6
Physicochemical Characterization of Macromolecules

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ALTHOUGH the x-ray diffraction method appears to be the only means capable of yielding the complete three-dimensional structure of large macromolecules (p. 94), an investigation of macromolecular structures in solution is important for two reasons. In a number of cases, the macromolecule is either not crystallizable or insufficiently crystallizable; consequently, x-ray diffraction methods cannot be applied. In the other cases, where x-ray structure determination is possible, physicochemical examination is necessary to show whether or not the configuration characteristic of the solid state remains intact in solution.

It is essential, therefore, that one find out from the physical methods as much as possible about these molecules in solution. These methods are complementary to the x-ray investigation which goes on in the solid state. In addition, they furnish unique information such as molecular weight and molecular-weight distribution. In general, the more that one can do to bridge these two approaches, the better.

Nature seems to deal primarily with polymeric structures in the production of macromolecules. Fortunately, there is not the chaos that might be present should all possible covalent bonds be joined together to make an infinite variety of large macromolecules. There has been, in fact, a great sorting out so that only three basic polymeric chains have been selected for wide use in living systems. Evolved over many millions of years are the polysaccharides, the polypeptide, and the nucleic-acid chains. These sometimes are tied together by cross-links, but this generally does not mar the basic simplicity of the long-chain structures which may, or may not, be folded in a particular way. It is these polymer chains, subject to many specific perturbations, that we wish to examine in the greatest detail that is practical.

SPECIAL PROBLEMS IN CHARACTERIZING MACROMOLECULES

One must consider at the outset three special and constantly recurring problems which arise in the characterization of macromolecules and have no simple counterparts in the field of small molecules. First to be mentioned is the fact that there may be a distribution of molecular weight. This does not usually occur in globular proteins, but it does in most other biological macromolecules. Consequently, the molecular weight is no longer a unique and simple number. One must deal instead with chains of varying lengths, but which are otherwise homogeneous. Each physical method that can be used for molecular-weight determination reflects a particular average of the molecular-weight distribution and as a result this average must be specified.

There are two widely used averages. The first of these is the number-average molecular weight—a very democratic form of averaging wherein each molecule is counted with a weight of one regardless of the actual weight. The second, the weight-average, corresponds not so much to democracy as to a more primitive arrangement wherein the importance of the molecule influences its count in accordance with its actual physical weight. The ratio of one of these to the other is a rough measure of the breadth of the molecular-weight distribution. This quality of having a distribution of molecular weight is sometimes referred to as "polydispersity."

Also to be considered is the problem of the specification of molecular size and shape. There are a variety of situations which arise from the basic polymeric chains. At one extreme, there can be found no periodic internal structure to the macromolecule whatsoever. It will become a randomly coiled molecule which is a rather common but degenerate form, since it does not have any specific configuration that is generally thought to be required for the functioning of many biological macromolecules. At the opposite extreme, many species of the three types of biological macromolecules can form helices (see Rich, p. 50), most commonly made of one, two, or three molecular strands, in which atoms are grouped in a periodic, inflexible array. Now, it is clear that the specification of size and shape must be treated differently in these two cases. For the case of the random coil, one will wish to measure the volume of space occupied by the molecule or some effective radius; in the case of the compact helical structure, one obviously will measure the length of the molecule and its mass, and this ratio of length to mass will characterize the particular helix. Therefore, the kind of parameter used to characterize the size of a macromolecule depends very much upon the form the molecule presents.

The final feature, which arises here and which has no counterpart with small molecules, is a space-filling concept in solution. If one had to deal only with dilute solutions of small compact spheres, then these particles could be considered as spaced at random with no overlapping and only rarely making contact. However, if these same molecules were not compact spheres, but random coils of the same mass, they would be expanded to such an extent that they would be continuously overlapping. This feature is maximal in the case of the

random-coil form, but it is significant for rigid, asymmetric macromolecules as well. The consequence of this is that, if physical measurements are to be used to characterize the individual properties of these molecules, the system must be diluted down to the point where molecules are clearly separated one from another. This requires a very high dilution which, in turn, puts a very severe requirement upon the sensitivity of the physical method to be employed. A number of physical methods which otherwise might be very attractive fail on this account. They are not sufficiently sensitive to respond to the very high dilutions necessary to allow the individual expression of the intrinsic properties of the single macromolecule.

THERMODYNAMIC METHODS

Keeping in mind these three special problems, a survey is undertaken now of what appear to be the most practical and general methods for obtaining quantitative information about the anatomical character of biological macromolecules. It is of interest to note that all of these methods have their origins in the work of eminent physicists at the turn of the century. The contributions of Einstein and Boltzmann appear in a number of places and our indebtedness to them is very great.

