Dielectric Dispersion of Deoxyribonucleic Acid. II[1]

by Shiro Takashima

Electromedical Division, Moore School of Electrical Engineering, University of Pennsylvania, Philadelphia, Pennsylvania *(Received July 12, 1965)*

Dielectric dispersion of deoxyribonucleic acid (DNA) solution is measured with a high-precision low-frequency bridge between 50 cps and 200 kcps. The measurements are carried out with DNA samples with widely different molecular dimension. It is found that the dielectric increment and the relaxation time of helical DNA are proportional to the square of the length of molecule. The dielectric increment and the relaxation time of coiled DNA are distinctly smaller than the values for helical DNA. This relationship seems to hold for different types of DNA. The measurements of flow birefringence is conducted simultaneously with the dielectric measurement. The rotary relaxation time is compared with the dielectric relaxation time. It is found that the agreement of both relaxation times is fairly good in a low molecular weight region. The disparity, however, becomes pronounced when DNA is larger, and the rotary relaxation time is greater than the dielectric relaxation time by a factor of 20.

Introduction

Dielectric dispersion of DNA was first measured by Allgen, Jungner, and Jungner.[2] They reached a conclusion, on the basis of their experimental results, that DNA had a permanent dipole in the transverse direction. They also concluded that electric polarization of DNA is caused by the rotation of the molecule around the major axis in its entirety. The dielectric dispersion of DNA was also studied by Jerrard and Simmons.[3] These previous measurements are, however, limited to a narrow frequency region and also limited to low molecular weight DNA samples. Recently, Takashima[4] extended the measurement to a low-frequency region. He measured the dielectric dispersion of various DNA samples with molecular weights ranging from 400,000 to 6,000,000 between 50 cps and 1 Mcps. He observed that DNA had a dielectric dispersion in a lower frequency region than previously observed. He found that the dipole moment and the relaxation time were proportional to the molecular weight of DNA. He concluded from these results that DNA had a dipole moment in the direction of the major instead of the minor axis. The present work is an extension of the previous experiments to provide further evidence for the conclusion described above.

It is known that DNA exhibits a negative flow birefringence. By measuring the extinction angle, we can calculate the rotary diffusion constant. This is in turn converted into the relaxation time. The conclusion drawn by Allgen and Jungner is based on the fact that the dielectric relaxation time they observed (10^{-7} sec) differs substantially from the rotary relaxation time observed by Edsall[5] (10^{-3} sec). As mentioned above, Takashima found a dielectric dispersion in a lower frequency region. The relaxation time he obtained is indeed in the range of 1 msec, which indicates that the dielectric relaxation time is close to the rotary relaxation time. In this experiment, measurements of dielectric dispersion and flow birefringence are carried out simultaneously. A more careful comparison of dielectric relaxation time with rotary relaxation time is attempted.

Experimental Section

A Wheatstone bridge designed by Schwan[6] was used.

(1) This work was supported by National Institutes of Health Grant GM-12083-01.

(2) L. G. Allgen, *Acta Physiol. Scand., Suppl.*, **22**, 76 (1950); G. Jungner, I. Jungner, and L. G. Allgen, *Nature*, **63**, 849 (1949).

(3) H. G. Jerrard and B. A. W. Simmons, *ibid.*, **184**, 1715 (1959).

(4) S. Takashima, *J. Mol. Biol.*, **7**, 455 (1963).

(5) J. T. Edsall, "Proteins, Amino Acids and Peptides," E. J. Cohn and J. T. Edsall, Ed., Reinhold Publishing Corp., New York, N. Y., 1943, p 450.

(6) H. P. Schwan, "Physical Techniques in Biological Research," Vol. 6, Academic Press, Inc., New York, N. Y., 1963, Chapter 6.

