Schoenberg, Riseman and Eirich[31] in high molecular weight polystyrene. The data are not consistent with the second possibility.

It does not seem likely that a theoretical solution of this problem will be achieved in the near future. Some light would be shed on the matter if an experimental study similar to this one could be carried out on some quite different molecule also behaving as a flexible rod, such as cellulose or cellulose derivatives.

In further support of the kinked rod model, it may be seen from Table III that the optical factor $(g_1 - g_2)$ decreases as the size of the molecule increases. This decrease with size, if it is not an inherent optical property of rods of dimension greater than the wave length of the light used, is plausibly attributable to the greater bending of the longer molecules.

When the limitation of flow birefringence calculation to the case of a rigid particle is considered, one must feel that the theoretical basis of light scattering as a tool for macromolecular studies is better founded. When the two methods are in disagreement, a reflection on flow birefringence is implied. However, this should not blind one to the much greater sensitivity of flow birefringence for the study of high molecular weight DNA preparations. For example, the differences between preparations V and S-VII appear to be tremendous by the standards of flow birefringence; by light scattering they are barely beyond experimental error.

An analysis by Doty and co-workers[5] of the flow birefringence and light scattering results discussed here, as well as viscosity and sedimentation data and electron microscope photographs, has led them to the conclusion that a model somewhere between a random coil and a kinked rod is capable of accounting for all the observed properties, while a rigid rod or ellipsoid leads to striking inconsistencies. Readers are referred to their paper for details.

Acknowledgments.—Though all the data reported here was obtained at Harvard University, a considerable amount of exploratory work on flow birefringence of DNA was performed by M. G. during the tenure of a National Institutes of Health Postdoctoral Fellowship at Brooklyn Polytechnic Institute. Particular thanks go to Dr. K. G. Stern for his help and advice at this time. Both of us would like to express our gratitude to Professor Paul M. Doty for his interest, help and encouragement. Part of the financial support of this work was provided by U. S. Public Health Service Grant G-3286. NOTE ADDED IN PROOF: We have been informed that Dr. N. S. Simmons, who provided the DNA sample referred to as N.S., designates his preparations by the letters A, B, C, ... and that the preparation used in this work was preparation B.

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(31) M. D. Schoenberg, J. Riseman and F. R. Eirich, *J. Coll. Sci.*, **5**, 393 (1950).

NOTES

Ultracentrifugal Determination of Molecular Weights of Small Molecules by the Archibald Procedure[1]

BY RAYMOND A. BROWN, DAVID KRITCHEVSKY AND MAURICE DAVIES

RECEIVED OCTOBER 27, 1953

Recent advances in analytical techniques have made it possible to carry on detailed structural studies of increasingly complex molecules. Substances whose sizes are intermediate to the macromolecules and the simple organic molecules are becoming the subject of intensive study. In these studies the molecular weight and homogeneity are often of considerable importance. Although there have been several methods devised for the determination of molecular weights of small molecules,[2a,b] there is at present no convenient established method for determining both the molecular weight and homogeneity of substances whose molecular weights are under 10,000.

Archibald[3] has derived a procedure for determining these parameters which is both convenient and applicable to substances of low molecular weight. Porath[4] has used the procedure to determine the molecular weight of bacitracin. However, the procedure has not been applied to any well defined substance and its reliability has not yet been evaluated.

A standard substance has been analyzed in the Spinco model E ultracentrifuge by means of the theory developed by Archibald, and the results are the subject of this communication. In order to check the results obtained by the Archibald procedure the sedimentation constant of the substance has also been determined by velocity sedimentation in the synthetic boundary ultracentrifuge cell of Pickels, *et al.*[2] These results have been supplemented by diffusion measurements.

Experimental

Material.—The saponin, digitonin, has been chosen as the standard substance. This material is a solid, colorless, non-ionizing substance whose formula weight has been reliably established as 1229.3.[5] The digitonin used in this work was the product available commercially from Hofmann–LaRoche. The melting point, 240° (S.236), corresponds to that given in the literature for the pure sub-

(1) Presented at the 1953 Fall Meeting of the American Chemical Society, Chicago, Ill., on September 6, 1953.

