which soundness is especially impressive since each set was obtained independently by two different pairs of observers. It also suggests the necessity of re-examination of the problem of structure and reactivity.

Acknowledgment.—Great indebtedness and gratitude is here acknowledged to Professor Louis P. Hammett of Columbia University for his characteristically careful examination and discussion of the results of this investigation with the senior author (J.D.G.) and to Mr. Daniel P. Ryskiewich of New York University for his uncomplaining and rapid determination and confirmation of the *p*H value of almost innumerable buffer solution samples during the entire course of this research.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

Sedimentation Coefficient Determinations with Some Sugars and Dextrins[1]

BY RUSSELL V. WEBBER

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The sedimentation coefficients of glucose, sucrose, raffinose, amyloheptaose, cyclohexaamylose and cycloöctaamylose were determined and converted to correspond to the sedimentation rate which would have been observed in water at 20°. The results were reproducible to ±0.03 Svedberg unit. They agreed well with the theoretical values which were computed from molecular weight and diffusion data. For the open chain compounds the dependence of sedimentation coefficient on molecular weight may be represented by the expression $s_{20,w} = 0.0087M^{0.56}$ Svedberg unit. For the cyclic compounds the sedimentation coefficients may appear to be somewhat larger than would be predicted by the above relationship for a molecule with open chain structure and the same molecular weight, but the point is not established with certainty.

Introduction

In a normal experiment for measuring a sedimentation rate with the ultracentrifuge the solute separates entirely away from the meniscus; when it does not the procedure is unreliable since the conditions for free diffusion in the sedimenting boundary do not exist in the ultracentrifuge cell.

Three approaches to the problem of obtaining the sedimentation coefficient, *s*, of slowly sedimenting solutes have been made. (1) Ultracentrifuge cells have been partitioned into top and bottom compartments. The amount of solute which moves through the partition during the experiment may be determined by analyzing the solutions in the two compartments; from which data the coefficient *s* can be computed.[2] (2) "Synthetic boundary cells" have been designed with which the sedimentation displacement of a freely diffusing boundary may be observed for a *short* period of time.[3] (3) Methods have been found for obtaining the sedimentation coefficient from the refractive index gradient which develops in the ultracentrifuge cell when the experiment is carried out in the usual way.[4-6]

In this paper a modified method based on procedure (3) is presented. It has been used to determine experimentally the sedimentation coefficients of several sugars and dextrins. The values obtained are compared with the theoretical figures which were computed from molecular weight and diffusion data.

(1) More complete details of this investigation may be obtained from the Ph.D. dissertation, 1954, of the writer filed in the library of the University of Wisconsin.

(2) A. Tiselius, K. O. Pedersen and T. Svedberg, *Nature*, **140**, 848 (1937).

(3) G. Kegeles, THIS JOURNAL, **74**, 5532 (1952); *cf.* also G. E. Pickles, W. F. Harrington and H. K. Schachman, *Proc. Nat. Acad. Sci.*, **38**, 943 (1952).

(4) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon Press, Oxford, 1940, p. 283.

(5) H. Gutfreund and A. G. Ogston, *Biochem. J.*, **44**, 163 (1949).

(6) R. L. Baldwin, *ibid.*, **55**, 644 (1953).

Theory

By applying the reasoning of Goldberg[7] to the case in which the concentration at the meniscus does not become zero during a sedimentation experiment, one arrives at the equation

$$c_t x_b^2 - c_{x_0} x_0^2 = \int_{x_0}^{X} x^2 \frac{dc}{dx}\, dx \qquad (1)$$

in place of his equation 59. Here x is the distance from the axis of rotation, x_0 is the position of the meniscus, x_b is the position the boundary would have if there were no diffusion of the sedimenting solute and if all the sedimenting particles had identical sedimentation coefficients, X is an arbitrary plane in the "plateau region" (*i.e.*, that part of the cell where concentration is independent of x), c is the concentration of the solute, with c_{x_0} corresponding to that at the meniscus and c_t to that in the plateau region at time t.