Measurements were carried out between 50 cps and 200 kcps. The bridge was designed for measurement with conductive materials and is suitable for DNA solution. The conductance of dilute DNA solution is usually 20 to 50 μmhos at a concentration of 0.01–0.03%. The magnitude of experimental error in lossy solutions was already discussed in detail by Schwan.[6]

The major source of experimental error with conductive solutions is due to electrode polarization. Two methods are applied to eliminate this effect. (1) The platinum electrodes are very carefully plated with platinum black according to the method developed by Maczuk and Schwan.[6] This is to increase the surface area of the electrodes. The measured capacity of the solution can be expressed by the following equation,[6] where C_s is the true capacity, C_p is the electrode capacity, ω is the angular frequency, and R is the resistivity of the solution. To make the second term of eq 1 small, either R or C_p must be made large. We cannot make the resistivity of DNA solution very large because the resistivity of water is at most 1 megohm and DNA is a highly charged molecule. Moreover, DNA is unstable at very low ionic strength and one must keep the ionic strength above a certain level. An increase in the electrode capacity is, therefore, a more feasible method of decreasing the second term of the equation. The effect of electrode polarization can still be considerable with conductive solutions, even with a good plating. This gives rise to a difficulty in determining the low-frequency dielectric constant. As will be mentioned later, the dispersion of DNA depends largely on the length of the molecule. The effect of electrode polarization is not serious for small DNA molecules because the dielectric dispersion is in a relatively high frequency region. For large molecules, however, further correction is essential. A dielectric cell was constructed in which the distance of the electrodes was variable. Measurements were repeated twice at two electrode distances, say at 10 and 1 cm. Electrode polarization is independent of the electrode distance and it can be eliminated by using the following equation;[6] C_s is the true capacity at 10 cm, C_1 and C_2 are the capacities at electrode distance 10 and 1 cm, respectively, and R_1 and R_2 are the resistivities at those electrode distances, respectively.

$$C = C_s + 1/[C_p^2\omega^2 R] \quad (1)$$

$$C_s = \left[C_1 - C_2\left(\frac{R_2}{R_1}\right)_{5kc}\right] \Big/ \left[1 - \left(\frac{R_2}{R_1}\right)_{5kc}\right] \quad (2)$$

The capacity and conductivity of the solution can be measured down to 100 cps with a reasonable accuracy. High molecular weight DNA, however, has an anomalous dispersion in a very low frequency region. The low-frequency plateau of the dielectric dispersion of these samples seems to appear even below 100 cps. The measurement of capacity of DNA solution becomes much more difficult below 100 cps. One cannot obtain the low-frequency plateau by extrapolating the dispersion curve without considerable arbitrariness. In a case like this, the use of a Cole–Cole plot[7] is very helpful. As shown in Figure 1, the Cole–Cole plot of DNA is symmetrical. Thus, we can estimate the low-frequency dielectric constant from the intersection of the circle with the abscissa. The value of the low-frequency dielectric constant obtained by this method is much more reliable and less arbitrary. In the present experiment, the low-frequency dielectric constant is always obtained by this method.

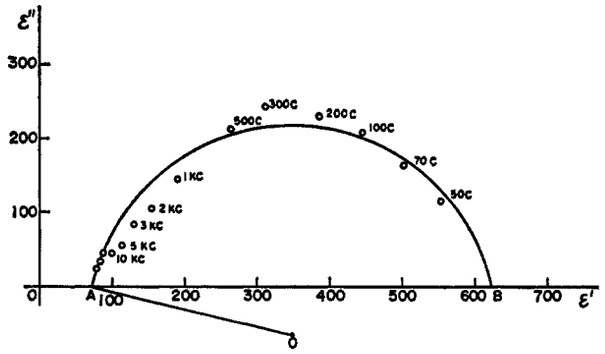

Figure 1. The Cole–Cole plot of salmon sperm DNA. The ordinate is the imaginary part and the abscissa is the real part of dielectric constant. The numbers in the figure are frequencies. A and B gives the high- and low-frequency dielectric constant.

The imaginary part of the dielectric constant (dielectric loss ϵ'') is calculated by the formula

$$\epsilon'' = (\kappa - \kappa_0)/2\pi f\epsilon_\gamma \quad (3)$$

κ_0 is the low-frequency conductivity in micromhos and ϵ_γ is the absolute value of dielectric constant of vacuum ($^1/_{36}\ \pi \times 10^{11}$). The dispersion of conductivity is shown in Figure 2. It is evident from eq 3 that a small error in the conductivity measurement can cause a considerable error in the dielectric loss at low frequency. The measurement of conductivity is performed with the error of ± 0.001 μmho. Although the temperature of the solution is controlled by circulating the thermostated water, the fluctuation of conductivity cannot be prevented. The correction method for the drift of conductivity is given in the previous publication.[8] The random error of the conductivity

(7) K. S. Cole and R. H. Cole, *J. Chem. Phys.*, 9, 341 (1941).

