[CONTRIBUTION NO. 136 FROM THE EXPERIMENTAL STATION OF E. I. DU PONT DE NEMOURS AND COMPANY, INC.]

The Molecular Weight of Linear Macromolecules by Ultracentrifugal Analysis. I. Polymeric ω-Hydroxydecanoic Acid[1]

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The most satisfactory results for the molecular weights of macromolecules have been obtained for the proteins by Svedberg and his associates using the ultracentrifuge.[2]

The proteins, however, appear to be exceptional among the macromolecular colloids, for, as shown by the viscosity and ultracentrifugal behavior of their solutions, many of them obey the kinetic theory for spherical particles reasonably well. Presumably, therefore, the dissolved units are nearly spherical in shape. On the other hand, macromolecular colloids in general display an anomalous behavior, which is particularly evident in connection with viscosity and with diffusion. For example, the specific hydrodynamic volume, calculated from viscosity,[3] may be many times greater than the specific volume of the colloid. Chemical and physical evidence indicates that the macromolecules in many cases exist in solution as highly elongated, perhaps rod-like, perhaps string-like units, and, as has been pointed out,[4] diffusion and sedimentation methods for molecular weight might be expected to give erroneous results. In order, therefore, to test the applicability of the ultracentrifugal methods to macromolecular colloids in general, an empirical study of the behavior of long-chain molecules was carried out with a particularly well-defined synthetic macromolecular substance of simple structure and known molecular weight.

The substance selected was a highly polymerized ω-hydroxydecanoic acid prepared and described by Carothers and Van Natta.[5] From chemical evidence it is practically certain that polymerization in this case involves self-esterification, with the formation of a linear polymer having the formula $HO—[(CH_2)_9—CO—O]_x—H$. The terminal carboxyl is the only acid group in the molecule. Consequently, the acid equivalent is equal to the molecular weight, which is thus determinable by titration with alkali. The specimen used in this investigation has a molecular weight, by titration, of 25,200; *i. e.*, the molecule consists of a chain of about 150 hydroxydecanoic acid residues. A study of the viscosity of solutions of this polymer as well as polymers of lower molecular weight in *sym*-tetrachloro-

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(1) Presented at the Denver Meeting of the American Chemical Society, August, 1932.

(2) See, for instance, Svedberg, "Colloid Chemistry," A. C. S. Monograph, Chemical Catalog Co., New York, 2d ed., 1928, and many papers in THIS JOURNAL, 1923–1933.

(3) Kraemer, Chap. XX, in Taylor's "Treatise on Physical Chemistry," 2d ed., 1931, p. 1615.

(4) Kraemer and Sears, *J. Rheology*, **1**, 238 (1930).

(5) Carothers and Van Natta, THIS JOURNAL, in press.

ethane has already been published by Kraemer and Van Natta,[6] who showed that the synthetic polyester resembles the high-viscosity type of intrinsic colloid rather than the simpler proteins.

Solutions of the polymer in *sym*-tetrabromoethane were subjected to ultracentrifugal analysis under conditions suitable for measuring diffusion, sedimentation velocity, and sedimentation equilibrium, and molecular weights were calculated in the usual way. Briefly, it was found that the molecular weight by the sedimentation-equilibrium method checked that by titration, whereas diffusion gave too large a value and sedimentation velocity gave too small a value. Calculation of the relation between osmotic pressure and concentration from the sedimentation equilibrium showed that van't Hoff's equation is not obeyed at concentrations exceeding 0.2% by weight or 0.00024 normal.

Experimental Details and Results

The ultracentrifuge used in this work was built under the direction of Professor Svedberg at the University of Upsala, and has been described by Svedberg and Sjögren.[7] The concentration gradients were determined by means of the refractive-index method, and the calculations were carried out in accordance with the methods developed in Svedberg's laboratory.[8]

The tetrabromoethane used as solvent was obtained from the Dow Chemical Co. Its physical properties were as follows: d_4^{20} 2.9596, refractive index $n_{436}^{20.7}$ 1.6598. The partial specific volume of the solute over the concentration range in question was determined pycnometrically and was found to be 1.013. The relation between refractive index and concentration, required in determining concentration gradients in the centrifuging cell, was established by measurements with the Pulfrich refractometer. dn/dc, the rate of change in refractive index with concentration in weight per cent., was 0.00577 for a wave length of 436 mμ.