By substituting the expressions

$$c_t = c_0 e^{-2s\omega^2 t} \qquad (2)$$

$$x_b = x_0 e^{s\omega^2 t} \qquad (3)$$

$$c_{x_0} = c_t - \int_{x_0}^{X} \frac{dc}{dx}\, dx \qquad (4)$$

into equation 1 and rearranging one obtains

$$e^{-2s\omega^2 t} = 1 - \frac{1}{c_0 x_0^2} \int_{x_0}^{X} (x^2 - x_0^2) \frac{dc}{dx}\, dx \qquad (5)$$

Here ω is the angular velocity of the rotor. Except for rearrangement this expression is identical with equation 3 of Baldwin.[6] It can also be obtained from equation 6a of Gutfreund and Ogston[5] by changing the order of integration in the double integral and integrating once. Taking logarithms of each side of equation 5 one obtains

$$\frac{-2s\omega^2 t}{2.303} = \log\left[1 - \frac{1}{c_0 x_0^2} \int_{x_0}^{X} (x^2 - x_0^2) \frac{dc}{dx}\, dx\right] \qquad (6)$$

When s is very small this equation may be replaced by the statement

(7) R. J. Goldberg, *J. Phys. Chem.*, **57**, 194 (1953).

$$2s\omega^2 t = \frac{1}{c_0 x_0^2}\int_{x_0}^{X}(x^2 - x_0^2)\frac{dc}{dx}dx \qquad (7)$$

The use of this equation for the computation of s introduces an error of less than 0.6% when $s < 0.4$ S, $\omega = 2000\ \pi$ radians per second (60,000 r.p.m.), and $t = 3600$ seconds (1 hour).

When the refractive index increment of the solute is a linear function of the concentration c_0 and dc/dx may be replaced by $(n_2 - n_1)$ and dn/dx, respectively, we may write

$$\frac{-2s\omega^2 t}{2.303} = \log\left[1 - \frac{1}{(n_2 - n_1)x_0^2}\int_{x_0}^{X}(x^2 - x_0^2)\frac{dn}{dx}dx\right] \qquad (8)$$

and

$$2s\omega^2 t = \frac{1}{(n_2 - n_1)x_0^2}\int_{x_0}^{X}(x^2 - x_0^2)\frac{dn}{dx}dx \qquad (9)$$

Here n is refractive index, n_1 being that of the solvent and n_2 that of the solution.

The sedimentation coefficient, s, described in these equations is the value which corresponds to the solute concentration in what we have termed the plateau region of the cell. Since s is a function of concentration it will be time dependent during a single experiment since c_t is changing according to equation 2.

When more than one component is sedimenting in the ultracentrifuge cell the preceding equations will hold for each individual component. By making the appropriate summations and neglecting the small change in the term $\sum_i c_i s_i / \sum_i c_i$ (where i refers to the individual components[8]), one arrives at expressions for a multicomponent system which are identical with equations 6, 7, 8 and 9 except that the s is now to be replaced by the weight average sedimentation coefficient, $\bar{s}$, of the original solution.

Experimental

The refractive index increments of the solutions were measured with the refractometer constructed by Dismukes and Alberty.[9]

The comparator used was a Gaertner Toolmakers Microscope. Two micrometers which moved in the x and y directions could be read to 0.002 mm. and a third which rotated the stage, could be read to one minute.

An oil turbine ultracentrifuge (Svedberg) equipped with Philpot–Svensson schlieren optical system served for the sedimentation experiments. In the optical path the usual diagonal knife edge was replaced by a diagonal hair of 0.0025 inch in diameter. A vertical hair was placed about one inch in front of the position of the photographic plate; its image aided in the alignment of the photographs on the stage of the comparator. All of the characteristic constants of the machine and its optical system were redetermined for use in the computations. All experiments were performed at a constant rotor speed of 60,000 r.p.m.

Special care was taken to determine the temperature of the rotor. The reading of the radiation thermocouple which is mounted in the casing of the ultracentrifuge was calibrated by the method of Biancheria and Kegeles,[10] using purified diphenyl ether in the cell under the normal operation conditions of a sedimentation experiment. The difference between the temperature of the cell, calculated according to the equation given by these investigators[11]

$$T = 26.80 + 0.02797 \times 10^{-6}\frac{\rho\omega^2}{2}(X^2 - X_0^2) \qquad (10)$$

and the reading of the thermocouple was found to increase with time during the first hour after the attainment of full speed, but was thereafter substantially constant. Since the photographs used in the computation of the results had to be taken during this hour period it was necessary to construct a curve which would give the thermocouple correction as a function of time.

The average temperature of the contents of the cell during a sedimentation experiment was obtained as follows. The photographs were taken at the end of the usual constant intervals of time. The temperature of the casing thermocouple was read at the beginning of each exposure. The temperature correction for the time corresponding to each exposure was obtained from the calibration curve and added to the corresponding thermocouple readings. The mean of these corrected temperature values was used for the average temperature of the cell during the experiment. Such a procedure is arbitrary.

