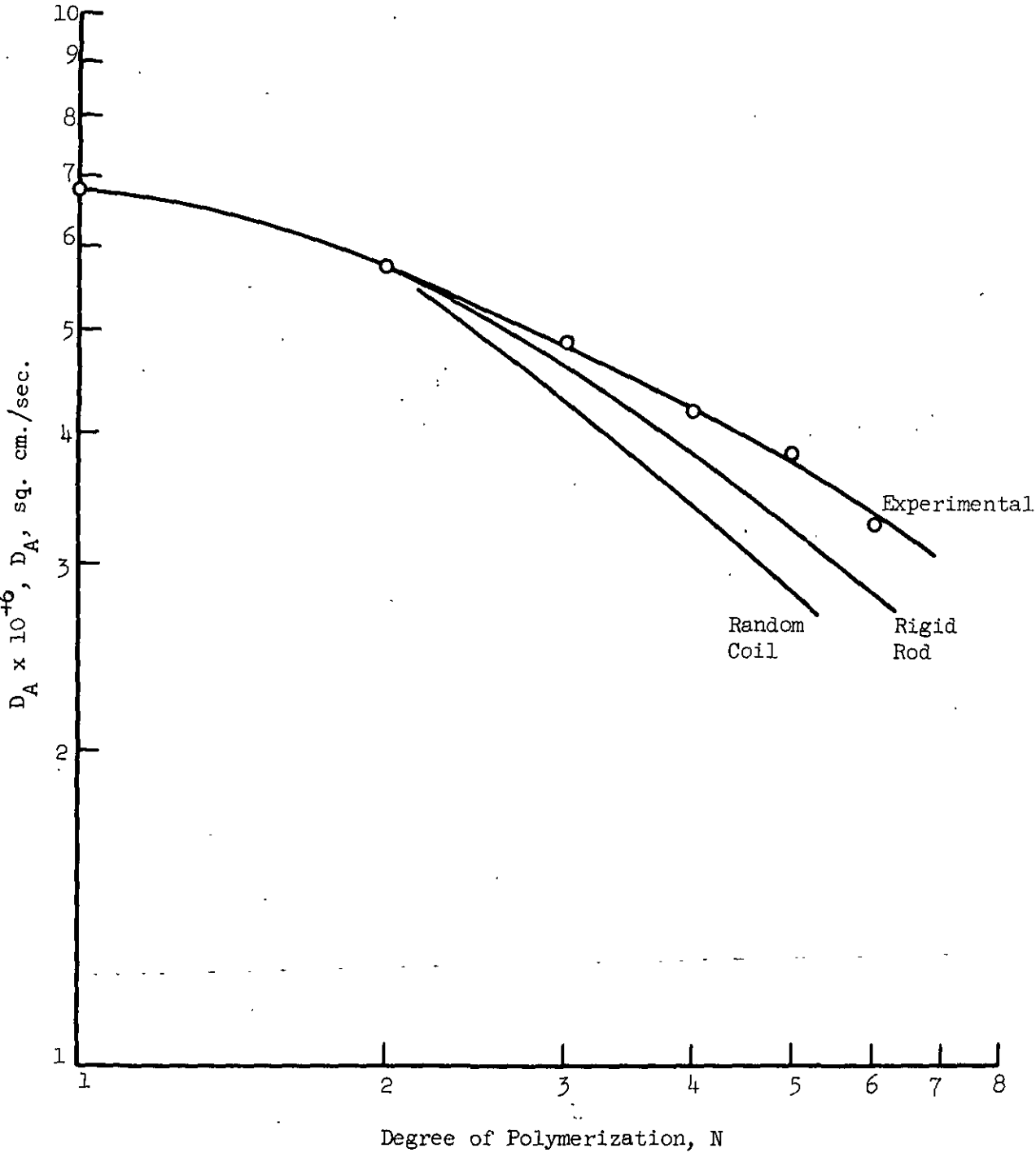

Figure 9. Mean Translational Diffusion Coefficient as a Function of Molecular Weight. Test of Theoretical Kirkwood-Riseman Equations for Rigid Rod and Random Coil Molecules

TABLE III

DIFFUSION COEFFICIENTS FOR THE CELLODEXTRINS IN WATER AT 30.00°C.
Comparison of Different Experimental Methods

	Method I Rayleigh Interferometer-Tiselius Cell		Method II Ultracentrifuge Transient State Analysis-Sedimentation Equilibrium		Method III Ultracentrifuge Rayleigh Interferometer-Boundary Cell[a]	
Cellodextrin	$\bar{C}$, moles/l.	$\underline{D}_A \times 10^6$, sq.cm./sec.	$\bar{C}$, moles/l.	$\underline{D}_A \times 10^6$, sq.cm./sec.	$\bar{C}$, moles/l.	$\underline{D}_A \times 10^6$, sq.cm./sec.
Cellobiose	0.01412	5.706±0.018			0.00923	5.713±0.016
	0.004324	5.739±0.012				
			0.00145	5.99		
	0.001342	5.944±0.060				
Cellotriose	0.006761	4.868±0.017				
	0.004828	4.864±0.009				
					0.00450	5.813±0.055
	0.003063	4.844±0.011				
	0.000939	4.908±0.022				
Cellotetraose	0.005008	4.202±0.009				
					0.00403	4.159±0.012
	0.000768	4.006±0.054				
Cellopentaose			0.00657	3.89		
					0.00300	4.032±0.020
	0.001037	3.817±0.022				
Cellohexaose					0.00103	3.179±0.084

[a]Obtained by James Tostevin.

The results obtained by Method I are considered to be the most accurate. The transient state method, Method II, yields results that are approximately 2% too high. Method III gives values that differ by as much as 5% from those of Method I. Apparently, refinement of this method is still needed before high accuracy results may be obtained. Ultimately, it should be nearly as accurate as Method I.

At the present time Method II appears to be the preferred method of obtaining diffusion coefficients in the ultracentrifuge, even with the limited accuracy of the schlieren optical system.

ANALYSIS OF FRINGE SKEWNESS

Because of the unsymmetrical method of pairing fringes in the Longsworth analysis scheme, the Rayleigh interference method is very sensitive to skewness of the refractive index curve. There are three common causes of skewness of the refractive index gradient curve. One is the concentration dependence of the refractive index increment discussed in the previous section. The other two are polymolecularity and concentration dependence of $\underline{D}$.

In the Longsworth scheme (23), two fringes separated by about $\underline{J}/2$ are paired. From their separation $(\underline{x}_{\underline{J}-\underline{j}} - \underline{x}_{\underline{j}})$ and the corrected time, a diffusion coefficient can be calculated from Equation (29). The mean concentration represented by these fringes is computed from Equations (41), (42), (43), and (44). In the process of moving through the fringe pairs a pattern of changes in $\underline{D}$ with concentration is noted. If a single pure solute is present, this pattern will be repeated for each photograph, that is, the skewness will not show a time dependence. In this case, a single experiment yields information on the concentration dependence of $\underline{D}$ over a range of about $\Delta\underline{C}/4$ to $3\Delta\underline{C}/4$, where $\Delta\underline{C}$ is the initial concentration

difference across the initial boundary. Figure 10 shows the results of measurements taken on three cellobiose solutions. One interesting observation to be made is that the curve seems to go through a minimum at about a concentration of about 7 to 9 moles per liter. This is not readily apparent from the data as shown in Table II. It should also be noted that the eight low and six high points in Experiment DB-12 all resulted from the earliest fringe picture. Apparently, the boundary still showed some skewness left over from the sharpening procedure.