(2) (a) H. Gutfreund and A. G. Ogston, *Biochem. J.*, **44**, 163 (1949); (b) E. G. Pickels, W. F. Harrington and H. K Schachman, *Proc. Natl. Acad. Sci.*, **38**, 943 (1952).

(3) W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).

(4) J. Porath, *Acta Chem. Scand.*, **6**, 1237 (1952).

(5) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, p. 580.

stance.[5-7] Crystallization from 85% ethanol did not alter this melting point.

Refractive Index Increment.—The refractive index increment of digitonin (0.126 cm.^{3}/g.) was estimated by comparing the areas produced on an electrophoresis plate in a Perkin–Elmer apparatus by a solution of Armour crystalline bovine serum albumin in distilled water and by a digitonin solution containing 63% ethanol by weight. Perlmann's[8] values for the refractive index increment and percentage nitrogen of bovine serum albumin were used for the calculation.

Partial Specific Volume.—The partial specific volume of digitonin in 63% by weight ethanol solutions was calculated from the weights of a pycnometer filled with ethanol solutions containing different concentrations of digitonin and found to be 0.69_9.[9]

Ultracentrifugal Analysis.—The Spinco model E ultracentrifuge equipped with an inclined wire was used for this study. An ethanol solution (0.70 ml.) containing 63% ethanol by weight and 0.658 g. of digitonin per 100 ml. was placed in a centrifuge cell with a 4° sector. The centrifuge was run with refrigeration at 52,640 r.p.m. for 576 minutes with pictures being taken at 32-minute intervals. The thermocouple temperature was 23.3° at the start and 21.6° at the end of the run. Taking into account the adiabatic cooling of the rotor[10] the average temperature during the run was assumed to be 21.7°. Upon completion of the run the cell was refilled with solvent and the run repeated. The centrifuge patterns were projected and traced on graph paper at a magnification factor of 11. Both the solution and the solvent patterns for the same elapsed time were traced on the same graph, care being taken to obtain a precise superposition of the reference lines. Curves for the base line and solution were constructed by bisecting the inclined wire image. The refractive index gradient due to the digitonin was assumed to be given by the difference between the solvent and solution patterns.

Distances on the tracings were converted to the refractive index gradient in the cell by the use of the appropriate optical constants.[11] The refractive index gradient curve was divided into equal intervals and the concentration gradient evaluated at the center of each interval by means of refractive index increment of digitonin. The concentrations of these points were calculated from equations I and II.

$$N_{j+1} = N_b - \sum_{i=1}^{j}\left(\frac{\partial N}{\partial R}\right)_i \Delta R_i - \frac{1}{2}\left(\frac{\partial N}{\partial R}\right)_{j+1} \Delta R_{j+1}; \quad j = 1, 2, \ldots K \quad \text{(I)}$$

$$\sum_{i=1}^{K} R_i N_i \Delta R_i = \frac{N_0}{2}[R_b^2 - R_a^2] \quad \text{(II)}$$

where R_a and R_b are the radial distances to the meniscus and bottom of the cell, respectively, N_0 is the initial concentration and N_b is the concentration at the bottom of the cell at the time of interest. Equation II is used to determine N_b. Some of the deviated light was intercepted by the bottom of the sector cup and a small portion of the gradient curve next to the bottom of the cell was not recorded on the film. This part of the curve was obtained by extrapolation. The velocity sedimentation analysis was done in the synthetic boundary cell supplied by Spinco. All runs were made with refrigeration at 59,780 r.p.m.

Results

Archibald has shown that one may obtain a parameter, δ, by extrapolating the function $\frac{1}{RN}\frac{\partial N}{\partial R}$ to R_a and R_b. δ is related to the molecular weight by equation III.

$$\delta = \frac{Mw^2(1 - \bar{V}\rho)}{RT} \quad \text{(III)}$$

If the substance is heterogeneous, the heavier molecules will be concentrated at the bottom of the cell and δ at this point will exceed the value found at the meniscus. The two values should be equal for a homogeneous substance. Table I lists the values

TABLE I

VALUES OF δ OBTAINED FROM $\frac{1}{RN}\left(\frac{\partial N}{\partial R}\right)$

