A Rotational Viscometer for Studying Non-Newtonian Solutions and Its Application to Pneumococcal Deoxyribonucleic Acid[1a]

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ABSTRACT: A rotational viscometer for studies of non-Newtonian solutions is described. The viscometer is similar in general design to that of Zimm and Crothers. It is a constant-stress viscometer capable of operating at very low shear stresses ($\sim 10^{-4}$ dyn/cm^2) with high accuracy and technical simplicity. It also provides for continuous variation of the shear stress or the shear rate while the experiment is in progress. Operation of the viscometer is based on the principles of electromagnetic induction. The instrument has been used to study the viscometric behavior of dilute DNA solutions, down to shear rates of approximately 10^{-2} sec^{-1}.

In studies of non-Newtonian solutions, a viscometer capable of operating at very low shear with high accuracy, low cost, and technical simplicity has been in constant demand. The viscometer elegantly designed by Zimm and Crothers[2] satisfies these requirements and is particularly useful for biological systems. Unfortunately measurements made with such an instrument built by us were low in precision because of wobbling of the rotor. It was also inconvenient to change the rate of shear. The wobbling effect was attributed to preferential interactions between the magnetic pellet in the rotor and the external rotating magnetic poles, and the difficulty of changing the shear rate was due to hysteresis saturation, as was recognized very early.

In the present communication we describe a rotational viscometer designed in this laboratory which retains the general features of the Zimm–Crothers instrument but is free from the above difficulties. The new viscometer employs an operational principle which, in essence, is that of electromagnetic induction. As a result, the rotor remains perfectly concentric with the stator; and the rotational speed (and hence the shear rate) can be changed continuously and conveniently through a powerstat without changing the entire rotor or, as suggested by Frisman, *et al.*,[3] the relative elevation of the rotor. An electrophoto device is used to record the rotation and reduce timing error. As will be demonstrated below, accurate measurements can thus be made. This work was completed in 1965.

It is noteworthy that another improved viscometer has been described by Gill and Thompson,[4a] who use the principle of the Cartesian diver to control the depth of the rotor for rapid changes of shear rate. Their design is considerably different from ours.

Very recently, Sloniewsky, Evans and Ander[4b] reported their efforts by an approach similar to ours. It is to be noted, however, that the viscometer here presented is operable down to much lower shear stress (approximately 100-fold) than the instrument reported by these authors.

Principles. Suppose a diamagnetic conducting ring to be placed in an alternating magnetic field. The planar surface of the ring is oriented perpendicular to the field. Inside the ring an electrostatic field is established and the ring is made to rotate by the action of a torque. At steady state, the ring rotates uniformly in the same direction as the inducing field. The mean value of the torque, T_m, can be expressed as[5]

$$T_m = \frac{1}{2}ABE_0\frac{RS}{R^2 + X_0^2S^2} \qquad (1)$$

where A is the area enclosed by the ring, B is the intensity of the inducing field, R is the resistance of the conductor, X_0 is the reactance, and E_0 is the root mean square electromotive force, both of the conductor at rest. The fractional slip S is defined as

$$S = \frac{n_1 - n_2}{n_1} \qquad (2)$$

where n_1 and n_2 are the angular velocity, in radians per second, of the external field and of the conducting ring, respectively. For a given set of conditions, A, B, E_0, R, X_0, and n_1 can be taken as constant. Thus T_m is a function of the fractional slip, or a function of n_2. If n_2 is adjusted such that $n_1 \gg n_2$ and $S \cong 1$, then T_m is a constant under the given set of conditions.

In the rotational viscometer designed, the conducting ring is inserted rigidly in the inner cylinder, called the rotor. After the solution to be measured is properly introduced into the annular space between the cylinders, the rotor is made to rotate by a rotating magnetic field generated by alternating current. The speed of the rotor is inversely proportional to the viscosity of the solution. The viscosity η, n_2, and T_m are related in the following fashion[6]

$$T_m = 4\pi L n_2 \eta \frac{a^2b^2}{a^2 - b^2} \qquad (3)$$

where a and b are the radius of the inner and the outer cylinder, respectively, and L is the length of the inner

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(1) (a) Taken in part from a dissertation submitted in partial fulfillment for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University, April 1967. (b) Marshall Research Laboratory, E. I. du Pont de Nemours & Company, Philadelphia, Pa. 19146.