If the solute is polymolecular or polydisperse consisting of materials of different D, the pattern of changes in diffusion coefficient as fringes are paired across the Rayleigh pattern will show a time dependence. Smaller, faster moving molecules will diffuse out ahead of the larger ones, spreading the edges of the boundary faster than the center.

A good example of this is found with cellotriose. Four successive experiments, DB-16, DB-17, DB-18, and DB-19 were conducted with this polysaccharide. In each experiment the cellotriose was in solution for approximately 24 hours. The results of the first experiment, DB-16, are shown in Fig. 11 and have the same general appearance as the cellobiose experiments.

After Experiment DB-16 was conducted, the sample was recovered from solution by freeze drying. This procedure was repeated for each succeeding cellotriose diffusion experiment. Three experiments later when Experiment DB-19 was conducted the fringe analysis yielded the diffusion coefficient pattern shown in Fig. 12. At short times, 536.25 seconds, the diffusion coefficient increases with increasing concentration. As the diffusion experiment proceeds the curves gradually rotate about a common point until the diffusion coefficient becomes a decreasing function of concentration as indicated by the measurements taken after 42,746

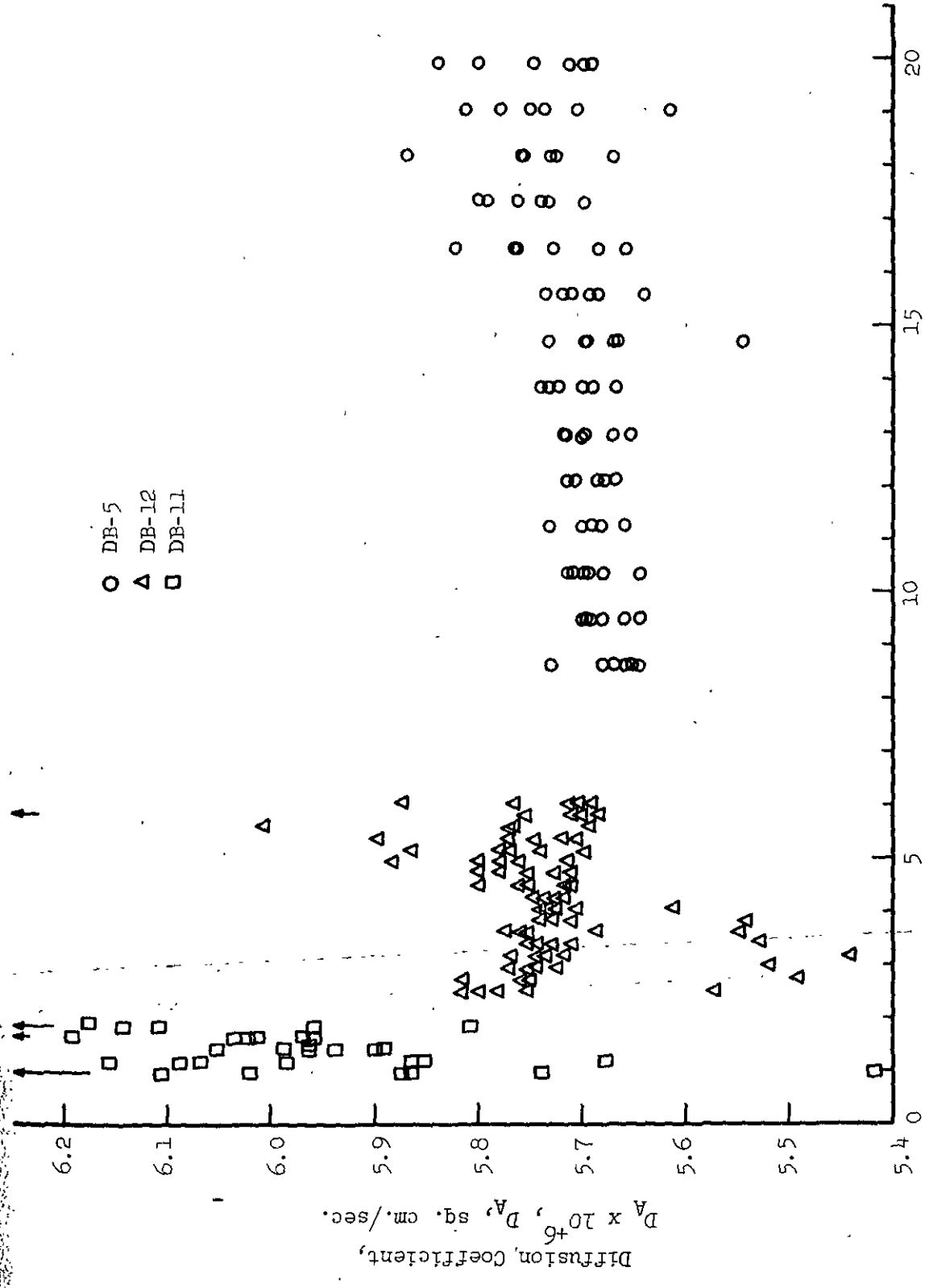

Figure 10. Diffusion Coefficient of Cellobiose as a Function of Mean Concentration