Osmotic Pressure

The thermodynamic methods, three in number, are considered first. Perhaps the widest known is that of osmotic pressures. It is one property of solution which, as it happens, gives a macroscopic response to the dilute macromolecular solutions that is sufficiently large to be practical. The osmotic pressure is a measure of the number of molecules of solute per unit volume in solution. Therefore, if the weight of the material per unit volume in solution is known, and if one measures the number by means of osmotic pressure, a number-average molecular weight can be obtained.

This was perceived in the latter part of the last century, but only recently has the exact and rigorous relation of the concentration-dependent behavior of osmotic pressure to that of gas pressure been demonstrated. This is important, because, in each one of these methods, particular attention must be paid to the way in which the physical property depends upon concentration, and so, in each case, measurements must be carried out on a series of dilute solutions and extrapolated to an infinitely dilute solution in order to isolate the property of the individual molecule.

In all of these thermodynamic methods, the concentration dependence bears a close analogy to that of the pressure in a gas. Thus, the pressure-volume relation of an imperfect gas has the following form

$$PV/nRT = 1 + B(n/V) + C(n/V)^2 + \cdots, \qquad (1)$$

where B and C are the second and third virial coefficients. The analogy with the concentration dependence of osmotic pressure is exact. Osmotic pressure, π, replaces gas pressure, P, and concentration (mass per unit volume) divided by molecular weight, c/M, replaces number of moles per unit volume, n/V. In this way,[1] one obtains the relation

$$\frac{\pi}{cRT} = \frac{1}{M} + \frac{B}{M^2}c + \frac{C}{M^3}c^2 + \cdots. \qquad (2)$$

At sufficient dilution, the terms in c^2 and higher will be negligible. Under these conditions, a plot of π/cRT against c yields a straight line whose intercept is the reciprocal of the number of average molecular weight and whose slope is equal to B/M^2.

In small molecules of gases, the virial coefficient often is thought of in terms of excluded volume—that is, the amount of space not available for the center of one molecule because of the volume of another molecule. In the domain of macromolecular solutions, this effect can become very much larger because of the extra-large volume-filling capacity of some forms of macromolecules. However, if the molecules have an attraction for each other, this over-all effect becomes smaller and even can become negative immediately before phase separation. On the other hand, if there is a large electric repulsion, the over-all effect can become very, very large making it impossible to establish the curve with precision.

The range in which the osmotic-pressure method can be applied is from molecular weights of about 10 000 upward to a value which depends upon the precision desired—usually, a few hundred thousand. The lower limit is set by membrane permeability.

As an illustration, some measurements on collagen solutions[2] are shown in Fig. 1. The molecular weight obtained from the reciprocal of the intercept at the concentration axis is 310 000, and the scatter shows the uncertainty is of the order of 15%. This is about the upper limit for practical use of this method in aqueous solutions. The second virial coefficient can be evaluated from the slope, and, in this case, is found to arise almost entirely from the excluded volume effects.

Light Scattering

Light scattering, the second thermodynamic method, is considered next. Concentrating only upon its thermo-

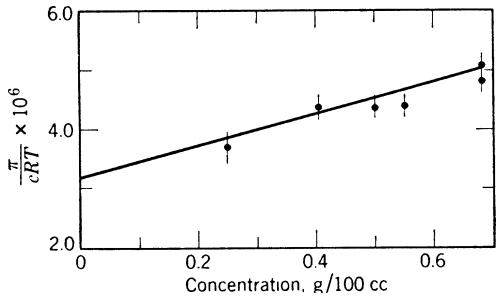

FIG. 1. Osmotic pressure of collagen solutions at 2°C.

dynamic aspect, compounded, of course, by the central electrostatic features which go along with dipole scattering, one finds again a close relation to the solution of the corresponding problem in gases.

Rayleigh's analysis of the problem of light scattering from dilute gases, worked out nearly a century ago, took the following form. The removal of light from an incident beam by scattering may be expressed quantitatively by means of the turbidity, τ, defined as

$$I = I_0 e^{-\tau l}, \tag{3}$$

where I_0 and I are the intensities of a parallel beam of light before and after passing through a length, l, of the gas. Alternatively, the scattering can be characterized by measuring its intensity at any angle θ, i_θ, and a distance, r. When θ is 90°, τ and i_{90} are related in the following manner:

$$\tau = \frac{16\pi}{3}\frac{i_{90} r^2}{I_0} = \frac{16\pi}{3} R_{90}, \tag{4}$$

where R_{90} represents the reduced form of the scattering intensity and is known as the Rayleigh ratio. This ratio was shown to be for any angle

$$R_\theta = \frac{2\pi^2 (n-1)^2}{\lambda^4}\frac{1}{\nu}(1+\cos^2\theta), \tag{5}$$

where n is the refractive index of the gas, λ_0 the wavelength of light in vacuum and ν the number of molecules per cc.