In order to test the reliability of the calibration procedure two independent determinations of the sedimentation coefficient of Armour bovine serum albumin were carried out. The logarithm of the distance from the axis of rotation to the maximum of the refractive index gradient was plotted as a function of time in the usual manner. The slope of the line was determined by the method of least squares and from it the sedimentation coefficient was computed. The temperature calibration curve was used in finding the average temperature of the cell during the experiment and in the correction to an hypothetical transport in water medium at 20°. The computation of $s_{20,w}$ made use of the standard equation

$$s_{20,w} = s_{t,s}\frac{\eta_{t,s}}{\eta_{20,w}}\frac{(1 - \bar{V}\rho)_{20,w}}{(1 - \bar{V}\rho)_{t,s}} \qquad (11)$$

Here $\bar{V}$ is the partial specific volume of the solute, η is the viscosity, and ρ is the density of the solvent. The subscripts refer to the actual solvent at the temperature at which the experiment was carried out (t,s), and to water at 20° (20,w). The $s_{20,w}$ values for the serum albumin as obtained are compared with other recent literature values in Table I.

TABLE I

SEDIMENTATION COEFFICIENT OF BOVINE SERUM ALBUMIN

Author(s)	$s_{20,w}$ Svedberg units	Concn., %
Shulman[12]	4.16[a]	1.0 (interpolated)
Kegeles and Gutter[13]	4.18[a]	1.15
Kegeles and Gutter[13]	4.19[a]	0.83
Taylor[14]	4.18[a]	1.0 (interpolated)
This work	4.16	1.0
This work	4.09	1.0

[a] A Spinco ultracentrifuge was used in all of these experiments. As suggested by Biancheria and Kegeles[10] the $s_{20,w}$ values have been corrected for the adiabatic cooling of the rotor; 0.08 S was added to each of the figures which occur in the literature.

Materials.—Sedimentation studies were carried out with glucose, sucrose, raffinose, cyclohexaamylose, amyloheptaose and cycloöctaamylose. The highest quality glucose and sucrose obtainable from the National Bureau of Standards was used. Raffinose pentahydrate, C.P. grade, was obtained from Pfanstiehl Chemical Company; all data, however, refer to the anhydrous material. Cyclohexaamylose and cycloöctaamylose in the form of propanol complexes and amyloheptaose in pure form were obtained from Dr. Dexter French, Ames, Iowa.

The specific refractive index increments of raffinose and amyloheptaose were determined experimentally. Corresponding data for glucose and for raffinose were calculated from data of Longsworth.[15] This investigator divided the number of fringes found in the integral fringe diffusiometer

(8) When two components having s's of 0.2 and 0.8 S are present in equal concentration and the rotor speed is 60,000 r.p.m., this term will decrease by 0.6% in one hour.

(9) E. B. Dismukes and R. A. Alberty, THIS JOURNAL, **75**, 809 (1953).

(10) A. Biancheria and G. Kegeles, *ibid.*, **76**, 3737 (1954).

(11) In the equation T is the temperature, ρ is the density of liquid diphenyl ether, X is the position of the liquid–solid meniscus and X_0 is that of the air–liquid meniscus.

(12) S. Shulman, *Arch. Biochem. Biophys.*, **44**, 230 (1953).

(13) G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951).

(14) J. F. Taylor, *Arch. Biochem. Biophys.*, **36**, 357 (1952).

(15) L. G. Longsworth, THIS JOURNAL, **75**, 5705 (1953).

by the concentration and obtained figures for glucose, sucrose and raffinose which are proportional to their respective refractive index increments. Taking the specific refractive index increment of sucrose reported by Gosting and Morris[16] as a reference, the increments for glucose and raffinose could then be computed. The values obtained for raffinose in the two instances agree very closely (Table II).

The value used for the partial specific volume of sucrose was that reported by Gosting and Morris. For all other compounds this quantity was computed from the partial molal volumes given by Longsworth.[17,15]

Solutions for all experiments with glucose, sucrose and raffinose, and for two experiments with amyloheptaose were made up by weight. The concentration of the solute in grams per milliliter at 25°, was calculated from the partial specific volumes and the density of water. The solutions of the samples which were in the form of a propanol complex were made up in the following way. From 20 to 30 mg. of the complex was weighed into a weighing bottle. A small jet of steam was directed onto one sample from a piece of glass tubing which had been drawn to an inside diameter of about one millimeter. The tip of the glass tubing was placed near the bottom of the bottle so that when a few drops of water had condensed the steam would then bubble through the solution and carry off the propanol. The flow of steam was continued until sufficient water had condensed to make the concentration of the solute approximately 1%. In this manner a solution free from propanol was obtained. The refractive index increment of the solute was then determined with the refractometer.

Preparation of the Cell.—When an experiment was to be performed the ultracentrifuge cell was first scrupulously cleaned and rinsed out with several small portions of the solution under investigation and filled to a level such that the meniscus would be just visible when the rotor was turning at full speed. The remainder of the cell was filled with paraffin oil. The cell was placed in the rotor which was then quickly accelerated to a speed of 60,000 r.p.m.