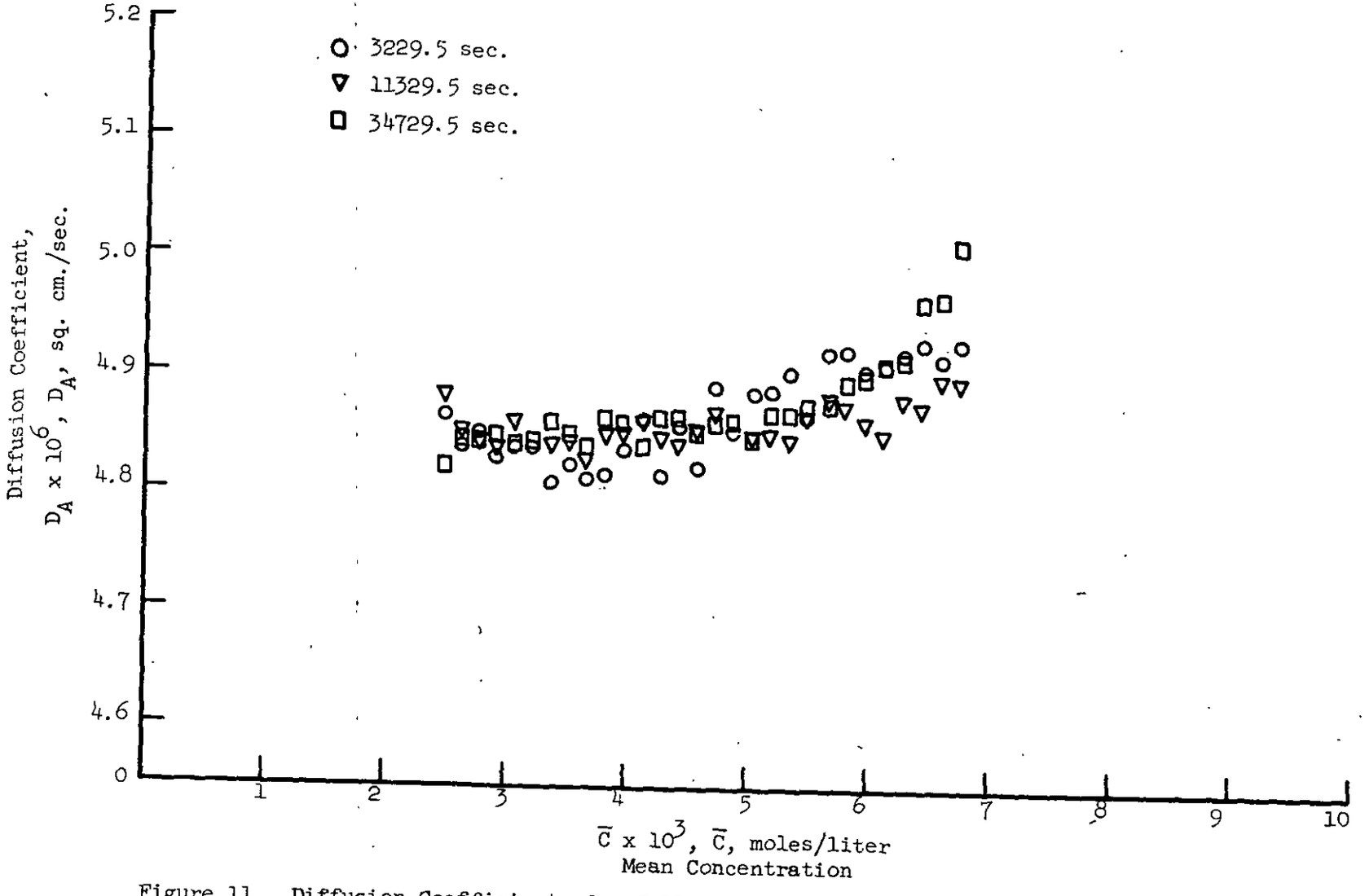

Figure 11. Diffusion Coefficients for Cellotriose as a Function of Mean Concentration for Experiment DB-16. For Clarity, Results from Only 3 of the Available 8 Patterns Are Shown

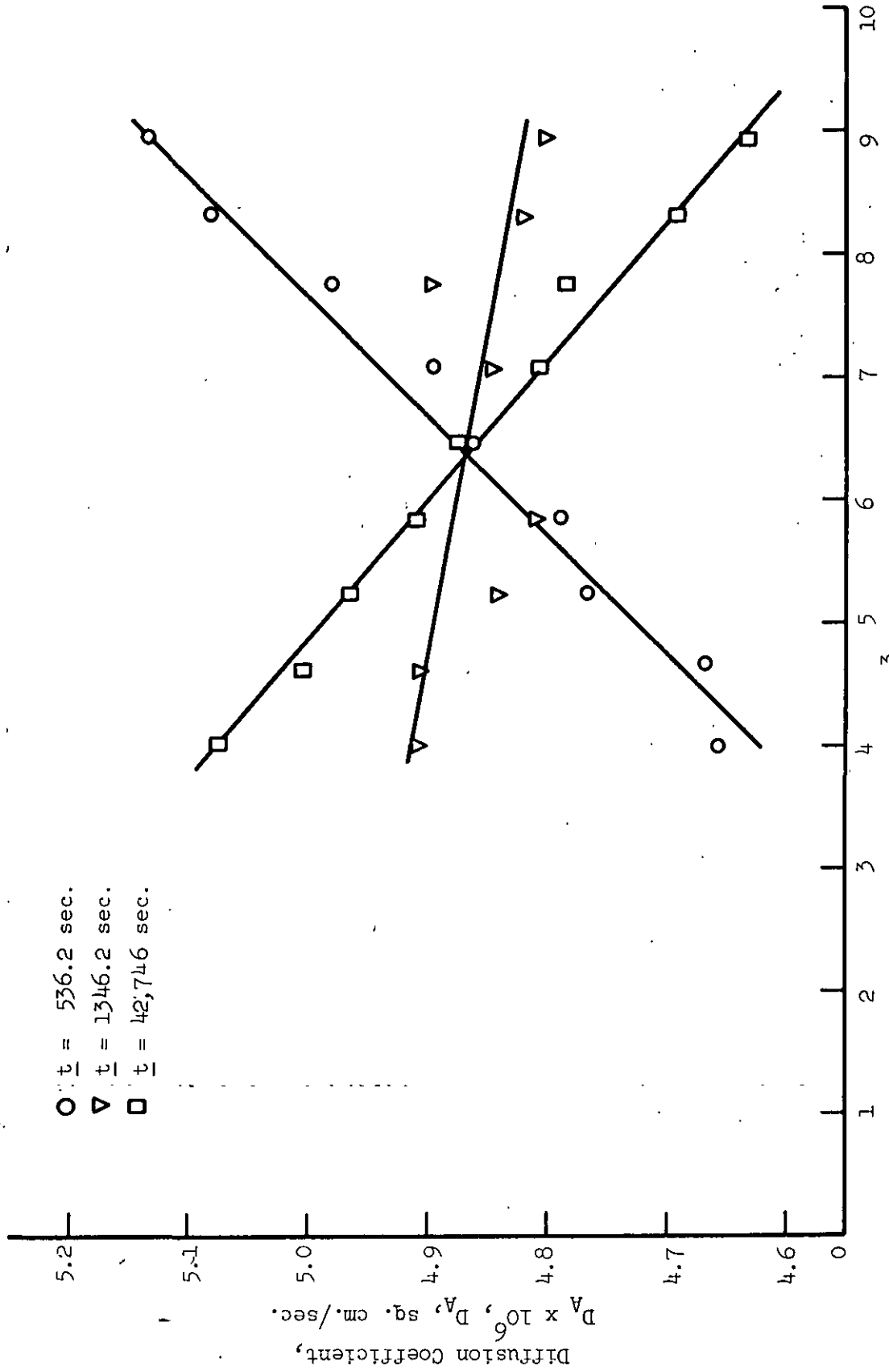

Figure 12. Diffusion Coefficients for Cellotriose as a Function of Mean Concentration for Experiment DB-19. For Clarity, Results from Only 3 of the Available 12 Patterns Are Shown

seconds of diffusion. It should be noted that the mutual center of rotation of the curves is located very close to the mean concentration and mean diffusion coefficient for the system.

The rather strange behavior of these curves in contrast to those shown in Fig. 11 is due to the polydispersity of the sample at this point. Apparently, during the experimental program the samples have become subjected to bacterial attack. As we have pointed out previously, the cellodextrins were inadvertantly subjected to bacterial attack during preparation but were recrystallized to constant melting and decomposition points. This procedure appears to have yielded a very pure product since the first experiment, DB-16, shown in Fig. 11 did not indicate any evidence of polymolecularity. Further bacterial action appears to have been initiated during the time the succeeding diffusion experiments were conducted.

Data obtained for cellotetraose and cellopentaose were also treated in this manner. The results of fringe analysis on these samples indicate that they were polydisperse and hence we were unable to obtain any information on the concentration dependence of these diffusion coefficients. If such information is to be obtained, small amounts of a bactericide will have to be added to the diffusion system.

Fringe analysis is an excellent means of checking the polymolecularity of a polysaccharide. In many instances the preparative chemist must rely on melting point determinations or chromatographic techniques. A diffusion experiment with subsequent fringe analysis would be a useful addition to these techniques and might even rival chromatographic analysis for detecting impurities.