The relevance of this to scattering from a solution of macromolecules is due to Einstein[3] and Debye.[4] At very great dilution, the same results hold except that the refractive index of the solvent, n_0, replaces unity in Eq. (5). This then can be rearranged to give:

$$R_{90} = \frac{2\pi^2 n_0^2 [(n-n_0)/c]^2}{N\lambda_0^4} cM = KcM. \tag{6}$$

From this it can be seen that the scattering as measured by R_{90} is proportional to the molecular weight (the weight average) and the proportionality factor is experimentally determinable.

This result holds for a collection of independent scattering centers. In real macromolecular solutions, the space-filling property, previously referred to, produces a concentration-dependent correlation between scattering centers. Einstein showed this could be taken into account in a manner that now can be expressed as follows:

$$K\frac{c}{R_{90}} = \frac{1}{M} + 2\frac{B}{M^2}c. \tag{7}$$

One sees here a complete analogy with osmotic pressure except for a factor of 2 and the type of molecular-weight average that is obtained. Again, scattering measurements must be made at a number of concentrations and upon extrapolation yield the desired intercept.

In contrast to osmotic pressure, the sensitivity of this method increases with molecular weight. However, when the molecular size becomes significant with respect to the wavelength of the light, the angular dependence of the scattered light is affected in a manner discussed in the next section, and this must be taken into account. Finally, it should be noted that, since osmotic pressure and light scattering provide different molecular-weight averages, their combined results provide a measure of the molecular-weight distribution.

Sedimentation Equilibrium

The third and final thermodynamic method is the various exploitations of sedimentation equilibrium that are found to be useful in the study of macromolecules. It is very likely that this will turn out to be the most versatile of all the tools which one can have for the physical investigation of macromolecules. The key to these methods is found in the high speed of the ultracentrifuge, which allows one to observe optically the distribution of macromolecules in a cell in which the gravitational field is increased to the order of 100 000 times that of gravity. The versatility of the modern ultracentrifuge allows one to carry out a number of quite different kinds of measurements. In one case, one may allow the field to be exerted on the molecules in the solution until they have redistributed themselves at equilibrium.[5] This would then correspond with the distribution of gas molecules in the earth's atmosphere, and the analysis of the situation can be readily adapted to yield an average molecular weight of the macromolecules distributed in the much greater field provided by the ultracentrifuge.

The basis of determining the molecular weight in this way can be seen if one recalls the relation between the pressure p and the height h in the earth's gravitational field g. That is,

$$\frac{dp}{p} = -\frac{Mg}{RT}dh. \tag{8}$$

In the ultracentrifuge cell, the pressure is replaced by the concentration, the molecular mass M is corrected for the buoyancy owing to the solvent by multiplying by $(1-\bar{v}p)$, where $\bar{v}p$ is the ratio of solvent to solute density, h is replaced by x, the distance from the center of rotation, and g is replaced by the centrifugal force w^2x. These exchanges lead to

$$\frac{dc}{c} = \frac{M(1-\bar{v}p)w^2 x dx}{RT}. \tag{9}$$

If there is only one macromolecular species present, its molecular weight can be determined if the concentration is known at only two places in the cell. When a distri-

bution of molecular weights exists, its weight-average molecular weight can be obtained most readily.[6] Again, the observed molecular weight is found to be dependent upon the concentration in a manner governed by the second virial coefficient.[6] Consequently, the reciprocal of the apparent value must be plotted against concentration and the true value of M_w determined from the reciprocal of the intercept at zero concentration.

Now, in actual practice this method is not widely used because a number of days are required to attain the equilibrium distribution of the macromolecules in the solution in most cases. However, the smaller the molecular weight of the macromolecule, the sooner such distribution is attained; consequently, it is practical for molecules whose molecular weights are only a few thousand. Waugh[7] has pioneered in devising a particular kind of ultracentrifuge cell containing a retracting partition, which greatly facilitates this kind of application. By determining the concentration of the two parts, above and below the partition, one can obtain the molecular weight.

Within the last three years, an alternative version of the sedimentation-equilibrium method has come into use that promises to have wide utility. It is the Archibald approach to equilibrium method.[8] Kegeles[9,10] has been chiefly responsible for recognizing and developing its potential. Its basis is both novel and simple. The condition on which the general method of sedimentation equilibrium is based is that at equilibrium the net flux of solute species across any plane within the solution and perpendicular to the radius is zero. Archibald pointed out about 10 years ago that this condition of no net transport through a boundary perpendicular to the direction of sedimentation was always valid at the bottom and top of the solution, that is, at the meniscus and the cell bottom. Consequently, very soon after speed is attained, one can determine the initial redistribution of solute near the meniscus, or near the bottom of the cell; and from this one can derive the weight-average molecular weight by use of Eq. (9), together with appropriate extrapolation. Operationally, one now has precisely the opposite situation, a very short run instead of a very long run.