Figure 1 presents experimental data for one run, the successive curves representing conditions during centrifuging at six-hour intervals. The position of the maximum locates the mean position of the boundary of the solute molecules as they move from the end of the cell, and from the rate of shift of the maximum, the sedimentation velocity is calculated. As the boundary moves, it becomes less sharp, owing to diffusion, and the width of the peak increases and its height decreases. From the rate at which this occurs, the diffusion coefficient is calculated. The maximum height of Curve D on Fig. 1 is labeled $Z_{\text{max.}}$. A height h from the bottom is laid off such that $h = Z_{\text{max.}}/\sqrt{e} = 0.606\ Z_{\text{max.}}$ The half-width of the curve u at this height is connected with the diffusion coefficient D by the equation

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(6) Kraemer and Van Natta, *J. Phys. Chem.*, **36**, 3175 (1932).

(7) Svedberg and Sjögren, THIS JOURNAL, **51**, 3594 (1929).

(8) Lamm, *Z. physik. Chem.*, **A138**, 313 (1928); **A143**, 177 (1929); Stamm, THIS JOURNAL, **52**, 3047 (1930).

$Dt = u^2/2$, where t is the time in seconds from the start of the run. In passing, it may be noted that the solute sedimented toward the center of rotation on account of the higher density of the solvent, contrary to the case for most ultracentrifugal investigations hitherto published.

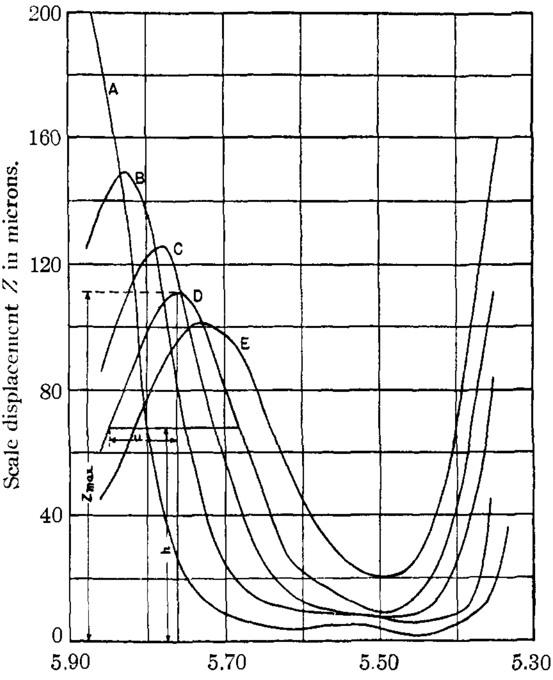

Fig. 1.—Curve A, 6 hours; Curve B, 12 hours; Curve C, 18 hours; Curve D, 24 hours; Curve E, 30 hours.

The values of the specific sedimentation velocity and the diffusion coefficient are given in Table I. From the former the apparent radius of the molecule, considered as a sphere, and the apparent molecular weight were calculated by means of Stokes's equation. Similarly, by means of Einstein's equation for the diffusion of spherical particles, an apparent molecular weight was calculated from the diffusion coefficient. A molecular weight value was also obtained by combining diffusion and sedimentation constants, as Svedberg and his associates commonly do, on the assumption that the frictional resistance offered by the liquid is the same for both cases. For this purpose the equation $M = RTs/D(1 - V\rho)$ is used. These various apparent molecular weights are also given in Table I.