In the present work we have used fringe analysis to detect the presence of small amounts of impurities resulting from bacterial action. These would have gone undetected if we had simply reported the mean diffusion coefficient as a function of concentration as we have done in Fig. 13. The general concentration dependence of diffusion coefficient is apparent but any information on sample purity is lost.

ION BINDING

In Quarterly Report Six (35) we presented the results of several experiments of the diffusion of cellobiose in the presence of sodium chloride. For purposes of reference these results are repeated here in Table IV and Fig. 14. It is apparent from an examination of Fig. 14 that the presence of 0.2958 molar sodium chloride has depressed the diffusion coefficient of cellobiose in water by about 1.5%. Since viscosity changes of the surrounding media can account for only 0.1% of this depression we are dealing with a small but very real ion-binding effect.

TABLE IV

DIFFUSION COEFFICIENTS FOR CELLOBIOSE IN 0.2958M NaCl
AT 30.00 ± 0.02°C.

Expt. Code	Compound	ΔC, moles/l.	$\bar{C}$, moles/l.	J	Δt, sec.	$D_A \times 10^6$, sq.cm./sec.	Std. Dev. $\times 10^6$, sq.cm./sec.	Cell Size, ml.
DB-13	Cellobiose	0.02778	0.01389	31.72	17	5.636	0.010	2
DB-14	Cellobiose	0.00832	0.00416	37.57	66	5.643	0.013	11
DB-15	Cellobiose	0.00286	0.00143	12.82	167	5.665	0.061	11

It is of interest to see how this small ion-binding effect will influence molecular weights of cellodextrins determined in the ultracentrifuge in the presence

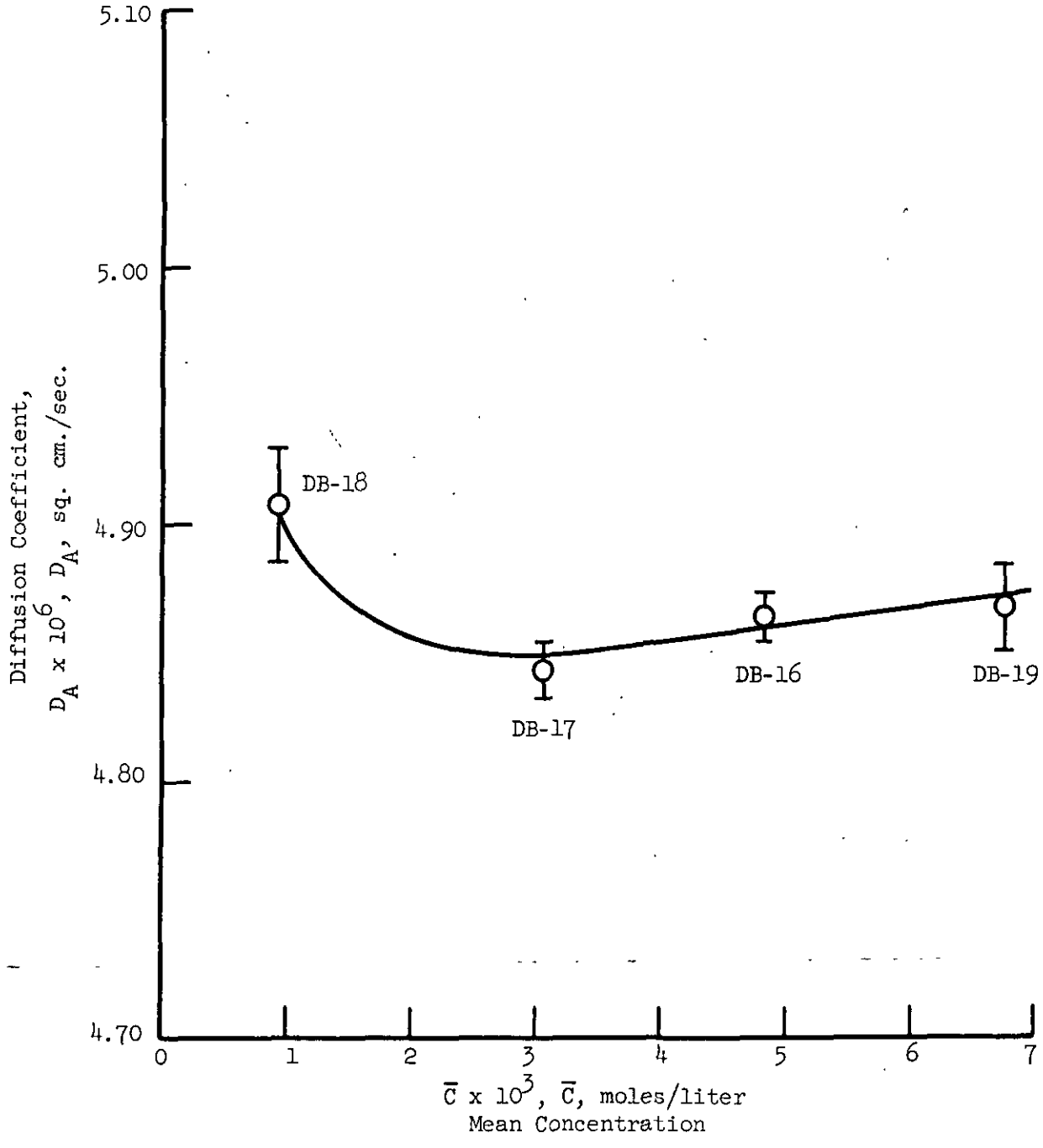

Figure 13. Diffusion Coefficients for Cellotriose as a Function of Mean Concentration