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cylinder. If R and X_0 can be measured accurately, the absolute value of the torque, and thus the absolute viscosity of the solution, can be calculated. These measurements are not necessary, however, if only the relative viscosity is desired. By measuring n_2 for the solution and for the solvent, the relative viscosity can be obtained.

In this design, there is no inherent magnetic interaction between the ring and the external field, and hence wobbling of the rotor is eliminated. Moreover, the intensity of the field can be changed by simply changing the alternating current supplied to the field, an operation which can be performed continuously and conveniently through the use of a powerstat.

Construction and Characteristics. The cylinders used are made of precision-bore glass tubings (Wilmad Glass Co.). The general features of the cylinders and the many precautions in preparing them are similar to those described in detail by Zimm and Crothers.[2] The conducting ring is made of aluminum and is snugly fitted inside the bottom of the inner cylinder.

The alternating magnetic field is generated from the laminated stationary core unit of a magnetic stir motor (Harshaw Scientific). After the armature is removed, the unit is attached rigidly to a base plate. The empty space of the core is just enough to allow proper insertion of the stator. The stator is positioned so that the aluminum ring is in the center of the core. Line current, after regulation by a constant voltage transformer (Sola Electric Co.), is supplied to the coil of the laminated core. This generates an alternating magnetic field, 60 Hz, in the core. The current supplied to the coil is measured by a multiple-range ammeter (Hall Mark Standards, Model SPFB). A current of 200 mA is needed to produce a shear rate of 10 $\sec^{-1}$ with water. By decreasing the current to about 10 mA, the shear rate is reduced to the order of 10^{-2} $\sec^{-1}$. Heat generated in the core is removed by circulating cooling water. The constant-voltage transformer used is of the CVS harmonic neutralized type which contains less than 3% total root mean square harmonic content in the output. The power of the transformer was carefully selected according to the load.

The inner surface of the rotor is plated with metallic silver in fine strips evenly spaced around the circumference. A light beam from a small bulb is focused on the rotor. As the rotor turns, it is transparent to the incident light at all positions except the silver strips where the light is reflected. The reflection is picked up by a photocell (Clairex CL-2) and the signal transmitted to a relay (Lafayette Electronics, KT-133A) which operates a counter. A precision timer is used to record the lapse of time for a certain number of revolutions. The time required for up to 100 revolutions of the rotor is usually measured to obtain an average rpm. The bulb and the photocell are fixed in the holder of the stator. By using this system, timing errors are drastically reduced. The system can also be made to operate automatically.

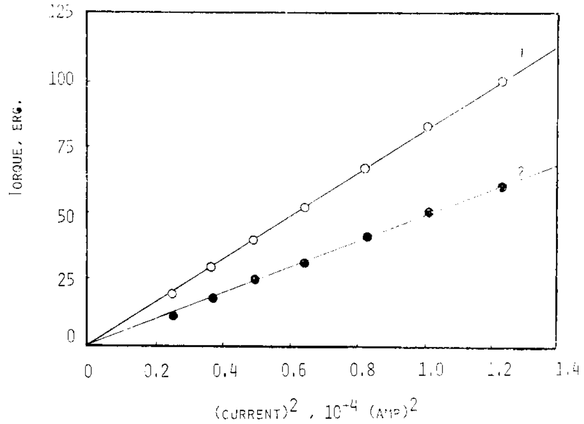

Figure 1. Relationship between torque and current: 1, water; 2, glycerol solution (relative viscosity 1.660).