This method appears to be applicable over a wide range of molecular weight, and fortunately it does well in the region of 1000 to 10 000 where most other methods fail. As one illustration of this, I have chosen some work that we have done on determining the molecular weight of polypeptides and correlating this with intrinsic viscosity.

Before the Archibald method was available, it had been possible to show that the weight-average molecular weights of poly-γ-benzyl-L-glutamate were related to the intrinsic viscosity in the expected manner. That is, on a double logarithmic plot, a linear relation was found as indicated by the open circles in Fig. 2. We were unable to carry measurements below 20 000, however, and extrapolation was precarious. When the Archibald results were obtained (filled circles), the proper extension down to molecular weights of 1000 could be made as shown.[11]

INTERFERENCE OF SCATTERED LIGHT AND MOLECULAR SIZE

If the macromolecules are quite small as compared to the wavelength of light, and if the incident light is vertically polarized, the scattering will be the same at all angles; that is, $R_\theta = R_0$. However, for larger molecules with dimensions exceeding 200 or 300 A, interference arises from light scattered from different parts of the same particle. As a consequence, the scattering is diminished, the effect increasing with the scattering angle θ. The effect vanishes at zero angle and, as a consequence, measurements over the accessible angular range (usually 30 to 135°C) can be extrapolated to give R_0 which is required for molecular-weight determination. The character of the angular dependence, however, is of particular interest because it reflects the distribution of matter within the scattering article. The angular dependence can be separated from the concentration dependence because the following relation is generally valid

$$\frac{Kc}{R_\theta} = \frac{1}{MP(\theta)} + 2Bc. \tag{10}$$

That is, the angular dependence can be shown to enter only as a function, $P(\theta)$, known as the particle-scattering factor equivalent to the square of the structure factor in x-ray diffraction. The experimental determination of $P(\theta)$ is used to obtain information of dimensions and shape through the relation

$$P(\theta) = 1 - \frac{\rho^2}{3}\left[\frac{4\pi \sin(\theta/2)}{\lambda'}\right]^2 + \cdots, \tag{11}$$

where λ' is the wavelength of light in solution and ρ is

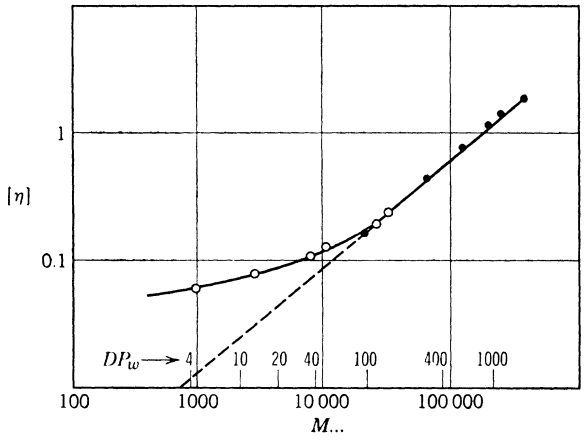

FIG. 2. A double logarithmic plot of intrinsic viscosity measured in dichloroacetic acid against weight-average molecular weight of poly-γ-benzyl-L-glutamate.

radius of gyration of the macromolecule. This radius of gyration is related to the dimensions of simply shaped particles. For example, the length of a rod-like molecule is $(12)^{\frac{1}{2}}\rho$. The root-mean-square end-to-end distance $(\langle r^2\rangle)^{\frac{1}{2}}$ of a randomly coiled polymer is given by $(6)^{\frac{1}{2}}\rho$. For quite large macromolecules, higher terms in Eq. (11) become significant in such a way that shape, as well as the size, can be independently determined or at least estimated.