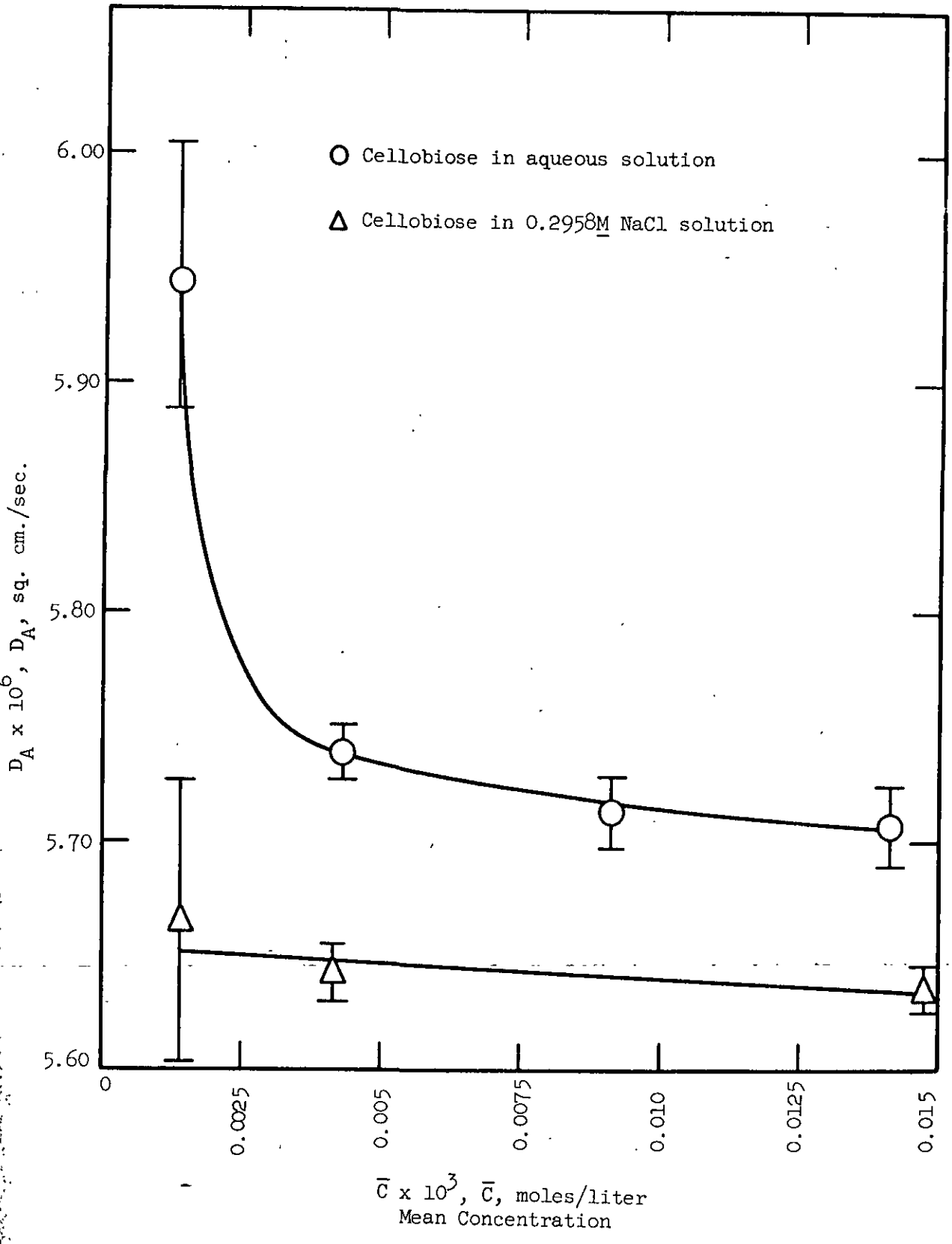

Figure 14. Diffusion Coefficients for Cellobiose as a Function of Mean Concentration

of sodium chloride as a supporting electrolyte. In order to do this, one may make use of the results obtained from the theory of sedimentation analysis (36). The system under consideration is the ternary system consisting of a solvent (1)–macromolecular solute (2)–solvent (3). These numbers will be affixed as subscripts to quantities appearing in the following equations. For our system, solvent (1) will be water, the macromolecular solute will be a cellodextrin molecule, and solvent (3) will be a sodium chloride electrolyte. We will be interested in the molecular weight determined in the ultracentrifuge from a sedimentation equilibrium experiment in the limit as the molality $m_2 \rightarrow 0$ and under the conditions that the ratio m_1/m_3 remains constant*. With these restrictions, the molecular weight determined in the ultracentrifuge is given by

$$\lim_{m_2 \rightarrow 0} M_2^{app} = M_2 \left[1 + \Gamma' \frac{(1 - \bar{v}_3 \rho)}{(1 - \bar{v}_2 \rho)} \right] \qquad (55)$$

where M_2^{app} is the apparent molecular weight in the presence of a supporting electrolyte, M_2 is the true molecular weight (i.e., in the absence of ion-binding effects), $\bar{v}_2$ is the partial specific volume of the solute in the absence of supporting electrolyte, $\bar{v}_3$ is the partial specific volume of the supporting electrolyte, and ρ is the density of the tricomponent solution. The binding coefficient, Γ', is the number of grams of supporting electrolyte bound per gram of macromolecule. The derivation of this equation assumes that the concentrations within the solution are known as a function of position in the gravitational field.

Assuming that Equation (51) is valid for cellobiose in the presence of sodium chloride, one can estimate M_2^{app} since the diffusion coefficients of cellobiose

*Molalities are used in this section since the resulting equations are simpler than those based on the concentration scale.

are known in water and in sodium chloride solution. The true molecular weight is known and the apparent molecular weight can be estimated so that Equation (55) can be used to calculate the ion-binding coefficient.

There is some question as to the proper values to use for the diffusion coefficients at infinite dilution. In describing the concentration dependence of sedimentation coefficients, an equation of the following form is often used (36)

$$\frac{1}{S} = \frac{1}{S_o}(1 + k_s C) \qquad (56)$$

where S_o is the sedimentation coefficient at infinite dilution, and k_s is a constant. According to Equations (1) and (2) this concentration dependence should also apply to diffusion coefficients since changes in S and D are due exclusively to changes in the friction coefficient. By analogy one can write,

$$\frac{1}{D} = \frac{1}{D_o}(1 + k_D C) \qquad (57)$$

where D_o is the diffusion coefficient at infinite dilution and k_D is a constant. An extrapolation of diffusion coefficients based on Equation (57) is shown in Fig. 15. Good agreement is observed for the diffusion of cellobiose in the presence of sodium chloride. For cellobiose in water the equation fails at low concentrations. Inasmuch as sedimentation equilibrium experiments are difficult to perform at concentrations of the order of 0.1% by weight (0.001 mole/l. for cellobiose) we have chosen to carry out the extrapolation based on the measurements taken at higher concentrations. With this in mind we obtain values for D_o of 5.65×10^{-6} sq. cm./sec. for cellobiose in the presence of sodium chloride and a D_o of 5.78×10^{-6} sq. cm./sec. for cellobiose in water. The corresponding apparent molecular weight calculated from Equation (51) is an M_2^{app} of 362. From this same equation, M_2 is calculated as 346.