The results for a sedimentation equilibrium run are shown graphically by Fig. 2, Curve I, representing an average of the displacements observed on the fifth, sixth and seventh days of centrifuging. The average devia-

TABLE I

ULTRACENTRIFUGAL ANALYSIS OF ω-HYDROXYDECANOIC ACID POLYMER IN *Sym*-TETRABROMOETHANE

	Expt. HDA-1	Expt. HDA-10	Expt. HDA-10 (corr. to 20°)
Temperature, °C.	20.2	40.2	....
Concentration, wt. %	0.199	0.108	....
Speed for sedimentation velocity (r. p. m.)	8600	12,000	....
Centrifugal force (× gravity)	4500	8900	....
Viscosity of solvent in poises	0.105	0.055	0.105
Relative viscosity of solution in tetrachloroethane[6]	1.63	1.25	....
Specific sedimentation velocity	6.98×10^{-14}	1.65×10^{-13}	8.65×10^{-14}
Diffusion coefficient (cm.²/sec.)	1.33×10^{-8}	4.13×10^{-8}	2.03×10^{-8}
Molecular weight from diffusion coeff.	8,400,000	2,400,000	....
Molecular weight from sed. velocity	5400	7600	....
Mol. wt. from sed. vel. + diff.	64,000	52,000	....
Mol. wt. from sed. equil. (speed 7200 r. p. m.)	....	27,000	....
Mol. wt. from combining wt.	25,200	25,200	....
Mol. wt. from viscosity (Staudinger's method)[6]	....	31,000	....

tion of individual points from this curve is of the order of one or two microns, with a maximum deviation of about 5 microns. The broken portions of the curve at either end are calculated scale displacements for a molecular weight of 26,000, which agrees with the solid curve in the center portion. Comparisons on a large-scale graph of calculated curves for molecular weights 26,000 and 28,000 and the observed data show that the

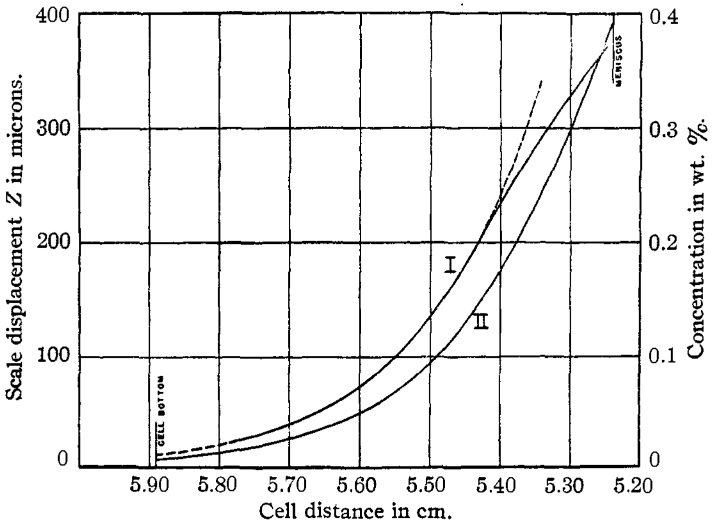

Fig. 2.—I, Scale displacement curve. II, concentration curve.

most probable molecular weight is about 27,000, and that figure has been included in Table I.

Discussion

Variation in Molecular Weights.—Upon comparison of the results in Table I, it is immediately evident that the different methods gave grossly different values for the molecular weight. Only the sedimentation-equilibrium value agrees with the combining weight. The molecular weight by diffusion is much too high, and the molecular weight by sedimentation velocity is too low.

As was pointed out above, all these values were calculated by means of equations for ideal cases, which for diffusion and sedimentation velocity are definitely restricted to spherical particles. The theory for sedimentation equilibrium indicates that it does not depend upon particle or molecular shape. Although the exact equations have not been worked out for non-spherical particles, it is known that departure from spherical form increases the frictional resistance of a particle of given volume moving through a viscous medium. Molecular weight values for such cases, calculated from diffusion by the equation for spherical particles, are therefore inevitably anomalously high, and values calculated from sedimentation velocity are inevitably anomalously low. The assumption of the same frictional coefficient for both cases, which amounts to solving the diffusion and sedimentation equations simultaneously for both frictional coefficient and molecular weight, equally inevitably gives an intermediate value of the molecular weight, which might be expected to be nearer the true value than the values from either diffusion or sedimentation velocity alone. In the light of these facts we may conclude that the hydroxydecanoic acid polymer is dispersed in solution as single molecules, and that the correct molecular weight is obtained by the sedimentation-equilibrium method. The molecule is far from spherical in form, and, therefore, its weight (or size) cannot be determined from the diffusion coefficient or the sedimentation velocity. At the concentrations studied, the friction coefficient is not the same for diffusion and sedimentation velocity, and the molecular weight cannot be determined by combining them, as is possible for spherical or near-spherical molecules. For instance, at 0.108% concentration, the friction corresponding to the diffusion coefficient ($f_D = RT/DN$) is about 4.5 times that for a spherical molecule of the same volume (*e. g.*, egg albumin), whereas the friction corresponding to the sedimentation constant [$f_S = (1 - V\rho)/s$] is 2.2 times that for a spherical molecule. The latter ratio is called by Svedberg the "Dissymmetry Number,"[9] and it indicates a degree of dissymmetry very much greater than any yet found among proteins of comparable molecular weight.

