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An Instrument for Measuring Retardation Times of Deoxyribonucleic Acid Solutions

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randomly coiled poly-L-lysine and poly-L-glutamic acid reported by Myer¹⁴ if the CD bands at 218 and 197 m μ are attributed solely to these two transitions, respectively. The results are $R_{n\pi^*} \approx 0.15$ D-BM and $R_{NV_1} \approx -1.26$ D-BM.

If only the $n\pi^*$, NV_1 , and NV_2 transitions are considered in the Woody and Tinoco¹⁶ description of the amide group, then the calculated rotational strength of the NV_1 transition agrees quite well with Myer's¹⁴ data. On the other hand, the same electronic description¹⁶ of the amide group overestimates $R_{n\pi^*}$ by a factor of 10. The electronic description of the amide group given by Basch, *et al.*,²⁰ leads to a calculated $R_{n\pi^*}$ in fair agreement with experiment, while the calculated magnitude of the NV_1 rotational strength is almost six times that obtained from Myer's¹⁴ experimental CD spectra. The magnitude of these discrepancies is similar to those encountered in previous^{13–18} optical activity calculations performed on polypeptides in their rigid conformations.

Although both descriptions of the electronic structure of the amide group predict the correct signs for the rotational strengths of the $n\pi^*$ and NV_1 transitions, neither leads to the correct magnitudes for both transitions. Schellman, *et al.*,¹⁹ calculated³¹ the

(31) They assumed that the $n\pi^*$ and NV_1 transitions adequately describe the electronic structure of the amide group and used the Woody and Tinoco¹⁶ set of electronic parameters after significant modification.

rotational strengths of the $n\pi^*$ and NV_1 transitions for a dipeptide and found that $|R_{NV_1}|$ should be at least an order of magnitude greater than $|R_{n\pi^*}|$. This finding is borne out by the calculations based on the Basch, *et al.*,²⁰ description and by experiment,¹⁴ while the electronic parameters given by Woody and Tinoco¹⁶ lead to $|R_{NV_1}| \gtrsim |R_{n\pi^*}|$. Since the GTO-SCF calculations of Basch, *et al.*,²⁰ yield an energy for the NV_1 transition which is 3 eV higher than observed experimentally, one should probably not be too surprised by the disparity between the calculated and experimental values of R_{NV_1} .

Neither description of the electronic structure of the amide group leads to a completely satisfactory prediction of the rotatory properties of randomly coiled poly-L-alanine, thereby precluding an unambiguous selection between the two. In addition, the validity of the present adaptation of the Tinoco method^{22,23} for calculating rotational strengths cannot be assessed further from the results presented here.

Acknowledgments. It is a pleasure for the author to acknowledge Drs. H. Basch and M. B. Robin for making available the details of their GTO-SCF calculations on the amide group and for many helpful discussions. Professor D. A. Brant is also gratefully acknowledged for providing the calculated conformational potential energies of randomly coiling polypeptides.

An Instrument for Measuring Retardation Times of Deoxyribonucleic Acid Solutions

R. E. Chapman, Jr., L. C. Klotz, D. S. Thompson, and B. H. Zimm¹

Department of Chemistry (Revelle College), University of California, San Diego, La Jolla, California 92037. Received August 11, 1969

ABSTRACT: A new instrument is described which measures retardation times for solutions of very large macromolecules in a creep–recovery type of experiment. Retardation times are closely related to relaxation times, and therefore should be useful parameters for determining molecular weights of macromolecules. Results of measurements on solutions of T2 bacteriophage DNA are presented. The molecular weights calculated by assuming that the DNA molecules are random coils agree well with the accepted value.

The “relaxation time” of a chain macromolecule is an interesting quantity that can be related to the molecular size. Theory² predicts that there are a number of relaxation times, τ_k , for a chain molecule given by a formula of the form

$$\tau_k = \alpha_k M \eta [\eta] / RT \quad (1)$$

where M is the molecular weight of the molecule, η is the solvent viscosity, $[\eta]$ is the intrinsic viscosity of the macromolecule, and α_k is a constant that depends on the index k and also to some extent on the structure of the chain. This formula has been verified experi-

mentally in a number of cases (for a review, see Ferry³). The longest relaxation time, τ_1 , is usually the most prominent in measured properties such as viscosity or flow birefringence.

Solutions of large deoxyribonucleic acid (DNA) molecules show pronounced relaxation effects.^{4,5} In particular, viscoelasticity, especially as manifested by the elastic recovery of the solutions from mechanical stress, is a familiar phenomenon to those who work with such solutions. D. S. T. noticed that this effect

(3) J. D. Ferry, “Viscoelastic Properties of Polymers,” John Wiley & Sons, Inc., New York, N. Y., 1961.