It has been shown that the torque established at the rotor is proportional to the product of B and E_0. Since E_0 depends on B which in turn depends on the current supplied to the coil, the torque is therefore proportional to the square of the current i measured by the ammeter. Thus a plot of T_m, the average torque acting on the rotor, *vs.* i^2 should give a straight line for Newtonian solutions. The slope of the line is inversely proportional to the viscosity of the solution (for example, see Figure 1). This provides a convenient way of calibration. It also enables one to detect any unstable rotation of the rotor. Wobbling, for example, would dissipate additional energy and reduce the angular speed. This would be indicated in the plot by points falling below the straight line. It is clear that the apparent viscosity and consequently the apparent intrinsic viscosity of a solution would be too high if measured in a viscometer with unstable rotations.

It is possible that at extremely low shear rates the quadratic dependence of torque on current may be varied since the load of the transformer is extremely low. The iron core of the magnet may also show unusual characteristics at very low current. In our experiments, the torque–current relationship was checked from time to time. The angular uniformity of the rotational speed of the inner cylinder was another item for routine check-up. We have made no attempts to extend the range of shear rate to below 10^{-2} $\sec^{-1}$ since all the requirements of stability are difficult to maintain. This does not mean, however, that the viscometer cannot, in theory, be used for lower shear rates. The upper limit of the shear rate is determined by the onset of turbulent flow.

Application. To illustrate the use of the viscometer, some results on the viscosity of dilute solutions of deoxyribonucleic acid (DNA) isolated from *Diplococcus pneumoniae* are presented. Methods of isolation and purification described by Roger, Beckmann, and Hotchkiss[7] were followed in this work. Sedimentation behavior, including both the concentration dependence and the speed dependence of the sedimentation coefficinet of the sample, has been studied in detail.[8]

The relative viscosities of DNA solutions of different concentrations (5–40 μg/ml) were measured. DNA concentration was measured by a Beckman spectrophotometer, Model DU. We used an extinction

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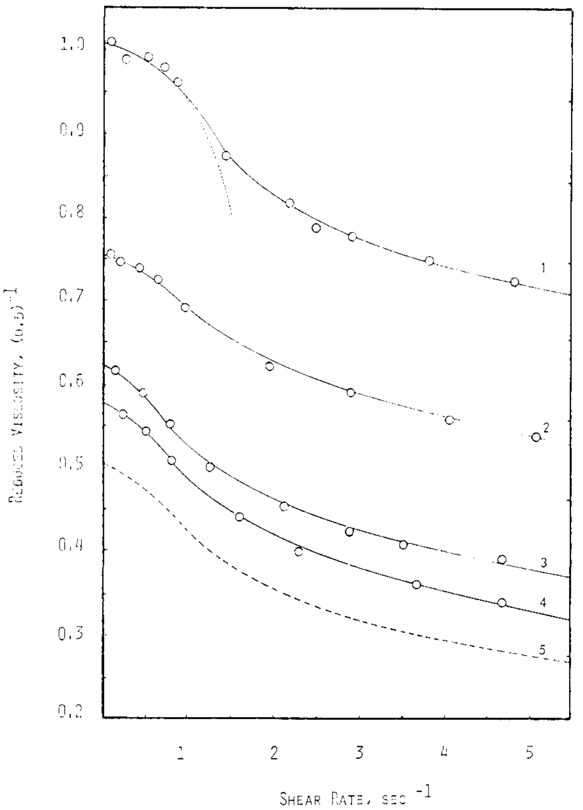

Figure 2. Shear rate dependence of the reduced viscosity of pneumococcal DNA in 0.15 *M* NaCl, pH 6.8. Curves represent different concentrations of DNA in optical density units: 1, 0.96; 2, 0.500; 3, 0.262; 4, 0.145; 5, zero concentration (extrapolated).