It was difficult to perform suitable experiments with cyclooctaamylose. Anomalous gradients often appeared during acceleration even after many "rinsing out" operations, in and out of the ultracentrifuge. It was found that the anomalous gradients were less likely to appear a second time if the experiment was repeated as soon as possible. Because of this difficulty more experiments with this compound were not attempted even though the results of the two that were completed do not agree very well.

Photographs.—Eastman Kodak spectroscopic plates, 103a-G, were used. Exposure time was 30 seconds in all cases. The plates were developed for five minutes with developer D-11 which was initially cooled to 18°. The first photograph of each experiment was taken from 5 to 15 minutes after full speed was attained; succeeding pictures were taken at 5, 10 or 15 minute intervals. In the subsequent use of these photographs, comparisons were always made with photographic records taken under identical conditions with solvent only in the cell. No significant change was ever observed in the position of the base lines.

It may be mentioned here that a refractive index gradient tended to blur the image of the diagonal hair; the greater the gradient, the greater was the blurring. The blurring was also increased by raising the angle of the diagonal hair. For convenience only angles of 4, 9, 14 and 19° were used. When a photograph was to be taken the diagonal hair was set at the highest of these angles which would give a well defined image. With a very low molecular weight compound such as sucrose, an angle of 19° could be used. In most experiments, however, the maximum angle was 9°.

Evaluation.—In the evaluation of the experiments all known precautions were taken in the precise alignment of the photographic plates on the stage of the Gaertner comparator, and with the subsequent measurements and computations. It was necessary to locate: (a) the positions of the two edges of the reference bar, (b) the top of the picture, corresponding to the top of the cell, (c) the center of the image of the meniscus, and its distance from the center of the reference bar to give x_0, and (d) the positions of the center of the image of the diagonal hair at integral numbers of half millimeters from the center of the reference bar in an over-all interval which extended from the meniscus to a point well beyond the limit of the refractive index gradient. (In the case of the base line pictures the corresponding interval extended from the top of the photograph to a point which was about two-thirds of the way to the bottom. These readings from the base line pictures were subtracted, interval by interval).

The position of the limit of the refractive index gradient could be located to within 0.5 mm. Displacements of the center of the image of the diagonal hair (due to the refractive index gradient in the cell) as a function of the position in the cell were thus made available. A plot of this displacement as a function of position on the photographic plate was made. Figure 1A is an example of such a plot. Frequently, arithmetical errors were detected from this plot by noting any marked deviation of the points from a smooth curve.

The displacements were converted to concentration gradient (or refractive index gradient). Curve I of Fig. 1B is an example of a plot of refractive index gradient as a function of position on the photographic plate. The value of x, and thence the value of $(x^2 - x_0^2)$ was computed for each point at which the refractive index gradient had been found, as in curve II of Fig. 1B. Since the readings were always taken at an integral number of half millimeters from the center of the reference bar the same table of x^2 could be used for all experiments. The value of dc/dx (or dn/dx) at each point was then multiplied by the corresponding value of $(x^2 - x_0^2)$ and the product was plotted against its respective position on the photographic plate. This curve goes to zero at both ends of the plot; at the meniscus $(x^2 - x_0^2) = 0$ and in the plateau region dc/dx (or dn/dx) $= 0$. An example of such a plot is shown in Fig. 1C. It may be noted that values of $(x^2 - x_0^2)$ were computed from a given figure for x_0; therefore, they fall on a perfectly smooth curve. The values of dc/dx (or dn/dx), however, are computed from the measured displacements and do not fall on a smooth curve as may be seen in Figure 1B. Any deviation from a smooth curve in the plot of the product is, therefore, due to errors in the values obtained for the displacements. One can see that a given error in a displacement near the meniscus will result in a relatively small percentage error in the product whereas the same error in a displacement at the other end of the plot will result in a much larger percentage error. Since the absolute error in measurements is the same for all displacements one would expect that near the meniscus the products would fall more closely to a smooth curve than at the other end of the plot. This is evident in Fig. 1C.

The area under the curve obtained by plotting dc/dx $(x^2 - x_0^2)$ (or dn/dx $(x^2 - x_0^2)$) as a function of position on the photographic plate was measured with a planimeter, and by application of the appropriate magnification factors was converted to the actual value of the integral in equation 6 or 7 (or equation 8 or 9). This value was then divided by $c_0x_0^2$ (or $(n_2 - n_1)x_0^2$). This procedure was repeated for each picture which was then taken during the experiment. If the sedimentation coefficient, s, being measured was less than 0.4 S, the value obtained from each picture for the right hand side of equation 7 (or 9) was plotted as a function of time. The slope $(=2s\omega^2)$ of the straight line through the points was found by the method of least squares and from it s was computed. If the sedimentation coefficient being investigated was greater than 0.4 S the values of the right-hand side of equation 6 (or 8) were plotted against time as in Fig. 2. As before the sedimentation coefficient was computed from the slope $(= 2s\omega^2/2.303)$ of the straight line through these points. The adjusted sedimentation coefficient, $s_{20,w}$, was then found by the use of equation 11.