(4) D. S. Thompson and S. J. Gill, *J. Chem. Phys.*, **47**, 5008 (1967).

(5) P. R. Callis and N. Davidson, *Biopolymers*, in press.

(1) Reprint requests should be addressed to this author.

(2) (a) P. E. Rouse, Jr., *J. Chem. Phys.*, **21**, 1272 (1953);
(b) B. H. Zimm, *ibid.*, **24**, 269 (1956).

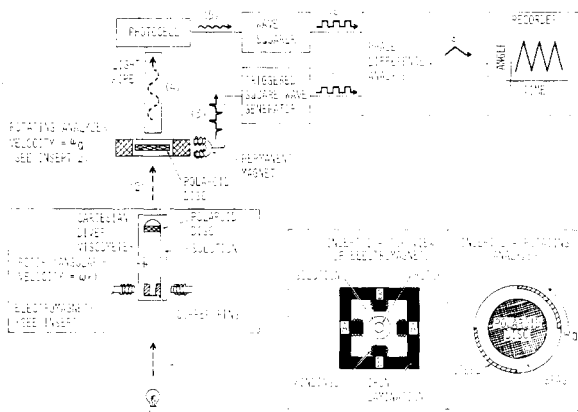


Figure 1. Schematic diagram of apparatus for measuring DNA retardation times. Dashed arrows represent light; solid arrows represent electrical current. The shapes of the arrow shafts show either intensity or current variation with time. All signal shapes are for a rotor moving with constant angular velocity. The numbers in parentheses refer to the description in the text.

is especially striking with the very large DNA of bacterial chromatin when observed in a Cartesian-diver rotating-cylinder viscometer;⁶ in this case the elastic recovery extended over a period of minutes. It occurred to us that this elastic or creep recovery could be easily and accurately measured with a refinement of this viscometer, and that it would lead, through eq 1, to useful information on the size and shape of the macromolecules. This paper reports the development of apparatus suitable for such measurements on solutions of large DNA molecules, and describes a preliminary application to the DNA from T2 bacteriophage.

Large DNA approximates a random coil to some extent, so it is not unreasonable to expect that eq 1 may hold. To this end, Thompson and Gill⁴ measured the longest relaxation time, τ_1 , for T2 DNA using optical birefringence to follow the relaxation. The numerical value of molecular weight computed from the measured relaxation time agreed very well with that obtained in other ways. More recently, Callis and Davidson⁵ using optical dichroism have measured the longest relaxation time of T4 DNA, T4 DNA half-molecules, λ_{b2b5e} DNA and λ_{b2b5e} half-molecules at different temperatures and in solvents of varying viscosity. They have essentially verified eq 1 for the dependence of relaxation time on molecular weight, solvent viscosity, and temperature.

In both the Thompson and Gill and the Callis and Davidson experiments the relaxation is followed after the steady-state flow of the solution is abruptly stopped. This procedure is called a stress-relaxation experiment in the parlance of the trade. (See Ferry,³ chapter 1, for example.) In our experiment, the relaxation is followed by observing the motion of the solution after the shear stress is suddenly removed. This is called a creep-recovery experiment and the characteristic exponential decay time is called a retardation time. The retardation time is closely related to the

relaxation time, as will be shown below, and therefore should be a useful parameter in the characterization of large DNA molecules.

The Instrument. General Principles of Operation.

The instrument is basically the rotating Cartesian-diver viscometer described by Gill and Thompson,⁶ modified so that the angle of rotation of the rotor may be measured as a function of time. The key to the use of this instrument for measuring retardation times is the fact that solutions of DNA molecules with long retardation times actually cause the direction of rotation of the Cartesian-diver rotor to reverse after the driving torque on the solution is suddenly removed. In contrast, if the rotor is suspended in pure solvent the rotational velocity of the rotor simply decays exponentially to zero due to frictional forces after the torque is removed. In a DNA solution, however, the direction of motion of the rotor is reversed as a result of the forces exerted on it by the DNA chains relaxing back to their equilibrium configurations. Under the appropriate conditions, the motion of the rotor in this reversed direction decays as a simple exponential in time, and its characteristic decay time is the retardation time.

Figure 1 shows a schematic diagram of the important components of the instrument. Unpolarized white light (1) passes through a Polaroid disk inside the Cartesian-diver rotor. For purposes of this description assume the rotor to be moving with a constant angular velocity, ω_r . The now polarized light passes through a rotating analyser Polaroid (angular velocity $\omega_a \gg \omega_r$) mounted in a brass and steel segmented ring (see insert 2) in which the brass and steel segments alternately span arcs of 90°. When a steel-brass of brass-steel interface passes near the poles of the permanent magnet, a spike of voltage (3) is induced in the coils surrounding the magnet poles.⁷ Thus, the voltage spikes (four per revolution of the analyser) provide an electrical means for measuring ω_a .