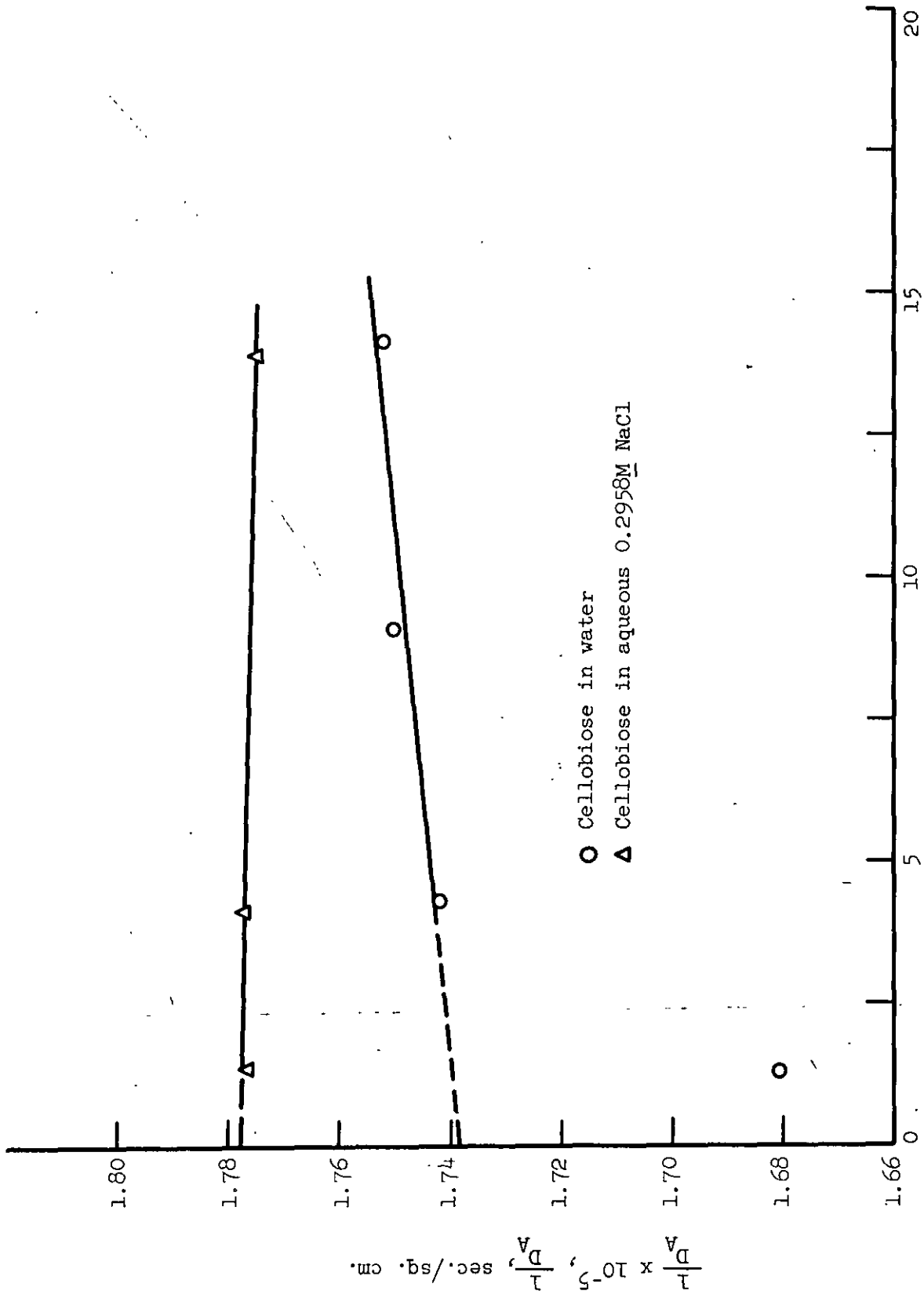

Figure 15. Extrapolation of Diffusion Coefficients of Cellobiose to Infinite Dilution

The partial specific volume of cellobiose $\bar{v}_2$ is taken as 0.6148 ml./g. and for sodium chloride $\bar{v}_3$ can be estimated to be 0.301 ml./g. If the density of the solution ρ is taken as 1.00 g./ml. then one can calculate the ion-binding coefficient to be $\Gamma' = 0.023$ g. NaCl/g. cellobiose.

In actual practice the effect of ion binding causes some additional difficulties in sedimentation experiments that are not represented by Equation (55). The Rayleigh and schlieren optical systems are based on the detection of refractive index gradients of the sedimenting species. This means that ion binding will cause changes in refractive index and these must also be considered. When this is done, Equation (55) must be written as (36)

$$\lim_{m_2 \to 0} M_2^{app} = M_2 \left[1 + \Gamma' \frac{(1 - \bar{v}_3\rho)}{(1 - \bar{v}_2\rho)} \right] \left[1 + \Gamma'(\Omega_3/\Omega_2) \right] \qquad (58)$$

where Ω_2 and Ω_3 are the specific refractive index increments, respectively, of solute and supporting electrolyte on the molal scale. The apparent molecular weight will therefore differ from that used in Equation (55) to calculate Γ'. Since Ω_2 and Ω_3 are experimentally accessible quantities this should cause no difficulty.

This rather convenient means of estimating binding coefficients has one serious drawback, namely, the neglect of hydration effects. We have no guarantee that the presence of supporting electrolyte has not altered the hydration of cellobiose and hence changed the diffusion coefficient. Until we are able to conduct reliable experiments in the ultracentrifuge it will be impossible to evaluate the validity of this calculational procedure.

NOMENCLATURE

ζ = friction coefficient of a structural unit

η = viscosity coefficient of the solution

η_o = viscosity coefficient of the solvent

λ = parameter in Kirkwood-Riseman theory, also the wavelength of light

μ = chemical potential

μ^o = reference state chemical potential

ρ = density of solution, g./ml.

ρ_a = density of air, g./ml.

ρ_o = density of solvent, g./ml.

$\emptyset_v$ = apparent specific volume, ml./g.

Γ = binding coefficient

Θ = one half the apex angle of a cone

$\Phi(\underline{z}^*)$ = Gaussian error function

$\Delta\underline{C}$ = concentration difference across a diffusing boundary in moles/liter

$\Delta\underline{n}$ = refractive index difference across a diffusing boundary

$\Delta\underline{t}$ = zero time correction for free diffusion experiment

Ω_2 = refractive index increment of solute 2 on the molal scale

Ω_3 = refractive index increment of solute 3 on the molal scale

$\nabla = \vec{\underline{i}}\frac{\partial}{\partial\underline{x}} + \vec{\underline{j}}\frac{\partial}{\partial\underline{y}} + \vec{\underline{k}}\frac{\partial}{\partial\underline{z}}$

a = thickness of Tiselius all along the light path

b = magnitude of the bond vector from the Kirkwood-Riseman theory

$\vec{b}_\ell$ = bond vector from Kirkwood-Riseman theory

f = friction coefficient of a molecule

f_o = friction coefficient of a molecule at infinite dilution

j = fringe number

k = Boltzmann's constant

k_D = concentration coefficient for diffusion

k_s = concentration coefficient for sedimentation

ℓ = integer from Kirkwood-Riseman theory

m = mass of solution

m_o = mass of solvent

m_2 = molality of solute, 2

n = an integer from Kirkwood-Riseman theory

t = true diffusion time, sec.

t' = experimentally observed diffusion time, sec.

$\bar{v}$ = partial specific volume of a solution, ml./g.

$\bar{v}_o$ = partial specific volume of solvent, ml./g.

$\bar{v}_2$ = partial specific volume of solute, 2, ml./g.

$\bar{v}_3$ = partial specific volume of solute, 3, ml./g.

$\bar{v}_N$ = partial specific volume of the cellodextrins, ml./g.

v_i = specific volume of a substance being weighed, ml./g.

v_w = specific volume of weights, ml./g.

x_j, x_{J-j} = position of Rayleigh fringes

y = activity coefficient on the concentration scale

z = parameter in Kirkwood-Riseman theory

$\underline{C}$ = concentration, moles/liter

$\overline{\underline{C}}$ = mean concentration, moles/liter

$\underline{C}_1, \underline{C}_2$ = concentrations at cell ends in a free diffusion experiment

$\underline{D}$ = diffusion coefficient

$\underline{D}_o$ = diffusion coefficient at infinite dilution

$\underline{D}_A$ = true area average diffusion coefficient

$\underline{D}'_A$ = area average diffusion coefficient based on times, $\underline{t}'$

$\underline{G}_1$ = glucose

$\underline{G}_2$ = cellobiose

$\underline{G}_3$ = cellotriose

$\underline{G}_4$ = cellotetraose

$\underline{G}_5$ = cellopentaose

$\underline{G}_6$ = cellohexaose

$\underline{J}$ = total fringe number

$\vec{\underline{J}}'_i$ = flow of solute species $\underline{i}$ referred to the local center of mass, g./sq. cm. sec.