The standard error of the slope of the straight line through the points in the plot of equation 6, 7, 8 or 9 (*e.g.*, Fig. 2) was computed[18] and was converted to the standard error of the $s_{20,w}$ in the same way that the slope itself was converted to $s_{20,w}$.

The sedimentation coefficients of glucose, sucrose and raffinose were calculated by the use of equation 7; of amyloheptaose by equation 6 in two experiments and by equation 8 in a third; and of cyclohexaamylose and cycloöctaamylose by the use of equation 8.

Since the available diffusion data for these compounds were

(16) L. J. Gosting and M. S. Morris, THIS JOURNAL, **71**, 1998 (1949).

(17) L. G. Longsworth, private communication.

(18) R. A. Fisher, "Statistical Methods for Research Workers," Oliver and Boyd Ltd., Edinburgh, 1946, 10th edition, p. 138.

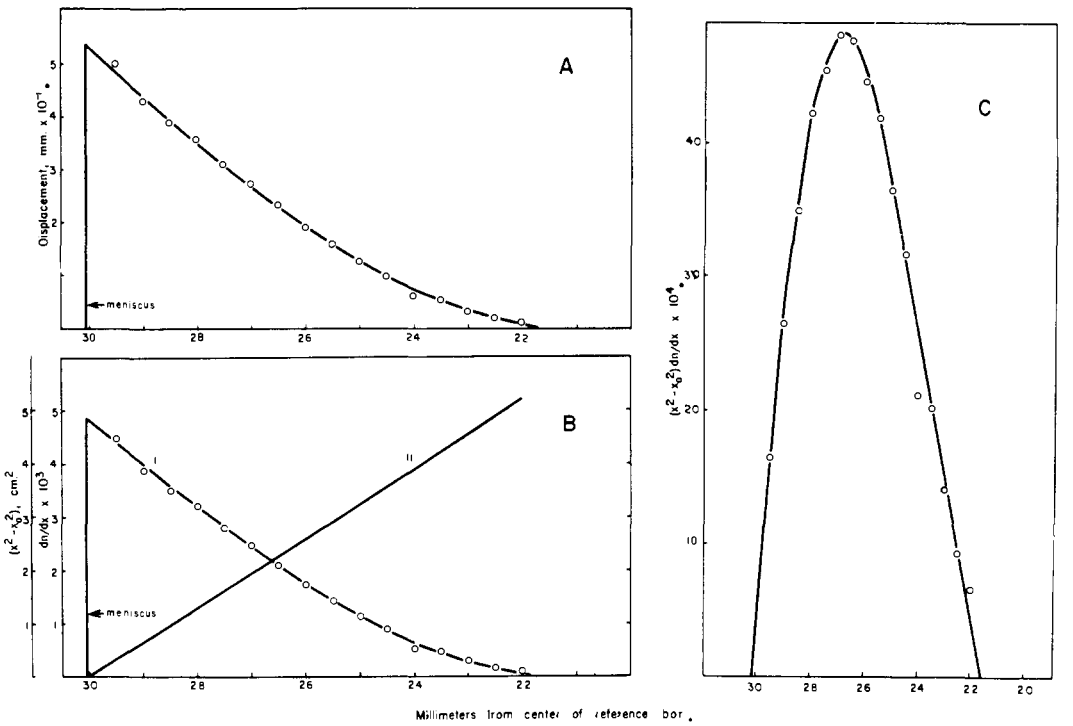

Fig. 1.—(A) The displacement of the center of the image of the diagonal hair; (B) dn/dx (curve I) and $(x^2 - x_0^2)$ (curve II); and (C) $(x^2 - x_0^2)dn/dx$ for amyloheptaose solution, expt. 127; all plotted as a function of distance from the center of the reference bar.

obtained at 25°, the theoretical s values at this temperature were calculated by the equation

$$s = \frac{MD(1 - \bar{V}\rho)}{RT} \qquad (12)$$

and converted to $s_{20,w}$ by equation 11.

In equation 12 M is the molecular weight, D is the diffusion coefficient and R is the molar gas content.