After the light passes through the analyser Polaroid (4), it enters a Lucite light pipe leading to a photomultiplier. (The photomultiplier is placed 30 cm away from the magnet to suppress hum pickup.) The light at this point has intensity that varies sinusoidally with time at frequency $(\omega_a + \omega_r)$. The light intensity is converted into electrical current (5), amplified and clipped to produce a square wave (6). The spikes from the pickup coils are also made into square waves (7) of frequency ω_a . The difference in phase

$$(\omega_a + \omega_r)t - \omega_a t = \omega_r t = \theta_r$$

between the two square waves is then converted into voltage by integrating their product. This voltage is fed into a strip-chart recorder to give rotor angle (with a plus or minus sign and plus an integral multiple of 90°) vs. time. During a retardation time measurement, of course, the output on the recorder will not be the saw-tooth pattern shown in Figure 1 but an exponential-like decay curve.

Some Details of Design. The Cartesian-diver viscometer is basically that constructed by Gill and Thompson⁶ except that we use a pair of alternating

(6) S. J. Gill and D. S. Thompson, *Proc. Nat. Acad. Sci. U. S.*, **57**, 562 (1967); D. S. Thompson, J. B. Hays, and S. J. Gill, *Biopolymers*, **7**, 571 (1969).

(7) B. H. Zimm, *Rev. Sci. Instr.*, **29**, 360 (1958).

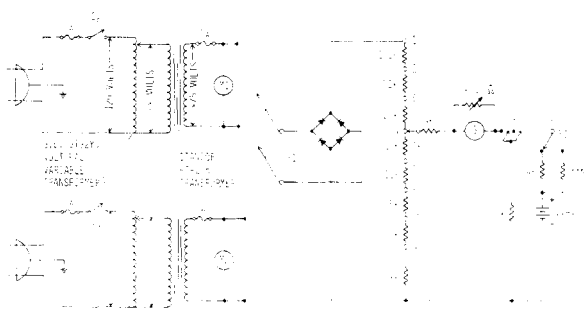


Figure 2. Magnet power supply and magnet voltage adjust circuit. M1 and M2 denote the two magnets, G is a galvanometer (sensitivity $0.18 \mu\text{A}/\text{division}$). See text for further details.

current electromagnets to drive the rotor instead of the synchronous motor, permanent magnet drive. The frame for the electromagnets (see insert 1 of Figure 1) was constructed from iron laminations obtained from several old transformers. The laminations were stacked and bolted together to a thickness of about 2 cm. Each pole piece (surface area about 6 cm^2) was wound with 200 turns of no. 20 Formex-coated magnet wire. In the figure insert, the two poles of one pair of magnet coils are lettered A, and the poles of the other pair are lettered B. One pair generates an eddy current in the copper ring inside the rotor, while the field from the second pair (which is one-third cycle out of phase with that of the first) interacts with the eddy current causing a torque to be exerted on the rotor. The range 1–20 V rms (0.1–3 A) on the two pairs of magnet coils spans a shear stress range of more than 0.01 – 1 dyn/cm^2 .

Since it is necessary to be able to reproduce exactly the current in each magnet in order to compare solvent rotation times to solution rotation times in the determination of intrinsic viscosity, we employ a potentiometer circuit which allows us to reproduce exactly a variety of magnet voltages. This circuit and the magnet power-supply circuit are shown in Figure 2. To set the magnet voltage, switches S_2 and S_3 are set to desired position, switch S_1 is set to the magnet whose voltage is to be adjusted, and then the variable transformer is adjusted until the galvanometer reads zero. In this way the current can be reset to give a time for one revolution of the rotor reproducible within 0.2%.

The electronics for measuring the phase differences between the two signals is essentially that described by Zimm.⁷

The output is recorded on an Esterline Angus Model No. S6015 strip-chart recorder with a 0.1-sec full-scale response time and 100-mV full-scale sensitivity. Full scale on the recorder at maximum sensitivity is equivalent to about a 2-deg rotation of the rotor.