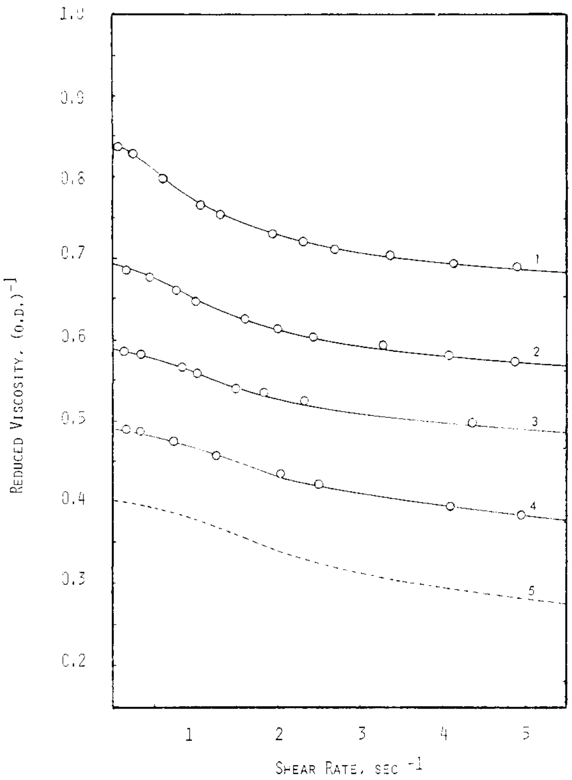

Figure 3. Shear rate dependence of the reduced viscosity of pneumococcal DNA in 1.0 *M* NaCl, pH 6.8. Curves represent different concentrations of DNA in optical density units: 1, 0.92; 2, 0.615; 3, 0.357; 4, 0.185; 5, zero concentration (extrapolated).

coefficient of 213 dl/g at 260 mμ for concentration conversion. All the solutions were phosphate buffered, pH 6.8, and contained 0.15 *M* NaCl. The reduced viscosity is plotted against the average shear rate, *G*, in Figure 2. It is evident that the solution viscosity of DNA depends strongly on the rate of shear in the region of low shear rates studied ($G < 5$ sec^{-1}). The reduced viscosity appears to fit eq 4 as first proposed by

$$\frac{\eta_{sp}}{C} = a_0 - a_1G^2 + a_2G^4 - a_3G^6 + \dots \qquad (4)$$

Kuhn and Kuhn[9] and by Saito.[10] The coefficients a_0, a_1, a_2, ... are constants characteristic of the polymer solutions. Application of this equation to rigid ellipsoidal particles has been fully illustrated.[11] In the limit of very low shear rate, terms higher than the quadratic can be neglected. The curve can thus be approximated by a parabola (see the dotted line, curve 1, Figure 2). A horizontal tangent at zero shear rate is predictable. The critical shear rate at which departure from a parabola becomes observable appears to be in the vicinity of 1 sec^{-1}. The curve levels off gradually and almost linearly at higher shear rates as the inclusion of higher terms become necessary. It is thus apparent that a viscometer operable at shear rates lower than this critical value is imperative for physical-chemical characterization of macromolecules of very high molecular weight or high asymmetry such as DNA. It is in this region where the shear-rate dependence of viscosity is most pronounced, and where critical information pertaining to molecular behavior is available. Obviously, any attempts to estimate the zero-shear viscosity by extrapolating the linear portion of the curve would result in large errors. The intrinsic viscosity of the pneumococcal DNA sample used was determined as 107 dl/g which is significantly higher than the best value obtained with a modified Ubbelohde viscometer, 85 dl/g, of the type described by Eigner.[12] Briefly, the modified Ubbelohde viscometer has four bulbs of different sizes located serially at different heights above the capillary. The lowest shear rate obtained was about 20 sec^{-1}. The concentration dependence of the reduced viscosity of pneumococcal DNA was also clearly established in this study (Figures 2 and 3).