As shown by the data of Table I, the friction coefficients for both dif-

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(9) Svedberg and Eriksson, THIS JOURNAL, **54**, 4737 (1932).

fusion and sedimentation velocity increase with concentration, the more rapid change occurring for diffusion. Similar observations were made by Stamm in his ultracentrifugal analysis of cellulose.[10] Since viscous friction of the solution is a linear function of concentration, one might expect the friction coefficients to be linear functions of concentration. At any rate, linear extrapolation of the two pairs of values provided by Table I to zero concentration gives a common intercept on the friction axis at $f_D = f_S = 7.03 \times 10^{-7}$, which is 1.6 times that for a spherical molecule of the same volume. Calculation of the corresponding diffusion coefficient and sedimentation constant for zero concentration gives values of 5.70×10^{-8} and 1.19×10^{-13}, respectively. Calculation of the molecular weight from these two values, on the basis of equal friction coefficients, gives a value of 26,000, in good agreement with the value from sedimentation equilibrium. Admittedly more detailed data will be required to prove definitely whether this is the proper way of correcting for the effect of concentration upon the diffusion coefficient and the sedimentation velocity.

An important question in judging the validity of the conclusions and inferences stated above concerns the homogeneity of the polymer with respect to molecular weight. It is, however, somewhat difficult to characterize this feature in a quantitative manner. Scale-displacement curves have been calculated for a uniform sample of molecular weight 26,000, and for a hypothetical mixture consisting of 50% molecular weight 26,000, and 25% each of 20,000 and 32,000 molecular weight, but on the scale of Fig. 2 the two curves would not be distinguishable. The sample, therefore, might be at least as inhomogeneous as the hypothetical mixture. The sedimentation velocity and diffusion curves (Fig. 1) appear to show that the sample is uniform. For a non-uniform sample, the peaks shown in Fig. 1 broaden out with time due to the combined effect of diffusion and the separation of the different particle sizes. Since there is no detectable drift in the diffusion coefficient, the observed broadening of these curves appears to be entirely accounted for by the diffusion. On the other hand, due to the non-spherical shape of the molecule, we are not able to calculate the sensitiveness of this effect as a measure for uniformity. In the absence of more sensitive tests for uniformity (perhaps sedimentation velocity with a very high centrifugal force), all that we can say with assurance is that this sample presents no evidences of marked non-homogeneity.

Osmotic Pressure vs. Concentration.—It may be seen in Fig. 2 that the experimental curve at one end falls distinctly lower than the theoretical curve for a homogeneous material, and at first sight it might appear that this represents some anomalous condition. By expressing the data in another form, however, it may be shown that the anomaly is of a sort frequently displayed by solutions of intrinsic colloids.

(10) Stamm, THIS JOURNAL, **52**, 3047 (1930).

Thermodynamically, the measurement of sedimentation equilibrium is equivalent to the determination of the osmotic pressure. Tiselius[11] has shown how the activity of the solvent or the solute may be calculated for any point in the centrifuge cell at sedimentation equilibrium. The corresponding osmotic pressures can be calculated by the usual methods.[12] Thus the relation between osmotic pressure and concentration can be determined for the range of concentrations represented in the sedimentation equilibrium. Curve II, Fig. 2, represents the variation in concentration with cell-distance at equilibrium, as calculated by integration from Curve I, using Lamm's equation ($Z = Gab(\mathrm{d}n/\mathrm{d}x)$), and the experimentally determined value of $\mathrm{d}n/\mathrm{d}c$ expressing the relation between refractive index and concentration. By integration of Curve II one obtains the amount of centrifugible solute in the cell, which in this particular case corresponded to 98% of the total solute present at the beginning of the run.