The ordinary procedure for carrying out an experiment is to set the magnets for the desired shear stress, allow the rotor to come up to its steady-state speed, then manually shut off the magnet power switches S_4 and S_3 and record the DNA relaxation. The data obtained from the measurement of the steady-state rotation speed, either on the recorder or visually by timing a number of revolutions of the rotor, allows us to measure the relative viscosity, η_{rel} , simply as

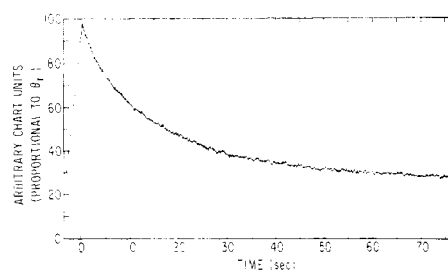


Figure 3. A photograph of a typical recorder tracing for T2 DNA in a solvent consisting of 75% glycerol–25% BBES buffer (see section on preparation of samples). The DNA concentration is $14.9 \mu\text{g/ml}$, $T = 25^\circ$, the shear stress at steady state rotation is 0.07 dyn/cm^2 and the steady-state angular velocity of the rotor is $7.32 \times 10^{-3} \text{ rev/sec}$. The figure shows both the steady-state motion of the rotor when $t < 0$ and the DNA relaxation when $t > 0$.

$$\eta_{rel} = \frac{t_r}{t_{r0}} = \frac{\omega_{r0}}{\omega_r} \quad (2)$$

where t_{r0} and t_r are the times per revolution of the rotor for the solvent and the solution, respectively, and ω_{r0} and ω_r are the angular velocities of the rotor for solvent and solution. (The more complicated expression used by Gill and Thompson⁴ reduces effectively to eq 2 in this case because of the high rate of rotation (60 rps) of the magnetic field.) A photograph of a typical recorder tracing for a T2 DNA relaxation in a solvent containing 75% glycerol is shown in Figure 3. The tracing at negative times gives the steady-state angular velocity of the rotor. The magnets are turned off at time zero, so that the tracing at positive times describes the DNA relaxation. Note that the rotor travels forward as a result of inertia for a short time after the magnets are shut off; then the force from the stretched DNA molecules reverses the motion of the rotor.

Classical Mechanical Analysis of the Motion. The motion of the rotor during a DNA relaxation experiment is obviously affected by its inertia and that of the surrounding solution. Thus, it is necessary to analyze the behavior of the rotor under conditions similar to those of a DNA relaxation experiment in order to see under what circumstances inertial effects can be neglected—or at least taken into account.

To approximate the behavior of the DNA we choose a single Maxwell element (see, for example, Alfrey,⁸ chapter A). This Maxwell element placed in parallel with a dashpot representing the solvent and both connected to an inertia, I , as shown in Figure 4, is a reasonable approximate model for the rotor in a viscoelastic solution.

Before analyzing the effect of inertia, we first derive the relationship between the relaxation time and the retardation time which we measure. To repeat, in the Thompson and Gill⁴ experiment and in the Callis and Davidson⁵ experiment, the solvent flow is abruptly stopped and the relaxation then optically observed. In the model in Figure 4 this procedure would correspond to stopping the inertia from moving during the relaxation while the spring, which has been previously

(8) T. Alfrey, Jr., "Mechanical Behavior of High Polymers," Interscience Publishers, New York, N. Y., 1948.

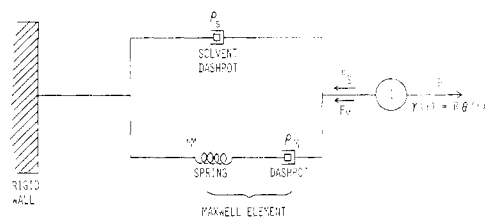


Figure 4. Spring-dashpot model for approximating the behavior of the rotor in a DNA solution. ρ_s is the frictional coefficient for the solvent, ρ_M is the frictional coefficient increment due to the DNA in the solution, and k_M is the constant for the spring simulating the springlike nature of the DNA. The circle of moment of inertia, I , represents the inertia of the rotor and surrounding solvent. F_0 is the applied stress on the rotor (from the magnets), F_s is the stress on the rotor due to the solvent alone, and F_M is the stress on the rotor due to the Maxwell element. $\gamma(t)$ is the strain along the line of action of the stresses, $\theta(t)$ is the angle of rotation of the rotor, and R is the radius of the rotor.

stretched by the applied stress, F_0 , relaxes against the Maxwell element dashpot, the solvent dashpot being unable to move. The relaxation time, τ_M , for this process is given by

$$\tau_M = \rho_M/k_M \quad (3)$$

where ρ_M is the frictional coefficient of the Maxwell element and k_M is its spring constant. In our experiment, in contrast, the stress F_0 is suddenly removed, and since the rotor is not constrained, the spring relaxes against both the solvent and Maxwell element dashpots. The characteristic exponential decay time for this process (in the case of no inertia) is the retardation time which we shall denote by τ_R .