Results

The results of the experiments are presented in Tables II and III. In Table II are included the specific refractive index increment, partial specific volume and diffusion coefficient data and the theoretically calculated sedimentation coefficients. In Table III are collected the results of the sedimentation experiments. From the data of column 7 one sees that the experimental sedimentation coefficients differ from the theoretical values by more than 0.04 S in only three cases. The greatest deviation is in the case of an experiment with cyclooctaämylose as a solute. It is believed that the sedimentation coefficient obtained from this experiment may be in error since it is so far from the theoretical value. It will be remembered that considerable difficulty was encountered in avoiding anomalous refractive index gradients in the sedimentation experiments with solutions of this compound. The low value obtained could be due to a slight but undetected anomalous refractive index gradient in the ultracentrifuge cell. Five of

TABLE II

PHYSICAL PROPERTIES OF SUGARS AND DEXTRINS

	Mol. wt. 1	Partial sp. vol. 2	Mean soln. concn. c, (wt. %) at which diff. coeff. was obsd. 3	Diffusion coefficient at concn. c and 25° 4	$s_{20,w}$, Svedberg units (calcd.) 5	Spec. refractive increment, g.$^{-1}$ ml.$^{-1}$ 6
Glucose	180	0.621[a]	0.390	6.728[d]	0.169	0.1421[a]
Sucrose	342	.618[b]	1.99	5.070[b,c]	.236	.1430[b,f]
Raffinose	504	.608[a]	0.380	4.339[d]	.305	.1467[a]
						.1468[g]
Cyclohexaamylose	972	.623[a,c]	.39	3.443[e]	.452	.....
Amyloheptaose[h]	1152	.620[c]	...	.....	...	.1435[g]
Cycloöctaamylose	1296	.621[a]	.39	3.000[e]	.529	.....

[a] Calculated from data of Longsworth.[15,17] [b] Gosting and Morris.[18] [c] Interpolated. [d] Longsworth.[15] [e] Longsworth.[17] [f] Average of values for several concentrations. [g] Experimentally determined. [h] Diffusion data for this compound are not available.

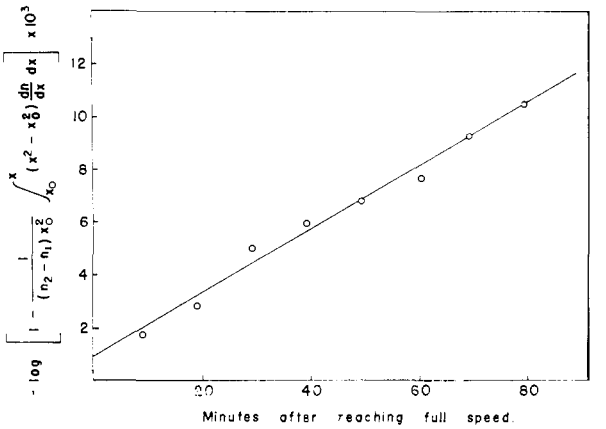

Fig. 2.—Logarithmic term of equation 8 as a function of time for a sedimentation experiment with amyloheptaose (Expt. 127).

TABLE III

RESULTS OF SEDIMENTATION VELOCITY EXPERIMENTS WITH SUGARS AND DEXTRINS

	Expt. no. 1	Concn., wt. % 2	$(n_2 - n_1) \times 10^3$ 3	$s_{20,w}$, Svedberg units 4	Stand. error 5	Av. $s_{20,w}$ for each compd. 6	Dev. of individual $s_{20,w}$ from theor. value 7
Glucose	79	4.94		0.11_2	0.00_9	0.13_4	-0.05_7
	126	4.21		$.15_6$	$.01_1$		$-.01_3$
Sucrose	55	1.99		$.22_4$	$.03_4$	$.22_8$	$-.01_2$
	56	1.99		$.23_2$	$.02_2$		$-.00_4$
Raffinose	57	2.05		$.30_0$	$.00_5$	$.27_7$	$-.00_5$
	74	2.09		$.25_5$	$.01_4$		$-.05_0$
	125	1.73		$.27_5$	$.01_2$		$-.03_0$
Cyclohexa-amylose	120	(0.99)[a]	1.42	$.48_9$	$.03_1$	$.48_0$	$.03_7$
	121	(0.99)[a]	1.42	$.47_0$	$.02_8$		$.01_8$
Amyloheptaose	80	1.02		$.44_2$	$.04_0$	$.48_8$	.....
	103	1.05		$.42_5$	$.03_6$		
	127	(1.19)[a]	1.71	$.47_6$	$.02_5$		.....
Cycloöcta-amylose	123	(0.91)[a]	1.31	$.53_4$	$.03_8$	$.49_2$	$.00_5$
	124	(1.01)[a]	1.45	$.44_9$	$.02_6$		$-.08_0$

[a] Calculated from $(n_2 - n_1)$ assuming specific refractive index increment is that of amyloheptaose.

the figures (absolute values) in column 7 are greater than the corresponding standard errors. All but one of these five figures are negative, thus showing a tendency for the experimentally determined sedimentation rate to be below the theoretical values. The mean of the experimental values for each compound differs from the theoretical value by more than 0.03 for only cycloöctoamylose and glucose.