Time, minutes	Meniscus	Bottom of cell
384	0.63	0.62
448	.59	.64
512	.66	.62
576	.60	.61
Average	.62	.62

of δ found at different times during the run. Figure 1 is a plot of $\frac{1}{RN}\left(\frac{\partial N}{\partial R}\right)$ *vs.* R and 576 and 384 minutes. There is excellent agreement between the values of δ found at the two ends of the cell. Archibald has

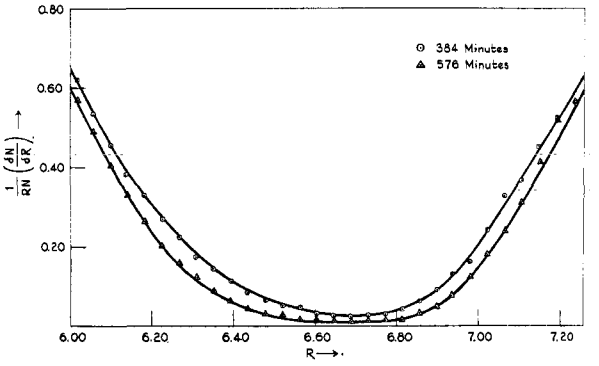

Fig. 1.

also shown that one may obtain sedimentation and diffusion constants by evaluating the integral $\int_{R_2}^{R_1} \frac{R}{N_0}\left(\frac{\partial N}{\partial t}\right) dR$ between any two points for which it does not vanish. The function $\frac{R}{N_0}\left(\frac{\partial N}{\partial t}\right)$ has been calculated from the 384- and 576-minute data and

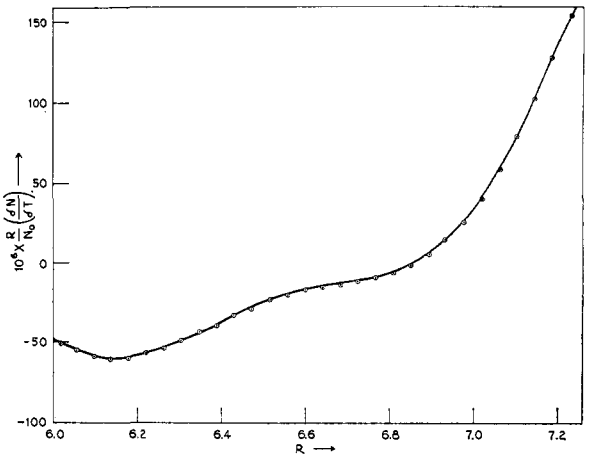

Fig. 2.

(6) H. Kiliani, *Ber.*, **24**, 339 (1891).

(7) K. Szahlender, *Arch. Pharm.*, **274**, 446 (1936).

(8) S. E. Perlmann and L. G. Longsworth, THIS JOURNAL, **70**, 2719 (1948).

(9) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, London, 1940, p. 57.

(10) D. F. Waugh and D. A. Yphantis, *Rev. Sci. Instr.*, **23**, 609 (1952).

(11) The centrifuge optical system has been calibrated by means of a special wedge cell supplied by Spinco.

the results are shown in Fig. 2. In agreement with theory, the total area under the curve is negligible, being less than 1% of the actual area without regard to sign. Measuring areas under the curve with a planimeter one finds

$$\int_{6.000}^{6.852} \frac{R}{N_0}\left(\frac{\partial N}{\partial t}\right) dR = 27.9 \times 10^{-6}$$

Using 0.62 for δ and this value, one finds 0.21×10^{-13} for the sedimentation constant and 1.0×10^{-6} for the diffusion constant. The values corrected to water at 20° are 0.41×10^{-13} and 2.5×10^{-6}, respectively. The results obtained with the synthetic boundary cell are shown in Table II. The diffusion constants were calculated from the half-widths at the inflection point of the centrifuge patterns.

TABLE II

Concn., g./100 ml.	Diffusion constant[a] $\times 10^6$	Sedimentation constant[a] $\times 10^{13}$
1.00	2.4	0.41
0.655	2.2	.38
0.353	2.4	.37

[a] Corrected to water at 20°.