For our simple model we can relate τ_M to τ_R easily. Let

$$\tau_R = \rho_R/k_M \quad (4)$$

where ρ_R is the total frictional coefficient against which the spring must relax. Since the solvent and Maxwell element dashpot act as a series combination (analogous to electrical resistances in parallel), we have

$$\frac{1}{\rho_R} = \frac{1}{\rho_s} + \frac{1}{\rho_M} \quad (5)$$

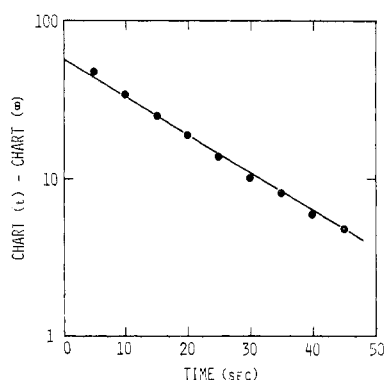


Figure 5. Semilog plot of a T2 DNA relaxation. The data were taken from the average of three recorder tracings similar to that shown in Figure 3. The DNA concentration is 14.9 $\mu\text{g/ml}$, the temperature is 25°, and the shear stress is 0.07 dyn/cm². The ordinate is in arbitrary chart units.

or

$$\rho_R = \frac{\rho_s \rho_M}{\rho_s + \rho_M} \quad (6)$$

This expression for ρ_R can be put in simpler form. First, frictional coefficients differ from viscosity coefficients only by the same constant factor determined exclusively by the apparatus geometry (see, for example, Landau and Lifshitz,⁹ p 60–61). Now, for the viscosity of the DNA solution we can clearly write

$$\eta_s \eta_{rel} = \eta_s + \eta_M \quad (7)$$

where η_s is the solvent viscosity, η_M is the viscosity increment due to the DNA, and η_{rel} is the relative viscosity. Since the viscosity coefficients are proportional to the frictional coefficients, it also follows that

$$\rho_s \eta_{rel} = \rho_s + \rho_M \quad (8)$$

or

$$\rho_M = \rho_s(\eta_{rel} - 1) \quad (9)$$

Substituting eq 9 into eq 6 yields

$$\rho_R = \rho_M/\eta_{rel} \quad (10)$$

so that from eq 3 and 4

$$\tau_R = \tau_M/\eta_{rel} \quad (11)$$

Thus $\tau_R = \tau_M$ at infinite dilution. In our experiments only the longest relaxation is observed over most of the course of an experiment as evidenced by the practical linearity of the semilogarithmic plot in Figure 5.

To derive the equations of motion for the model in Figure 4, specifically to derive an expression for $\theta(t)$, we first apply Newton's law (for definition of symbols see caption of Figure 4)

$$R(F_s + F_M + F_0) = I \frac{d^2\theta}{dt^2} \quad (12)$$

where $F_0 = 0$ in the case of relaxation. Now

$$F_s = -\rho_s \frac{d\gamma}{dt} = -R\rho_s \frac{d\theta}{dt} \quad (13)$$

so that eq 12 becomes

$$\frac{I}{R} \frac{d^2\theta}{dt^2} + R\rho_s \frac{d\theta}{dt} - F_M = 0 \quad (14)$$

We cannot yet solve this for $\theta(t)$ since F_M , the stress from the Maxwell element, is also a function of time. To find a second differential equation we concentrate on the Maxwell element. For this series combination of spring and dashpot, the displacements add, and the force on each element is the same and equal to F_M , that is

$$F_M = -k_M \gamma_{MS} = \rho_M \frac{d\gamma_{MS}}{dt} \quad (15)$$

$$\gamma = \gamma_{MS} + \gamma \quad (16)$$

The subscripts MS and MD stand for Maxwell element

(9) D. Landau and E. M. Lifshitz, "Fluid Mechanics," Addison-Wesley Publishing Co., Inc., Reading, Mass., 1959.

spring and Maxwell element dashpot, respectively. Substituting $\gamma = R\theta$ for each γ and then differentiating yields

$$\frac{d\theta}{dt} = \frac{d\theta_{MS}}{dt} + \frac{d\theta_{MD}}{dt} \quad (17)$$

Combining eq 15 and 17 gives

$$\frac{d\theta}{dt} + \frac{1}{RK_M} \frac{dF_M}{dt} + \frac{1}{R\rho_M} F_M = 0 \quad (18)$$

It is desirable to cast eq 14 and 18 in terms of experimentally observable quantities. To this end, it is convenient to let $\theta_{MS} = -F_M/RK_M$ be the other independent variable instead of F_M . If we define δ as

$$\delta = \frac{R^2\rho_S}{I} \quad (19)$$

and use eq 4, 8, 9, and 10, we can rewrite eq 14 and 18 as

$$\frac{d^2\theta}{dt^2} + \delta \frac{d\theta}{dt} + \frac{\delta(\eta_{rel} - 1)}{\eta_{rel}} \frac{1}{\tau_R} \theta_{MS} = 0 \quad (20)$$

$$\frac{d\theta}{dt} - \frac{d\theta_{MS}}{dt} - \frac{1}{\eta_{rel}\tau_R} \theta_{MS} = 0 \quad (21)$$

The quantity δ is important because relaxation of the pure solvent gives

$$\theta(t) = \frac{\omega_r}{\delta} \{1 - e^{-\delta t}\} \quad (22)$$

where ω_r is the steady-state angular velocity before the magnets are shut off. Since δ can be determined from experiments on solvent alone, all the constants in eq 20 and 21 can be determined from experiment.