$\overline{\underline{J}}_i$ = flow of solute species $\underline{i}$ referred to the cell fixed reference frame, g./sq. cm. sec.

$\underline{K}_1, \underline{K}_2, \underline{K}_3$ = empirical constants

$\underline{L}$ = length of a rigid rod molecule

$\underline{L}_o$ = length of a monomer unit in the rigid rod molecule

$\underline{L}_{ik}$ = phenomenological coefficient

$\underline{M}$ = molecular weight

$\underline{M}_a$ = molecular weight of an anhydroglucose unit

$\underline{M}_H$ = atomic weight of hydrogen

$\underline{M}_{OH}$ = molecular weight of an OH-group

$\underline{M}_2$ = molecular weight of solute, 2

M_2^{app} = apparent molecular weight of solute, 2

N = degree of polymerization and number of anhydroglucose units in a cellodextrin molecule

N_a = Avogadro's number

N_m = number of monomer units in a polymer segment

N_s = number of segments in a polymer molecule

P = pressure as a subscript, per cent solute by weight

R = gas constant

R_i = $\rho_a(v_i - v_w)$

S = sedimentation coefficient

S_o = sedimentation coefficient at infinite dilution

T = absolute temperature

$\vec{V}$ = velocity of the mass fixed reference frame relative to the cell fixed reference frame, ml./mole

$\bar{V}_a$ = partial molar volume of an anhydroglucose unit, ml./mole

$\bar{V}_c$ = partial molar volume due to co-operative motion between anhydroglucose units and between polymer segments, ml./mole

$\bar{V}_e$ = partial molar volume associated with the ends of a polymer chain, ml./mole

$\bar{V}_N$ = partial molar volume of a cellodextrin molecule, ml./mole

$\bar{V}_s$ = partial molar volume of a polymer segment, ml./mole

Z^* = reduced cell co-ordinates

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Schachman, H. K. Ultracentrifugation in biochemistry. New York, Academic Press, 1959. 272 p.

2. Flory, P. J. Principles of polymer chemistry. Chap. VII. Ithaca, Cornell Univ. Press, 1953. 672 p.

3. Einstein, A., Z. Elektrochem. 14:235(1908).

4. Gosting, L. J. Measurement and interpretation of diffusion coefficients of proteins. In Advances in protein chemistry. Vol. II. p. 429. New York, Academic Press, 1956.

5. Prigogine, I. Introduction to thermodynamics of irreversible processes. Springfield, Ill., C. C. Thomas. 115 p.

6. Denbigh, K. G. The thermodynamics of the steady state. London, Methuen, 1951. 103 p.

7. Onsager, L., Phys. Rev. 37:405(1931).

8. Onsager, L., Phys. Rev. 38:2265(1931).

9. Dunlop, P. J., and Gosting, L. J., J. Am. Chem. Soc. 75:5073(1953).

10. Gutter, F. J., and Kegeles, G., J. Am. Chem. Soc. 75:3893(1953).

11. Gosting, L. J., and Akeley, D. F., J. Am. Chem. Soc. 74:2058(1952).

12. Gosting, L. J., and Morris, M. S., J. Am. Chem. Soc. 71:1998(1949).

13. Lyons, P. A., and Sandquist, C. L., J. Am. Chem. Soc. 75:3896(1953).

14. Riseman, J., and Kirkwood, J. G. The statistical mechanical theory of irreversible processes in solutions of macromolecules. In Eirich's Rheology. Vol. 1. Chap. 13. New York, N. Y., Academic Press Inc., 1956. 761 p.

15. Jermyn, M. A., Australian J. Chem. 10:55(1957).

16. Miller, G. L., Dean, J., and Blum, R., Arch. Biochem. Biophys. 91:21(1960).

17. Alm, R. S., Acta Chem. Scand. 6:1186(1952).

18. Wolfram, M. L., and Dacrons, J. C., J. Am. Chem. Soc. 74:5331(1952).

19. Bauer, N. Determination of density. In Weisberger's Technique of organic chemistry. Physical methods. Part I. New York, N. Y., Interscience.

20. Bauer, N., and Lewin, S. Z. Determination of density. In Weisberger's Technique of organic chemistry. Vol. I, Part I, 3rd ed. p. 131. New York, Interscience, 1960.

21. Kraemer, E. O. The partial specific volume factor. In Svedberg and Pederson's The Ultracentrifuge. Oxford, 1940.

22. Tables of probability functions. Vol. 1. Federal Works Agency, Work Projects Administration (sponsored by National Bureau of Standards). 1941.

23. Longsworth, L. G., J. Am. Chem. Soc. 74:4155(1952).

24. Longsworth, L. G., J. Am. Chem. Soc. 75:5705(1953).

25. Longsworth, L. G., J. Am. Chem. Soc. 69:2510(1947).

26. Schachman, H. K., and Harrington, W. F., J. Polymer Sci. 12:379(1954).

27. Inagaki, H., and Teramoto, A., Makromol. Chem. 48:185(1961).

28. Fox, T. G., Gratch, S., and Loshaek, S. Viscosity relationships for polymers in bulk and in concentrated solution. In Eirich's Rheology. Vol. 1. Chap. 12. New York, N. Y., Academic Press Inc., 1956. 761 p.

29. Bueche, F., J. Chem. Phys. 21:1850(1953).

30. Polson, A., J. Phys. Colloid Chem. 54:649(1950).

31. Polson, A., and van der Reyden, D.J., Biochem. et Biophys. Acta 5:358(1950).

32. Van Holde, K. E., and Baldwin, R. L., J. Phys. Chem. 62:734(1958).

33. Kurath, S. F., and Swanson, J. W., The molecular properties of naturally occurring polysaccharides. The effect of ion binding on the molecular properties of low molecular weight polysaccharides. Report Three, A Quarterly Report to The Pioneering Research Committee, Pioneering Research Program. Appleton, Wis., The Institute of Paper Chemistry, June 23, 1961.

34. Tostevin, J. E. Private communication.

35. Bump, D. D., Kurath, S. F., and Swanson, J. W., The molecular properties of naturally occurring polysaccharides. The effect of ion binding on the molecular properties of low molecular weight polysaccharides. Report Six, A Quarterly Report to the Pioneering Research Committee, Pioneering Research Program. Appleton, Wis., The Institute of Paper Chemistry, June 29, 1962.