(8) J. Maczuk, Intramural Report of University of Pennsylvania, 1957. This method is described also in ref 4.

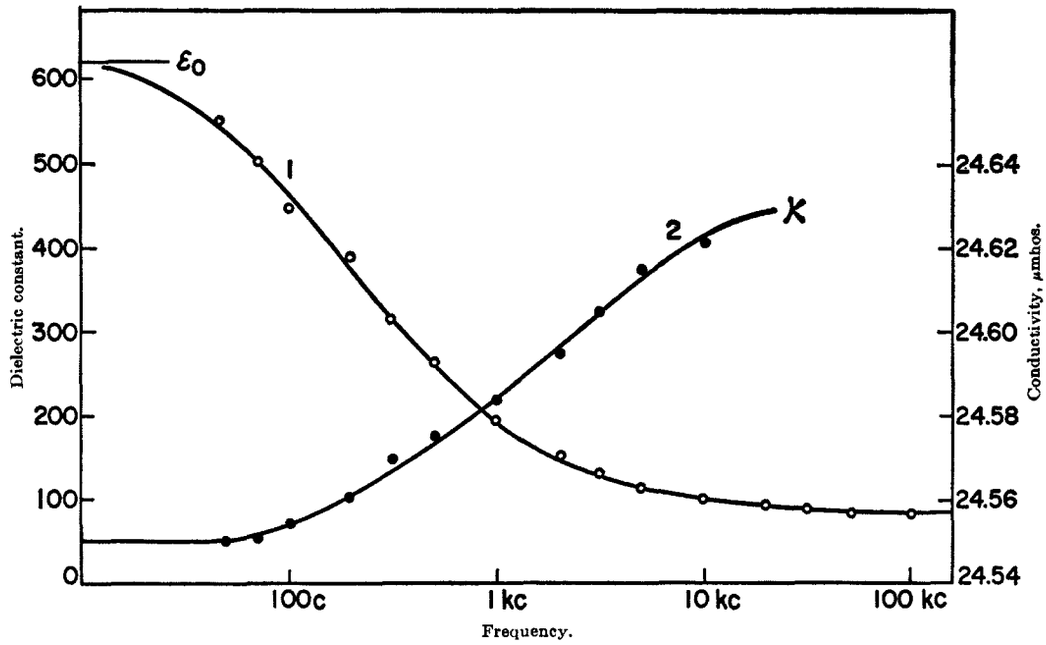

Figure 2. Dielectric dispersion of salmon sperm DNA: curve 1, dielectric constant; curve 2, conductivity. The concentration is 0.01%. The horizontal line designated ϵ_0 indicates the low-frequency dielectric constant obtained from Cole–Cole plot.

reading is sufficiently small for the determination of the dielectric loss.

The flow birefringence and the extinction angle of DNA solution are measured with a Rao birefringence apparatus Model-B-22. The extinction angle χ is related to a parameter α[9] in the equation by Boeder[10] and that by Peterlin and Stuart.[11]

$$\chi = \frac{1}{2}\tan^{-1}\frac{6}{\alpha} = \frac{\pi}{4} - \frac{\alpha}{12}[1 - f(\alpha, a, b)] \quad (4)$$

The value of α is tabulated in the paper by Edsall, *et al.*,[12] for various axial ratios. The rotary diffusion constant is related to the parameter α by eq 5, where β

$$\alpha = \beta/\theta \quad (5)$$

is the velocity gradient. The rotary relaxation time is calculated from the rotary diffusion constant by eq 6. The length of DNA is calculated by using the

$$\tau = \theta/2 \quad (6)$$

Perrin equation[13]

$$\theta_b = \frac{3kT}{16\pi\eta a^3}\left(2\ln\frac{2a}{b} - 1\right) \quad (7)$$

where θ_b is the rotary diffusion constant around the minor axis, η is the viscosity of the solvent in poises, a and b are the semimajor and semiminor axes in centimeters. The axis a is not necessarily the fully stretched length of DNA and the b is not necessarily the radius of double helix. Since the Perrin equation cannot be solved analytically, a computer IBM 1710 is used to obtain the solution for the length of DNA molecule.[14]

Calf thymus and salmon sperm DNA were used in this experiment. The length of calf thymus DNA ranged from 10,500 to 1500 A and that of salmon sperm DNA was from 7400 to 1300 A. A 20-kc sonic oscillation was applied to produce smaller DNA samples. Viscosity and rotary diffusion constant were measured each time. Viscosity was measured with a Rao couette-type viscometer with varying shearing stress. The viscosity was obtained by extrapolating the consistency curve to zero shear.