Equations 20 and 21 are solved subject to the initial conditions for creep recovery

$$\begin{aligned} \theta(0) &= 0 \\ \left(\frac{d\theta}{dt}\right)_0 &= \omega_r \end{aligned} \quad (23)$$

$$\theta_{MS}(0) = \eta_{rel}\tau_R\omega_r$$

yielding

$$\theta(t) = \omega_r \left\{ C + \frac{1 - (A + B)C}{2B} e^{-(A - B)t} + \frac{(A - B)C - 1}{2B} e^{-(A + B)t} \right\} \quad (24)$$

where

$$A = \frac{1}{2\eta_{rel}\tau_R} + \frac{\delta}{2} \quad (25)$$

$$B = \left\{ \left[\frac{1}{2\eta_{rel}\tau_R} + \frac{\delta}{2} \right]^2 - \frac{\delta}{\tau_R} \right\}^{1/2} \quad (26)$$

$$C = \frac{1}{\eta_{rel}} \left\{ \frac{1}{\delta} - \eta_{rel}(\eta_{rel} - 1)\tau_R \right\} \quad (27)$$

In its present form, eq 24 is not easy to use because the retardation time terms and the inertial, δ , term are mixed up in a complicated fashion in the exponentials. To put eq 24 in more useful form, eq 26 is first rearranged into

$$B = \left[\frac{1}{2\eta_{rel}\tau_R} - \frac{\delta}{2} \right] \left\{ 1 - \frac{4\delta\eta_{rel}\tau_R(\eta_{rel} - 1)}{[1 - \delta\eta_{rel}\tau_R]^2} \right\}^{1/2} \quad (28)$$

Under conditions where the second term of the square root in eq 28 is much less than one, we can approximate

$$\sqrt{1 - X} = 1 - \frac{X}{2} - \frac{X^2}{8} \quad (29)$$

Anticipating the results for T2 DNA in a solvent containing 50% glycerol $\tau_R \approx 4$, $\delta \approx 6$, so that for $\eta_{rel} \approx 2$

$$X = \frac{4\delta\eta_{rel}\tau_R(\eta_{rel} - 1)}{[1 - \delta\eta_{rel}\tau_R]^2} \approx 0.1 \quad (30)$$

In a 75% glycerol solvent, $\delta \approx 20$, $\tau_R \approx 20$, so $X \approx 0.01$. Hence for T2 DNA in 50–75% glycerol, the approximation 29 is good.

Using this approximation for B , we calculate the parameters $A + B$ and $A - B$ which govern the exponential decays in eq 24

$$A + B = \frac{1}{\tau_R\eta_{rel}} \left\{ 1 - \frac{\delta\tau_R\eta_{rel}(\eta_{rel} - 1)}{1 - \delta\eta_{rel}\tau_R} - \frac{[\delta\eta_{rel}\tau_R(\eta_{rel} - 1)]^2}{[1 - \delta\eta_{rel}\tau_R]^3} \right\} \quad (31)$$

$$A - B = \delta + \frac{\delta(\eta_{rel} - 1)}{1 - \delta\eta_{rel}\tau_R} + \frac{[\delta(\eta_{rel} - 1)]^2\tau_R\eta_{rel}}{[1 - \delta\eta_{rel}\tau_R]^3} \quad (32)$$

Because $A - B > 5$ and $A + B < 0.5$ for T2 DNA in the glycerol solvents, $\theta(t)$ after the first second will depend only on the $\exp[-(A + B)t]$ term, which in turn is mainly a function of τ_R , the retardation time.

In the 75% glycerol solvent where $\tau_R \gg 1$, we can further approximate $A - B$ and $A + B$ to obtain a very simple expression for $\theta(t)$

$$\theta(t) = -\omega_r(\eta_{rel} - 1)\tau_R \{1 - e^{-t/\tau_R}\} \quad (33)$$

Equation 33 corresponds to relaxation in a system free of inertia. In fact, the inertia appears only in the δ term, so by eq 19, increasing the solvent viscosity by using glycerol is equivalent to reducing the inertia. Under this condition of no inertia, eq 33 shows that the exponential decay of the motion yields τ_R directly.