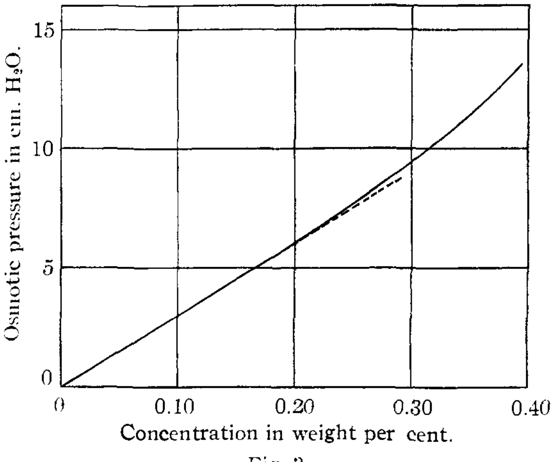

Fig. 3.

From Curve II of Fig. 2, the solid curve of Fig. 3, representing the empirical relation between osmotic pressure and concentration, was calculated. The broken curve is a straight line, corresponding to van't Hoff's equation for a molecular weight of 27,000. It is evident that the sedimentation equilibrium "anomaly" can be interpreted as due to a deviation from the laws of ideal dilute solutions at concentrations higher than about 0.2% by weight or 0.00024 normal. Such behavior has frequently been observed in the osmotic behavior of intrinsic colloids.[13] The viscosity-concentration data for the same polymer also show a departure from linearity above 0.2%.[6]

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(11) Tiselius, *Z. physik. Chem.*, **124**, 449 (1926).

(12) Lewis and Randall, "Thermodynamics," New York, 1923, p. 213.

(13) Freundlich, "Kapillarchemie," 4th ed., 1932, Vol. 11, p. 321; Grard, *J. chim. phys.*, **29**, 287 (1932).

Comparison of Viscosity, Diffusion and Sedimentation.—Non-ideal behavior in diffusion and sedimentation is probably due to the same structural features that give rise to non-ideal viscous behavior. The results of this investigation confirm the suggestion of Kraemer and Sears[4] that ultracentrifugal analysis should be carried out at concentrations sufficiently low to give a linear relationship between relative viscosity and concentration, and that even under these conditions, diffusion and sedimentation may be expected to be anomalous whenever the ratio of the specific hydrodynamic volume to the partial specific volume of the solute is much greater than unity. For the case in question, this ratio is 74. The apparent specific volume, calculated from diffusion on the assumption of a spherical molecule, is about 90 times the partial specific volume, so it would seem that the hydrodynamic conditions associated with flow and diffusion are closely related. For sedimentation velocity, the apparent specific volume is only eleven times the actual volume at 0.1% concentration. Detailed study of these interrelations and the influence of concentration probably would give considerable information concerning molecular shape, and perhaps would answer the question as to whether a linear macromolecule in solution is approximately straight or is more or less coiled up.

We wish to acknowledge our indebtedness to Professor The Svedberg for the opportunity of discussing our results with him.

Summary

Polymeric ω-hydroxydecanoic acid, a typical high-viscosity type of linear macromolecular colloid, has been subjected to ultracentrifugal analysis. The molecular weight calculated from sedimentation equilibrium agreed with the chemical value, and is believed to be correct. Diffusion coefficient gave much too high a molecular weight, whereas sedimentation velocity gave much too low a value. According to the osmotic pressure *vs.* concentration relations, as calculated from the sedimentation equilibrium, van't Hoff's law does not apply at concentrations exceeding 0.00025 normal. It is believed that rubber, cellulose and cellulose derivatives and similar macromolecular colloids would show a similar behavior.

WILMINGTON, DELAWARE

RECEIVED AUGUST 7, 1933
PUBLISHED OCTOBER 6, 1933