Discussion

Considering for the moment only the behavior of the open chain compounds, we have plotted, Fig. 3, the adjusted sedimentation coefficient $s_{20,w}$ as a function of $M^{0.56}$. The straight line is accurately represented by the relationship, $s_{20,w} = 0.0087M^{0.56}$.

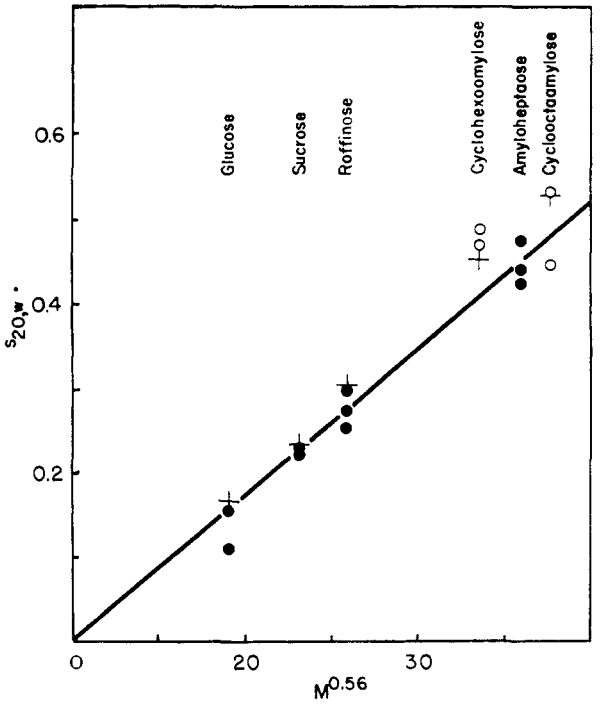

Fig. 3.—$s_{20,w}$ plotted as a function of $M^{0.56}$: O, cyclic compounds; ●, non-cyclic compounds; +, calculated from M and D.

The theoretical value expected for the exponent in the sedimentation coefficient–molecular weight relationship ($s = KM^a$) is 0.67 for spheres, 0.4–0.5 for random coiled chains, and zero for free draining molecules.[19] It is seen that the actual experimental exponent for the open-chain compounds lies between the value for spheres and that for random coiled chains, thus excluding the possibility of the free-draining model.

It is interesting to compare the value of the exponent obtained in these experiments with that found by Williams and Saunders[20] in the case of a dextran sample. These bacterial polysaccharides are known to answer the description of a branched chain molecule. They obtained a linear relationship when sedimentation "constant" (*i.e.*, the limit of s as the concentration of the solute approaches zero) was plotted against $M^{0.5}$. Their dextran fraction possessed molecular weights in the range 10,000 to 200,000. This suggests that in the case of polysaccharides the dependence of s on M does not vary a great deal all the way from a fairly large and complex molecule down to a monomeric unit.

One might expect that the cyclic polysaccharides would behave more like spheres than the open chain compounds. Except for the one experiment with cycloöctaamylose, the points for the cyclic compounds, also included in Fig. 3, seem to lie above the straight line shown, in spite of the tendency for the experimentally determined sedimentation coefficients to be below the theoretical values. Even so, the accuracy of the experiments is hardly sufficient to warrant definite decision.

It should be kept in mind that the sedimentation coefficients were obtained at a number of different concentrations and that except for sucrose, these concentrations were not the same as those for which the diffusion coefficients were observed (*cf.* Table II). The effect of this factor on the differences between the theoretical and experimental sedimentation coefficient is negligible. For instance, Longworth's data show that by reducing the mean concentration of cycloöctaamylose from 0.39 to 0.20 g. per 100 ml. the diffusion coefficient is increased by only about 0.3%. Thus at the concentration used in the sedimentation experiments (*i.e.*, 1 g. per 100 ml.) the value of D would be expected to differ from that at a concentration of 0.39 g. per 100 ml. by less then 1%. The correct theoretical value for the sedimentation coefficient of this compound which should be compared with the experimental results in Table III, might be something less than 0.005 S lower than that given in Table II. This effect would be expected to be of the same order of magnitude in the case of cyclohexaamylose as in that of cycloöctaamylose. Assuming the same dependence of diffusion coefficient with concentration as that found for sucrose by Gosting and Morris[16] it can be shown that this same discrepancy is less than 0.005 S in the case of raffinose and less than 0.01 S in that of glucose. Since these

(19) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, Chapter XIV.