Application to T2 DNA Solutions. Preparation of Samples. Samples of T2 DNA were prepared according to the method of Massie and Zimm¹⁰ (see also Crothers and Zimm¹¹). Stock solutions of concentration about 100 $\mu\text{g}/\text{ml}$ were prepared from the bacteriophage. Concentrations of DNA were determined spectrophotometrically assuming that 55.2 $\mu\text{g}/\text{ml}$ gives an extinction coefficient of unity. Intrinsic viscosities were measured in a pH 9 borate buffer, BBES,¹² which is 0.01 M $\text{Na}_2\text{B}_4\text{O}_7$, 0.001 M Na_3EDTA , 0.178 M NaCl . This buffer is practically equivalent in sodium ion to the widely used "standard saline citrate," but DNA keeps better in it.

For retardation time measurements, DNA solutions

(10) H. R. Massie and B. H. Zimm, *Proc. Nat. Acad. Sci., U. S.*, **54**, 1641 (1965).

(11) D. M. Crothers and B. H. Zimm, *J. Mol. Biol.*, **12**, 525 (1965).

(12) H. R. Massie and B. H. Zimm, *Biopolymers*, **7**, 475 (1969).

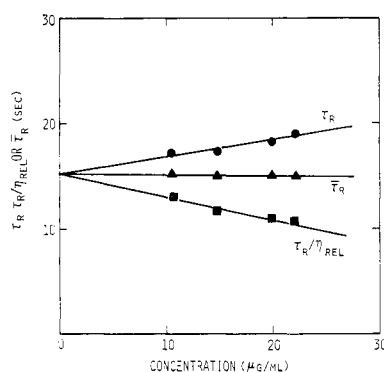


Figure 6. Concentration dependence of retardation times plotted as τ_R (●), as τ_R/η_{rel} (■), and as $\bar{\tau}_R$ (▲) which is defined in the text. The solvent is 75% glycerol–25% BBES and the temperature is 25°.

of varying concentrations from 10 to 30 $\mu\text{g/ml}$ in either 50% glycerol–50% BBES buffer (weight per cent) or 75% glycerol–25% BBES buffer were prepared by weighing. The solution components were mixed by rolling the sample tubes mechanically for 0.5 hr at no more than 30 rev/min to avoid breaking the DNA. All retardation-time experiments were carried out at 25°.

Determination of δ . For experiments in which inertia cannot be neglected it becomes necessary to determine δ for the solvent in question. As indicated by eq 22, δ can be found directly by performing relaxation experiments on the solvent alone. However, δ is much greater than one reciprocal second for the glycerol solvents used, so the solvent relaxations are too fast for our recorder. As it turns out, δ is near unity and easily measurable in solvents of viscosity near that of water. Since the inertia of the moving system is determined completely by the inertia of the rotor and that of the solvent, it is possible to determine δ for a glycerol mixture by performing relaxations on some less viscous liquid of the same density as the glycerol mixture and converting the value of δ found, by the ratio of the viscosities (see eq 19). Potassium bromide solutions are convenient for this purpose. Table I lists pertinent data for KBr and glycerol solutions, along with corresponding δ values.

Results. The retardation times for a sample of T2

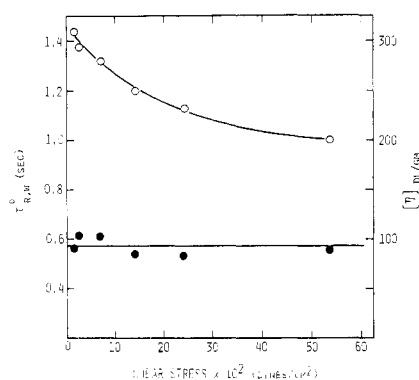


Figure 7. Shear stress dependence of reduced retardation times (●) and intrinsic viscosity (○). The solvent is 75% glycerol–25% BBES and the temperature is 25°. See text for the definition of $\tau_{R,W}^0$.

TABLE I^a
VALUES OF VISCOSITY AND OF δ FOR SOLVENT MIXTURES

Rotor	Solvent mixture	Density, g/ml	Viscosity, cp	δ , sec ⁻¹
1	16.0% KBr	1.12	0.860	0.88
	50% glycerol–50% BBES	1.12	5.37	5.50
2	22.8% KBr	1.19	0.856	0.89
	75% glycerol–25% BBES	1.19	22.0	22.8

^a The δ for the KBr solutions is measured; that for the glycerol solutions is calculated from the KBr value and the viscosity. See text.

DNA in 75% glycerol–25% BBES as a function of concentration for a typical shear stress are shown in Figure 6. The data are plotted three ways: as τ_R which is the data directly from the recorder tracing, as τ_R/η_{rel} and as

$$\bar{\tau}_R = \tau_R[\eta]c/(\eta_{rel} - 1) \quad (34)$$

where η_{rel} , of course, is obtained before shutting off the magnet current from the steady-state speed of the rotor using eq 2. The form of eq 35 is suggested by eq 1. All three types of plots should extrapolate to the same value of τ_R at zero concentration.