As an illustration of these methods, one can consider a particular fraction of cellulose nitrate.[12] Osmotic-pressure measurements plotted according to Eq. (1) yielded a value of 234 000 for M_n. Light-scattering measurements showed substantial angular dependence. A double extrapolation against concentration and $\sin^2(\theta/2)$ in accordance with Eqs. (10) and (11), known as a Zimm plot, showed that $M_w = 400\,000$ and $(\langle r^2\rangle)^{\frac{1}{2}} = 1500$ A (Fig. 3). This indicates a rather broad molecular-weight distribution for a sample that has already been fractionated and the size is indicative of a quite extended random coil. This is evident when the following is considered. Each monomer unit has a molecular weight of 294 and a length of 5.15 A. Hence, the number of units (degree of polymerization) making up a chain of 400 000 molecular weight is 1350. Completely extended, this would be 6950 A in length. By comparing this with $(\langle r^2\rangle)^{\frac{1}{2}} = 1500$ A, the extent of coiling is readily visualized. If there were no hindrances to rotation at the glycosidic linkages, the value of $(\langle r^2\rangle)^{\frac{1}{2}}$ would be several times smaller. The extent to which this molecule is extended because of steric and potential hindrances to rotation is the highest yet found for single chains. One can appreciate that this "stiffness" is put to good use in the biological role of this material since this will not only give rigidity to the crystallites of cellulose, but will make firm the amorphous regions in between the crystallites. Moreover, this natural tendency toward rod-like behavior in localized regions of the chain is effective in lowering the entropy of melting, and thereby contributes to the high melting point and hence the negligible solubility of cellulose.

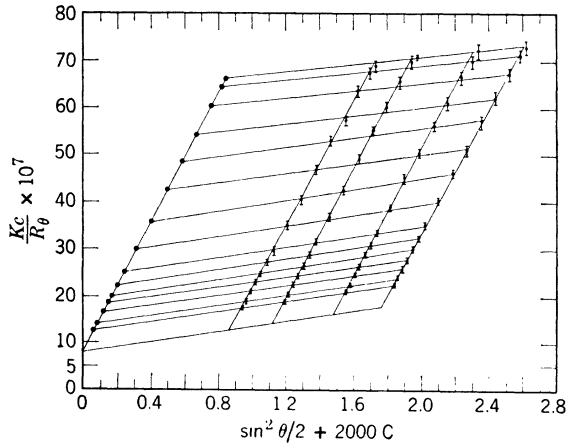

FIG. 3. Cellulose-nitrate fraction Ab in acetone at 25°C.

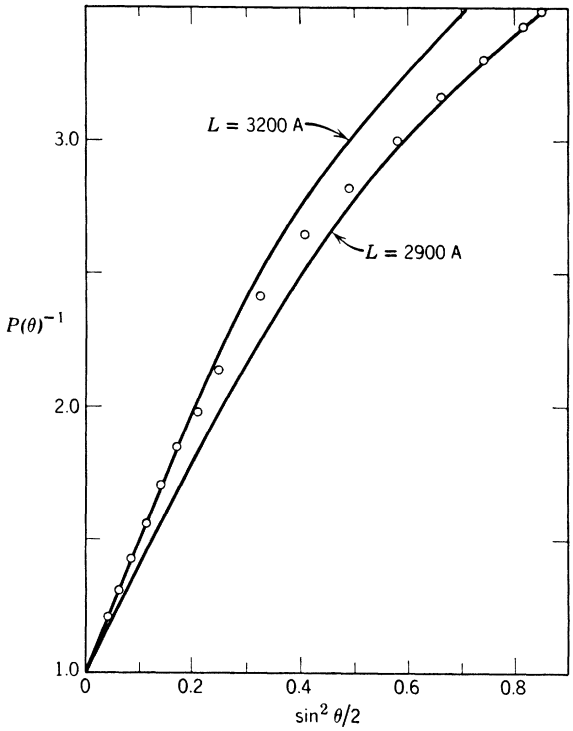

FIG. 4. Reciprocal particle-scattering factor of tobacco mosaic virus solution: sample *A*-4, experimental points,—theoretical scattering curves.

The Zimm plot for tobacco mosaic virus is shown in Fig. 4. It can be seen that the experimental points are bounded by the theoretical scattering curves for rods (having negligible diameter) of 2900 and 3200 A. Boedtker and Simmons[13] were able to conclude from these data that the length was 3000±50 A. In contrast to the complete linearity of the plot for the randomly coiled cellulose derivative in the previous figure, one finds here a pronounced downward curvature characteristic of rod-shaped scattering elements. From the molecular weight of 39.5 million obtained, from the intercept and the length and density, an effective diameter of 150 A can be computed. Thus, the shape and dimensions of this virus are completely determined from light-scattering measurements.

An application of light-scattering studies to collagen[2] is shown in Figs. 5 and 6. In the former, the Zimm plot for the native-collagen molecule in solution is shown. The downward curvature indicates a rod-like shape and the quantitative interpretation of the results show that the molecule is 3000 A long and has a molecular weight of 360 000. The diameter in this case is only 13.5 A and, as described by Rich (p. 50), consists of only three polypeptide chains in a helical arrangement. As in other cases which are dealt with in the paper by Rich, this macromolecule is essentially a one-dimensional crystallite. Hence, it should melt on raising the temperature.