Discussion

A value of 0.62 for δ corresponds to a molecular weight of 1310. There is a difference of 6.5% between this value and the formula weight of 1229. The fact that a repeat run gave a value of 1300 with equal values of δ at the two ends of the cell suggests that this difference is not entirely due to experimental error.[12] There is an indication that the sedimentation constants determined in the synthetic boundary cell and consequently the apparent molecular weight may depend on concentration. Precipitation of the digitonin at the bottom of the cell prevented an accurate analysis of a run made with 1% solution. However, δ appeared to be of the order of 0.7 at this concentration.

The agreement between the values of δ found at the two ends of the cell suggest that if there is any dependence of the apparent molecular weight upon concentration, it is not due to aggregation but to some other deviation from ideal behavior.[13] Dr. L. G. Longsworth of the Rockefeller Institute has measured the diffusion constant of a 0.4% digitonin solution in 63% ethanol at 24.92° by the Rayleigh interference fringe method.[14] Although the peak was definitely non-Gaussian it was reasonably symmetrical. One would infer from the lack of skewing that the diffusion constant did not vary markedly over the concentration interval of the boundary. The observed diffusion constant in the 63% ethanol solution was 1.10×10^{-6}. This corresponds to a value of 2.35×10^{-6} in water at 20°.

The diffusion constants obtained in the synthetic boundary cell are in reasonable agreement with the value obtained by interferometry. There is a larger error in the first value obtained by the Archibald procedure. The value (2.6×10^{-6}) obtained in the second experiment is in even poorer agreement.

The molecular weight (1310) obtained by Dr. Longsworth's diffusion constant and the velocity sedimentation constant measured at a concentration of 0.65% is in excellent agreement with those obtained by the Archibald procedure.

In general, one would expect the molecular weights determined by the Archibald procedure to be more precise than the diffusion and sedimentation constants. The latter measurements involve the calculation of a small difference between two large numbers which is subject to large experimental errors.

There are several advantages to the Archibald procedure. If one estimates the partial specific volume and refractive index increment, a determination of the molecular weight and heterogeneity of a low molecular weight material may be made in a 2° sector cell with only a 3-mg. sample.

A preliminary analysis of peptides may thus be obtained with very small quantities of material. Once the order of magnitude of the molecular weight of a peptide is known, a precise value may be calculated from the amino acid composition. The Archibald procedure should prove a valuable tool in peptide chemistry.

Acknowledgments.—We wish to thank Dr. Longsworth for making the diffusion measurements. The centrifuge work was done with the technical assistance of Mr. Gilbert Richmond and Miss Mary Englert.

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(12) The experiments with digitonin and work with several peptides would indicate that the molecular weight values are reproducible to 2-3%.

(13) In a two-component solvent, there will be small differences in solvent density at the two ends of the cell. However, the resulting errors in δ are less than 1%.

(14) L. G. Longsworth, THIS JOURNAL, **74**, 4155 (1952).

Dimethylphosphinoborine Trimer. Mass Spectra and Thermal Decomposition[1]

BY R. E. FLORIN, L. A. WALL, F. L. MOHLER AND EDITH QUINN

RECEIVED FEBRUARY 26, 1954

In connection with the recent description[2] of the dimethylphosphinoborine trimer

$$\overline{P(CH_3)_2BH_2P(CH_3)_2BH_2P(CH_3)_2BH_2}$$

it is of interest to report the results of some mass spectrometric and pyrolytic studies on this quite unusual compound. We wish to thank Dr. A. B. Burg for kindly supplying the sample used in this work.

1. Mass Spectra

Measurements were made with a model 21-103 Consolidated Mass Spectrometer using standard operating conditions except that some spectra were obtained with the ionizing voltage reduced to low values. Crystals of the phosphinoborine trimer were evaporated directly into the reservoir of the mass spectrometer. There was no evidence of any appreciable impurity in the compound.

(1) The research reported in this paper was performed in part under the sponsorship of the Ordnance Corps, Department of the Army.

(2) Anton B. Burg and R. I. Wagner, THIS JOURNAL, **75**, 3872 (1953).