It is mildly surprising that the $\bar{\tau}_R$ plot is the flattest, that is, shows the least dependence on concentration. Thompson and Gill⁴ found this type of plot to be flattest for their T2 DNA relaxation time measurements, but since the relaxation time is related to the retardation time of our simple model by $\tau_M = \eta_{rel}\tau_R$, we might have expected our $\bar{\tau}_R$ plot to slope downward with increasing concentration. Nevertheless, the $\bar{\tau}_R$ plots were the flattest of the three types over the range of shear stresses examined, and therefore provide the best type for extrapolating data to zero concentration.

We define a reduced retardation time, $\tau_{R,W}$, by

$$\tau_{R,W} = \tau_R \frac{\eta(\text{H}_2\text{O})}{\eta_s} \quad (35)$$

where $\eta(\text{H}_2\text{O})$ is the viscosity of water and η_s is the viscosity of the glycerol and BBES solvent used in the experiment. Since by eq 1 the relaxation time and hence the retardation time should depend linearly on solvent viscosity, the reduced retardation time allows us to compare relaxations in solvents of varying viscosity.

We define $\tau_{R,W}^0$ as a reduced retardation time which has also been extrapolated to zero concentration. Presented in Figure 7 is a plot of $\tau_{R,W}^0$ vs. initial shear stress for a sample of T2 DNA in 75% glycerol–25% BBES. The intrinsic viscosity as a function of shear stress is also plotted in the figure. Within experimental error, $\tau_{R,W}^0$ is essentially independent of shear stress, the average value being 0.57 sec.

This result is in reasonable agreement with the relaxation time of 0.45–0.52 sec obtained by Thompson and Gill⁴ for T2 DNA. Our results are directly comparable to theirs since relaxation times should equal retardation times at zero concentration. Actually, the agreement is better than indicated since the Thompson and Gill measurement was carried out on a T2 DNA

TABLE II
CALCULATED MOLECULAR WEIGHTS
OF VARIOUS SAMPLES OF T2 DNA

Intrinsic viscosity, ^a dl/g	Reduced retardation time, τ_{R,W^0} sec	Solvent, viscosity, cp	Molecular weight (eq 1), $\times 10^{-6}$	Molecular weight (from intrinsic viscosity ^d), $\times 10^{-6}$
305	0.57	22.0	115	111
320	0.57	5.37	110	120
294	0.48	5.37	101	106
290 ^b	0.50 ^{b,c}	0.89	107	104

^a Measured in aqueous buffer BBES. ^b From Gill and Thompson.⁴ ^c Relaxation time. ^d From empirical formula of Crothers and Zimm.¹¹

sample of $[\eta] = 290$ dl/g, and ours was carried out on a sample $[\eta] = 305$ dl/g. Two other preparations of T2 DNA of $[\eta] = 320$ dl/g and $[\eta] = 294$ dl/g gave average values over the same shear stress range as in Figure 7 of $\tau_{R,W^0} = 0.57$ sec and $\tau_{R,W^0} = 0.48$ sec, respectively. These two experiments were carried out in 50% glycerol–50% BBES.

Our results indicate that $[\eta]$ is the same for both glycerol mixtures and in purely aqueous buffer solvents.

The results can best be compared by calculating apparent molecular weights from eq 1 using a value of the constant α_1 predicted by theory;¹⁸ for DNA, with a viscosity–molecular weight exponent¹¹ of 0.65, this value of α_1 is 0.449. The results for our three samples and that of Thompson and Gill⁴ are shown in Table II. Also shown are the values derived from

the empirical formula relating intrinsic viscosity to molecular weight.¹¹ The new results corroborate the earlier finding⁴ that the agreement of the molecular weights calculated by the different methods is surprisingly good.

In summary, our initial experiments indicate that retardation times may be useful for molecular weight determinations of large DNA molecules. The instrument described here has certain advantages; among these are the ability of measuring relative viscosity and retardation time almost simultaneously on the same sample and of being able to perform these measurements over a wide range of shear stresses, including very low stresses. It is also possible to measure a third quantity, the amount of recoil of the rotor, which should be related to the average amount of deformation of the chain molecules under the shear stress. In regard to this, the total amount of recoil found in our experiments does not fit well with that predicted by our simple model, eq 33. We always find less recoil than predicted. This discrepancy could be explained by the presence of other rapid modes of relaxation, which would require the inclusion of additional Maxwell elements in the model. A more extensive theory providing for this possibility has been worked out and is being prepared for publication.

One disadvantage of this instrument is that, except for especially large molecules, high viscosity solvents must be used in order to minimize the inertial effect. This, however, has not turned out to be a serious problem for measurements on large DNA.

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