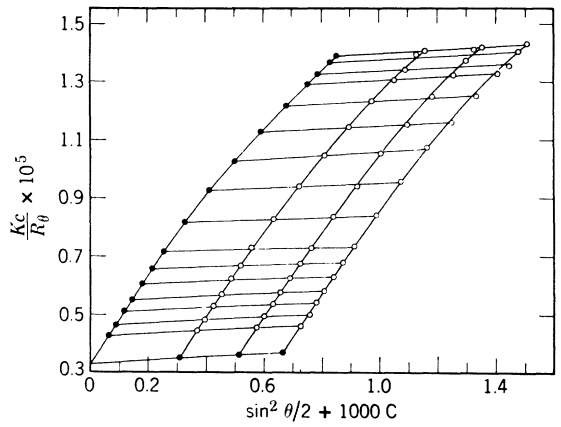

FIG. 5. Collagen (ichthyocol) in citrate buffer (*p*H 37) at 15°C. $M_w = 310\,000$; $P = 955$ A; $L = 3300$ A; $M/L = 94$.

Upon melting, the secondary bonding holding the three chains together disappears and the "melted" or denatured state should consist of the three polypeptide chains in randomly coiled configurations. This configurational transition can be observed directly in light scattering. In Fig. 6, the reciprocal envelopes are plotted before and after heating. The intercept is seen to be nearly three times higher indicating a correspondingly lower molecular weight, and the slope is likewise greatly diminished indicating a much smaller spatial extent. The curvature is also gone, consistent with the assumption of a randomly coiled configuration.

Light-scattering studies of deoxyribonucleic acid ((DNA) have been particularly useful in showing the nature of these molecules in solution. These macromolecules are so highly extended in space that very great dilution is required in their physical characterization, and as a consequence few methods could be properly applied. A typical light-scattering result is shown in Fig. 7. The slope relative to the intercept is seen to be much greater than those encountered before. For this case, its interpretation as a coiled molecule leads to an estimate of 7000 A for the average end-to-end length and a molecular weight of 8 000 000. The contour length of this molecule is about 40 000 A on the basis of the Watson-Crick model. Thus, it is only very modestly curved and the mild downward curvature reflects its intermediate status between a coiled and rod-like shape. Actually, the size of this molecule is greater than present light-scattering techniques can handle because the extrapolation should be based on measurements down to 5°C in order to eliminate the effects of polydispersity on the final answer. Thus far, however, the errors have not been serious because the polydispersity has been such as to justify a linear extrapolation from the higher angles, but this good fortune cannot be expected to hold in general.

The use of the angular dependence of scattered light in this manner requires only the ratio of particle size to wavelength to be such that the interference is first order. This situation holds in the region of x-rays as well, and as a consequence the scattering of long x-rays can lead to the assignment of dimensions of the order of hundreds of A, just as light scattering deals with dimensions of a few thousands A. Time does not permit an examination of this, but reference should be made to the work of Beeman[14] and Luzatti[15] in this connection.

HYDRODYNAMIC METHODS

The motions of a macromolecule in solution can be resolved into those of translation and rotation. If the motion is simply that of diffusion, one terms it translational or rotatory diffusion and characterizes it by a diffusion constant D for translation and Θ for rotation. If the motion arises from an imposed gradient, two other situations occur. If the imposed force is that of a gravitational gradient as in the ultracentrifuge, sedimentation is observed and one characterizes it by a sedimentation constant s. If the imposed force arises from a hydrodynamic gradient, the molecule is caused to rotate with a definite bias instead of at random. The dissipation of energy that this produces gives rise to an increase in viscosity over that of the solvent. The fractional increase is known as the specific viscosity, and, when divided by concentration and extrapolated to zero concentration, it becomes known as the intrinsic viscosity $[\eta]$.

These four types of motion are all the result of a certain amount of resistance to an applied force (or couple). This resistance is hydrodynamic in nature and arises from the size and shape of the molecule. On very general grounds, it can be argued that for a given molecule the resistance can be characterized by a frictional factor f, which is the same in all four cases. Consequently, two developments are possible. Theoretical

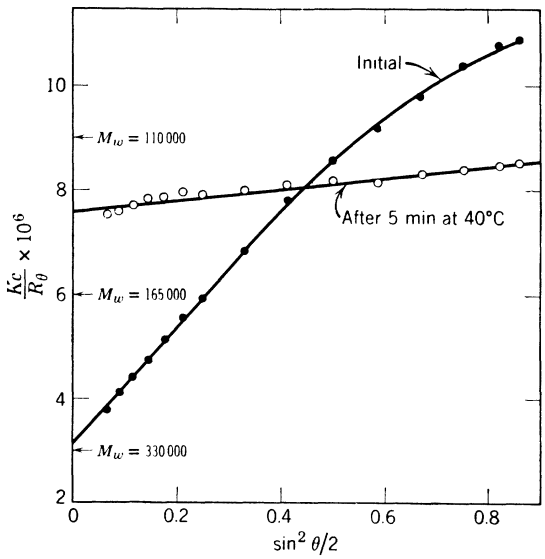

FIG. 6. Denaturation of collagen in solutions. ● = before denaturation; ○ = after denaturation.