36. Williams, J. W., Van Holde, K. E., Baldwin, R. L., and Fujita, H., Chem. Rev. 58:715(1958).

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APPENDIX I

FORTRAN PROGRAMS FOR THE CALCULATION OF DIFFUSION COEFFICIENTS FROM RAYLEIGH FRINGE PATTERNS

```
C     THIS PROGRAM GIVES CALCULATIONS OF APPARENT DIFFUSION COEFFICIENTS
C     FROM RAYLEIGH DIFFRACTION FRINGE DATA BY LONGSWORTHS METHOD.
C     SUBSEQUENT PAIRINGS MAY BE MADE DROPPING THE FIRST AND LAST
C     FRINGES USED IN THE PREVIOUS COMPUTATION.
C     APPARENT DIFFUSION COEFFICIENTS AND THE RECIPROCAL OF TIME ARE
C     PUNCHED ON CARDS FOR USE IN A LEAST SQUARES PROGRAM.
C     A TOTAL OF NOT OVER 80 FRINGES MAY BE INCLUDED IN THE DATA.
      C0=1.7787391
      C1=.802853
      C2=.014605998
      B1=2.0262682
      B2=.378538
      B3=.0036995827
      SP=2./SQR(3.14159)
C     IF DATA IS IN CENTIMETERS, CM=1.0
C     IF DATA IS IN INCHES, CM=.3937
      READ 1, CM
C     F=MAGNIFICATION FACTOR
      READ 1, F
C     FS=TOTAL NUMBER OF FRINGES
C     J=NUMBER OF FRINGES INCLUDED IN DATA
C     NPRS=NUMBER OF DIFFERENT PAIRINGS OF DATA
C     NPTNS=TOTAL NUMBER OF FRINGE PATTERNS
      READ 1, FS, J, NPRS, NPTNS
C     TC1, TC2,TC3, AND TC4 ARE TIME CORRECTIONS, NEEDED FOR CORRECT
C     DIFFUSION COEFFICIENTS FOR EACH RESPECTIVE PATTERN
```

```
51      READ 1, TC1, TC2, TC3, TC4
        NP=0
C       INDEX=CODE NUMBER OF THE EXPERIMENT
C       NBR=CODE NUMBER OF THE FRINGE
C     T=TIME
11    READ 1,INDEX, NBR
      READ 1, T
      PRINT 1, INDEX, NBR
       PRINT 1,T
       T=T+TC1+TC2+TC3+TC4
       PRINT 1, T
       PRINT 1,
       NP=NP+1
       M=J/2
       K=M
       N=1
       DIMENSION A(80),H(80),FJ(80),Z(80),R(80)
       DIMENSION DZ(40),DR(40),DELH(40),DELZ(40)
       DIMENSION DEV(40)
       DO 4 I=1,J
C     A(I)=FRINGE NUMBER
C     H(I)=FRINGE POSITION
       READ 1, A(I),H(I)
       H(I)=H(I)/CM
       FJ(I)=(2.*A(I)-FS)/FS
       IF(FJ(I))2,3,3
```

```
2       FJ(I)=-FJ(I)
3       S=LOG(2./(1.-FJ(I)))
        S=SQR(S)
        Z(I)=S-(S**2.*C2+S*C1+C0)/(S**3.*B3+S**2.*B2+S*B1+1.)
        S=SP/EXP(Z(I)**2.)
        Q=-(FJ(I)**2.+Z(I)*FJ(I)*S+S**2./2.-1.)/2.
        R(I)=Q/S
C       IF SENSE SWITCH 1 IS ON, THE COMPLETE TABLE OF STATEMENT 42
C       WILL BE PRINTED OUT
        IF(SENSE SWITCH 1)42,4
42      PRINT 1,A(I),H(I),FJ(I),Z(I)
4       CONTINUE
        PRINT 1,
6       MN=J-N+1
        S=0.
        U=0.
        DO 9 I=N,K
        L=I+M
        DELH(I)=H(I)-H(L)
        IF(DELH(I))14,15,15
14      DELH(I)=-DELH(I)
15      DELZ(I)=Z(I)+Z(L)
        DR(I)=R(L)-R(I)
        Q=DELH(I)/DELZ(I)
C       DAQ = DIFFUSION COEFFICIENT FOR THE FRINGE PAIR CONSIDERED
        DAQ=(Q**2.)/(4.*T*F**2.)
```

```
      S=S+Q
      U=U+DAQ
C     IF SENSE SWITCH 2 IS ON, THE NORMALIZED FRINGE SEPERATIONS
C     AND THE CORRESPONDING DIFFUSION COEFFICIENTS WILL BE PRINTED OUT.
      IF(SENSE SWITCH 2)8,9
8     PRINT 1,Q,DAQ
9     CONTINUE
      PRINT 1,
      Q=M
C     YF=AVERAGE VALUE OF NORMALIZED FRINGE SEPERATIONS
35    YF=S/Q
C     DAQY=AVERAGE VALUE OF DAQ
      DAQY=U/Q
      DO 26 I=N,K
      S=DELH(I)/YF
      DEV(I)=S-DELZ(I)
26    CONTINUE
C     IF SENSE SWITCH 3 IS ON, THE VALUES OF THE FRINGE DEVIATIONS
C     AND THE CORRESPONDING DELTA R(Z*) VALUES WILL BE PRINTED OUT.
      IF(SENSE SWITCH 3)29,41
29    DO 27 I=N,K
27    PRINT 1, DEV(I),DR(I)
      PRINT 1,
41    CONTINUE
C     DA=DIFFUSION COEFFICIENT.  IT SHOULD AGREE WITH DAQY
      DA=(YF**2.)/(4.*T*F**2.)
```

```
      RCPT=1./T
      PUNCH 1,RCPT,DA
      IF(N-1)16,16,17
16    PRINT 1,YF,DAQY,RCPT,DA
      GO TO 18
17    PRINT 1,YF,DAQY,DA
18    PRINT 1,
      IF(NPRS-N)10,10,7
7     N=N+1
      M=M-1
      GO TO 6
10    IF(NPTNS-NP)12,12,11
12    STOP
      END
```

```
C     THIS PROGRAM PERFORMS A LEAST SQUARES EXTRAPOLATION OF
C     THE DIFFUSION COEFFICIENTS CALCULATED IN THE PROGRAM FOR
C     THE ANALYSIS OF RAYLEIGH FRINGES TO INFINITE TIME
C     DAPP IS THE APPARENT DIFFUSION COEFFICIENT
C     RCIPT IS THE RECIPROCAL OF THE TIME
C     NO IS THE NUMBER OF PAIRINGS
C     NPAT IS THE NUMBER OF FRINGE PATTERNS ANALYZED
      DIMENSION DAPP(100),RCIPT(100)
21    READ,NO,NPAT
      ND=NO*NPAT
      DO 20 NB=1,ND
20    READ,RCIPT(NB),DAPP(NB)
12    LS=1
      NA=ND-NO+1
25    RSQ=0.
      RS=0.
      DSQ=0.
      DS=0.
      RDS=0.
      DO 22 NB=LS,NA,NO
      RSQ=RSQ+RCIPT(NB)**2.
      RS=RS+RCIPT(NB)
      DSQ=DSQ+DAPP(NB)**2.
      DS=DS+DAPP(NB)
      PRINT,RCIPT(NB),DAPP(NB)
22    RDS=RDS+DAPP(NB)*RCIPT(NB)
```

```
      P=NPAT
      RMN=RS/P
      DMN=DS/P
      VARR=(RSQ-(RS*RS/P))/(P-1.)
      VARD=(DSQ-(DS*DS/P))/(P-1.)
      B=(RDS-DS*RS/P)/(RSQ-RS**2./P)
      VAR=(P- 1.)*(VARD-B*B*VARR)/(P-2.)
      DCORR=DMN-B*RMN
      DELT=B/DCORR
      PRINT,
C     VAR IS THE VARIANCE
C     B IS THE SLOPE OF THE LINE
C     DELT IS THE ZERO TIME CORRECTION
C     DCORR IS THE DIFFUSION COEFFICIENT AT INFINITE TIME
      PRINT,VAR,B,DELT,DCORR
      PRINT,
      PRINT,
      IF(NO-LS)24,24,23
23    LS=LS+1
      NA=NA+1
      GO TO 25
24    PAUSE
      GO TO 21
      STOP
      END
```