The zero concentration sedimentation coefficient, $s_{20,w}^0$ of the sample was 31.8 Svedbergs. By combining this value with the intrinsic viscosity, the molecular weight of the pneumococcal DNA sample was calculated to be 22.0×10^6 according to the Flory–Mandelkern–Scheraga equation[13,14]

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$$M^{2/3} = \frac{s^{0}_{20,\mathrm{w}}[\eta]^{1/3}\eta_0 N}{10^{13}\beta(1 - \bar{v}\rho)} \qquad (5)$$

where M is the molecular weight, $[\eta]$ is the intrinsic viscosity, $\bar{v}$ is the partial specific volume of the solute, η_0 and ρ are, respectively, the viscosity and density of the solvent, N is Avogadro's number, and β is a universal constant (2.50×10^6 for random coil molecules). Many equations for calculating the molecular weight of DNA from hydrodynamic measurements have been published in the past 15 years.[15] For example, Crothers and Zimm[16] suggest eq 6 and 7.

$$0.665 \log M = 2.863 + \log([\eta] + 5) \qquad (6)$$

$$0.445 \log M = 1.819 + \log(s^{0}_{20,\mathrm{w}} - 2.7) \qquad (7)$$

From our $[\eta]$ and $s^{0}_{20,\mathrm{w}}$ the molecular weights calculated for our sample are 24.3×10^6 and 23.8×10^6, respectively. The agreement among the three calculations seems satisfactory.

The shear-rate dependence of the reduced viscosity also changes with the salt concentration of the solution. In fact the effect of salt on the intrinsic viscosity of DNA has been known for a long time.[17,18] Different results obtained have led to different interpretation of the molecular properties of DNA.[19] In the present study it was found that increasing the salt concentration from 0.15 to 1.0 M results in a 20% decrease of the intrinsic viscosity. This observation is similar to those of Scruggs and Ross,[20] who reported a continuous decrease of the intrinsic viscosity of T4 DNA on adding salt over the range 0.1–1.0 M NaCl.

The effect of added salt can be attributed either to a change of molecular conformation or to a change of intermolecular interactions, or both. Any effect of salt on the intrinsic viscosity of the DNA solution would be due to the change of molecular conformation.

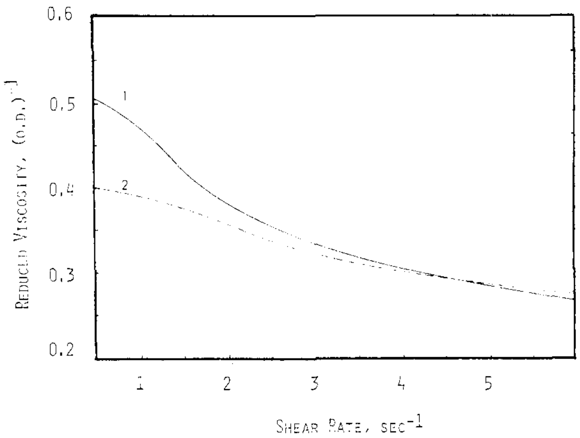

Figure 4. Effect of salt on the non-Newtonia intrinsic viscosity of pneumococcal DNA, pH 6.8; 1, 0.15 M NaCl; 2, 1.0 M NaCl.

The broken line in Figure 2 shows the intrinsic viscosity at various shear rates. Figure 3 shows the results for similar solutions in 1.0 M NaCl. The two intrinsic viscosity curves are superimposed in Figure 4. A marked salt effect on the non-Newtonian behavior in the region of very low shear rates is evident. This difference may well be overlooked by other viscometers of high operating shear rates. The shear-rate dependence of viscosity is milder at the higher salt concentraion. It can therefore be concluded that DNA molecules in 0.15 M NaCl are more rigid and extended than in 1.0 M NaCl. Physically this is possible because the phosphate charges in the DNA backbone are less shielded in the former solution, permitting stronger electrostatic repulsions between the phosphate groups.

Acknowledgments. This paper is dedicated to the late Professor Charles O. Beckmann, Columbia University, who aroused my interests in polymer chemistry and extended to me his generous guidance and encouragement throughout the course of this work. I also wish to express appreciation to Professor M. Roger of Rockefeller University and Professor T. I. Taylor of Columbia University for many helpful discussions.

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