investigations can aim at calculating the frictional factor for various macromolecular models as a function of dimensions and provide, in this way, a basis of obtaining dimensions from measuring one or more of the foregoing quantities. On the other hand, the measurement of any two quantities provides at least the possibility of eliminating f and obtaining the molecular weight. This is illustrated in the well-known Svedberg equation. Here, the expressions for D and s are combined to eliminate f as shown.

$$D=kT/f, \qquad s=M(1-\bar{v}\rho)Nf, \tag{12}$$

$$M=\frac{sRT}{D(1-\bar{v}\rho)}. \tag{13}$$

Thus, if the partial specific volume $\bar{v}$ and the solvent density ρ are determined, the molecular weight can be obtained from measurements of s and D.

The difficult and time-consuming nature of the measurement of D, particularly for chain-like and rod-like macromolecules, has led to a search for other means of achieving the same result by using the much more easily measured quantity, the intrinsic viscosity $[\eta]$, in its place. This can be done rigorously for ellipsoids, making use of the work of Perrin and Simha. The result is

$$M=\left[\frac{s[\eta]^{\frac{1}{3}}\eta_0 N}{\beta(1-\bar{v}\rho)}\right]^{\frac{3}{2}}, \tag{14}$$

where the constant β is a slowly varying quantity dependent only upon the axial ratio. Its values range from 2.12 for spheres to 3.50 for infinite axial ratio. The axial ratio can always be determined from $[\eta]$ with enough precision to permit the correct choice of β.

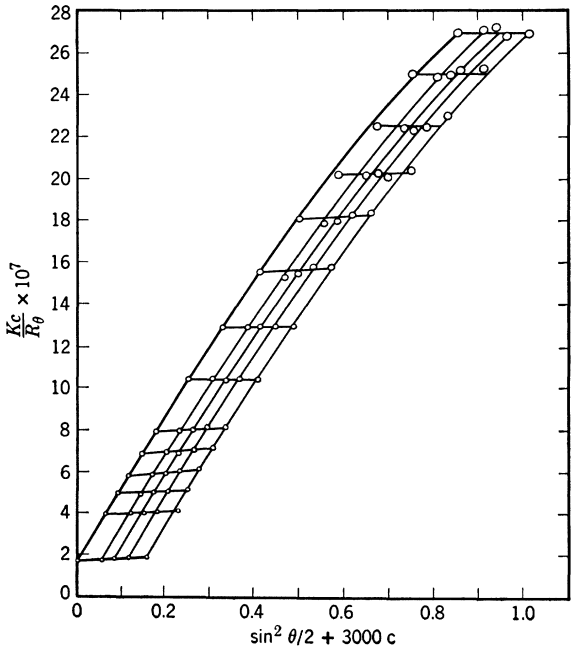

FIG. 7. Light scattering of DNA.

The usefulness of intrinsic-viscosity determinations is by no means limited to Eq. (14). Quite the contrary. Its application stems from a series of impressive theoretical derivations that have been made relating it to size and shape. The first of these was owing to Einstein who showed that the intrinsic viscosity of compact (unpenetrated by solvent) spheres in solution was 2.5 times their specific volume in units of cc/g. This value represents a lower limit: almost all values observed for macromolecules are much larger. For example, 4 to 10 for a number of globular proteins, 10 to 100 for a number of proteins known to be asymmetric, and extremely high values in the case of collagen (1200) and deoxyribonucleic acid (7000). Later theories showed that these higher values could all be accounted for in terms of two effects: asymmetry of the macromolecule, or solvent immobilization. The latter effect could arise from hydration owing to the binding of water (or other solvent) at specific sites on the macromolecule or by loosely incorporating it with the domain of the macromolecule through swelling. Thus, a typical randomly coiled polymer pervades a domain that is perhaps 100 times or more greater than it would occupy in a compact form; yet, the solvent in this domain moves with the polymer and is, therefore, hydrodynamically immobilized. As a consequence, the intrinsic viscosity measures this volume of immobilized material, and, in a rough way, its numerical value expresses in units of cc/g the ratio of the volume of the domain to the mass of the polymer molecule. Extremely useful developments in the last few years have led to the recognization of the relation between intrinsic viscosity (and sedimentation constant as well) and the mean end-to-end length, $(\langle r^2\rangle)^{\frac{1}{2}}$, of polymer chains.[16] As a consequence, the size of such polymer molecules can be determined from the intrinsic viscosity, if their molecular weight is known. Moreover, the large variations in intrinsic viscosity observed when the solvent or temperature is changed are seen to be the result of swelling and shrinking of the polymer coils. Finally, the previously observed empirical relation between intrinsic viscosity and molecular weight for a series of homologous polymers,

$$[\eta]=KM^a, \tag{15}$$

becomes understandable from this point of view. The exponent in this relation is a constant with a value between 0.5 and 1.0 for any given polymer-solvent system. These limiting values correspond to tightly coiled molecules with many intramolecular contacts (0.5) and to very highly swollen molecules (1.0), with most actual cases distributed in between.