(20) J. W. Williams and W. M. Saunders, *J. Phys. Chem.*, **58**, 854 (1954).

errors are so small and their values could not be known with precision, no attempt was made to correct for them.

By the procedure described in this paper it is possible in one experiment to estimate a sedimentation coefficient in the range 0.2 to 1 S to within 0.05 S of its true value. If three or more experiments are carried out and the results are averaged, one could rely on the value obtained to ± 0.03 S with some certainty. This corresponds to an error of 15% in a coefficient of 0.2 S and of 6% in one of 0.5 S.

Such data may be combined with diffusion data to obtain an estimate of the molecular weight of molecules of small and intermediate size such as are often isolated from biological sources. The error in this estimate would be nearly the same as that in the sedimentation coefficient, if the material were monodisperse. In the case of polydisperse or paucidisperse materials the estimate would be much poorer. In cases where such a molecule is known to be polymeric in character and the equivalent weight of the monomeric unit is known, the molecular weight of the polymer could be determined with sufficient accuracy to provide the degree of polymerization to a reasonable degree of certainty.

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MADISON, WISCONSIN

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, UNIVERSITY OF TOKYO, AND THE TOKUGAWA INSTITUTE FOR BIOLOGICAL RESEARCH]

Dielectric Properties of Hemoglobin. I. Studies at 1 Megacycle

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Dielectric properties of hemoglobin, oxyhemoglobin, carboxyhemoglobin and methemoglobin were studied by the resonance method using a frequency of 1 megacycle. A characteristic change in the dielectric increment was observed when hemoglobin was subjected to progressive oxygenation, and indeed it was found that at temperatures lower than 30°, the curve relating dielectric increment to oxygen partial pressure showed two distinct maxima. This fact indicates that in the process of oxygenation, there exist four steps having alternately increasing and decreasing effects on the dipole moment of the hemoglobin molecule. At 37°, however, the first maximum of the curve disappeared, thus giving rise to a single peak curve. Assuming that the consecutive steps of increasing and decreasing increment values correspond to the formation of intermediary compounds of progressing oxygenation, an O_2-saturation curve of the whole hemoglobin molecule was constructed based on the data obtained at 15°. It was found that the curve thus drawn coincided almost completely with the O_2-saturation curve which has been determined spectrophotometrically at the same temperature. These results confirm the fact that the four consecutive steps in the increment–O_2 curve correspond to the formation of intermediary compounds of different grade of oxygenation. The effect of dielectric constant of the medium upon the dielectric increment of the hemoglobin oxyhemoglobin system also was investigated.

I. Introduction

Although the dielectric properties of carboxyhemoglobin have been studied by Errera[2] and later by Oncley,[3] no investigation in the same line has ever been attempted for other derivatives of hemoglobin. The acid–base titration studies performed by German and Wyman[4] have shown that in the molecules of hemoglobin and oxyhemoglobin there are some ionizable groups closely linked to hemes. Since the states of these groups are not independent of the chemical state of hemes, we may suspect that the modifications in hemes brought about by their reaction with oxygen or carbon monoxide might possibly lead to a change in the charge distribution of the hemoglobin molecule. It may not be extravagant to speculate that such a change in the charge distribution would be detectable as a modification in the dipole moment of the whole hemoglobin molecule or in its behavior in dielectric dispersion. Thus, we attempted in this work to measure the effect of oxygen and carbon monoxide on the dielectric properties of hemoglobin.

One of the difficulties in measuring the dielectric constants of proteins in aqueous solutions is the elimination of electrolytic impurities which enhance the electric conductivity and render the dielectric measurement at low frequencies difficult or even impossible. It has been found[5-8] that protein solutions usually show anomalous dispersion at frequencies in the neighborhood of a few megacycles, below which the dielectric measurements must be carried out.[9] Therefore, a great deal of effort was made to remove the electrolytic impurities by repeating the purification until the conductivity of the solution was rendered negligible in

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(3) J. L. Oncley, THIS JOURNAL, **60**, 1115 (1938).

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(5) J. D. Ferry and J. L. Oncley, THIS JOURNAL, **60**, 1123 (1938).

(6) J. D. Ferry and J. L. Oncley, *ibid.*, **63**, 272 (1941).

(7) M. A. Elliott and J. W. Williams, *ibid.*, **61**, 718 (1939).

(8) H. O. Marcy and J. Wyman, *ibid.*, **64**, 638 (1942).

(9) The lower the frequency, the larger is the value of correction term for conductance.