In the case of rigid particles, the exaltation of the intrinsic viscosity arises from the increased energy dissipation resulting from the end-over-end rotations in solution. The analysis of this problem, particularly by Simha,[17] showed that the intrinsic viscosity was a func-

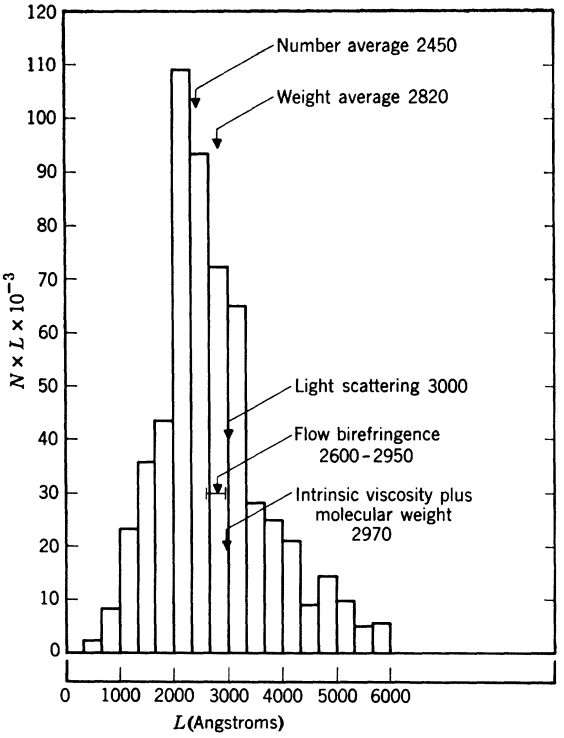

FIG. 8. Weight distribution of lengths of ichthyocol macromolecules as measured from electron micrographs compared with results from other methods. Total number represented is 238 [from C. E. Hall and P. Doty, J. Am. Chem. Soc. **80**, 1269 (1958)].

tion only of the axial ratio of the particle and that this dependence rapidly approached that of the square of the axial ratio. In many typical proteins, one is faced with the possibility that the intrinsic viscosity reflects both molecular asymmetry and moderate amounts of hydration. The resolution of this problem has been worked out by Oncley.[18]

The final point to make on intrinsic viscosity is about its ease of measurement relative to the other three hydrodynamic properties.

The determination of the rotatory-diffusion constant from streaming birefringence of flow is a rather specialized technique and is limited to rigid particles. In these cases, provided the length of the molecules exceeds a few hundred angstroms, the length and often the length distribution can be measured with considerable accuracy.

As a single illustration of the methods discussed in this section, as well as a demonstration of their self-consistency, the results on soluble collagen are presented in Table I.[2] The intrinsic viscosity was 1150 cc/g, the sedimentation constant was 2.96 Svedbergs and the distribution of rotatory-diffusion constants corresponded to a range in length from 2500 to 2950 A.

This brief discussion of the physical determination of the characteristics of macromolecules would not be complete without the mention of a new technique that has, within the last two years, become practical for asymmetric, rigid macromolecules. This is the perfection of the direct viewing of such macromolecules in the electron microscope by Hall.[18] Thus, nucleic acids, some proteins, and even simple polypeptides in the α-helical configuration have been measured and found to be in agreement with the results of other physical methods applied in solution.[19] As one illustration of this, shown in Fig. 8 is the weight distribution of the lengths of collagen molecules compared with the average values shown in Table I. The agreement is seen to be quite good. It is possible that the distribution was somewhat broadened as a result of some damage in spraying and shadowing the electron-microscope preparation, but the effect has been modest. F. O. Schmitt deals with the way in which these macromolecules are united in collagen fibrils and the extent to which the dimensions found here are compatible with his studies (p. 349).

TABLE I.

Method	Mol. Wt.	Length A	Diameter A
Osmotic pressure (M_n)	310 000	...	...
Light scattering (M_w)	345 000	3100	13.0
Intrinsic viscosity and M_w	...	2970	13.6
Sedimentation and viscosity	300 000	...	12.8
Flow birefringence and viscosity	350 000	2900	13.5

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