DNA was dissolved in freshly deionized water. The pH of the water was carefully examined each time. Distilled water becomes acidic on storage. Since DNA is unstable at low ionic strength, the pH of the water must be maintained close to neutral to avoid the denaturation. If DNA was dissolved in acidic water, it did not exhibit flow birefringence nor large dielectric increment. This indicates denaturation,

(9) This parameter should not be confused with the Cole–Cole parameter, α.

(10) P. Boeder, *Z. Physik*, **75**, 273 (1932).

(11) A. Peterlin and H. A. Stuart, *ibid.*, **112**, 1, 129 (1939).

(12) H. A. Scheraga, J. T. Edsall, and J. O. Gadd, *J. Chem. Phys.*, **19**, 1101 (1951).

(13) F. Perrin, *J. Phys. Radium*, **7**, 5, 497 (1934).

(14) The author is grateful to Mr. B. Pennock for his help for the programming.

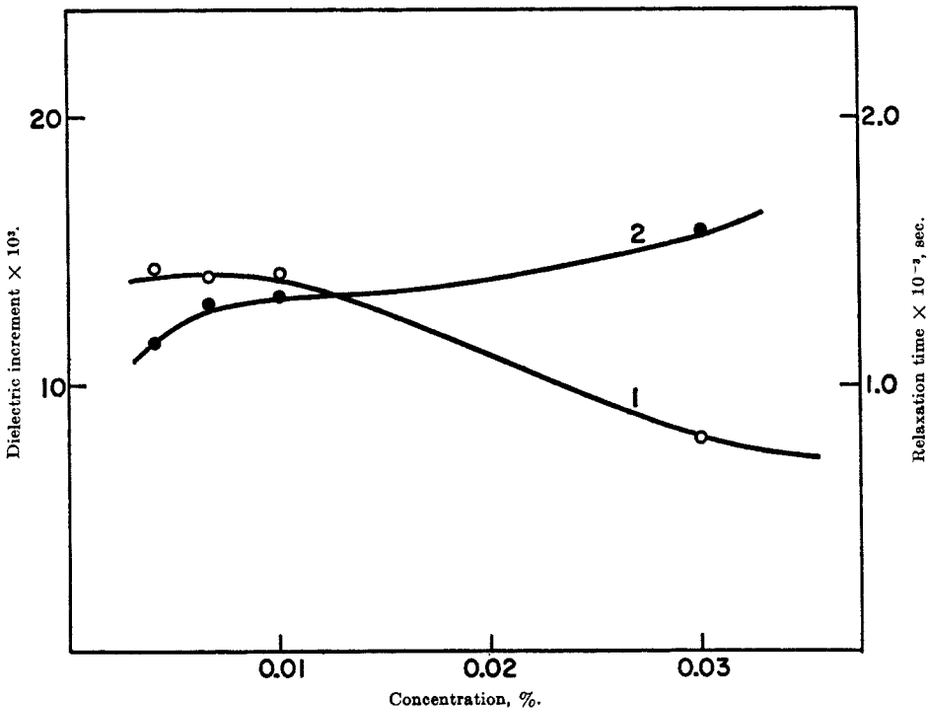

Figure 3. Dependence of specific dielectric increment and relaxation time on concentration, grams per 100 ml. Specific dielectric increment is $\Delta\epsilon$ per gram per 100 ml: curve I, dielectric increment; curve 2, relaxation time.

probably strand separation. It was found that calf thymus DNA is more stable than salmon sperm DNA. The concentration of DNA was 0.01% unless otherwise stated.

Results

(*a*) *Native DNA.* The dielectric dispersion of salmon sperm DNA is shown in Figure 2. The dielectric constant of DNA solution rises far above the dielectric constant of water and is still increasing at 50 cps. Thus, it is difficult to estimate the low-frequency dielectric constant from the dispersion curve with a reasonable accuracy. As explained in the previous section, the use of the Cole–Cole plot is very helpful for obtaining the low- and high-frequency dielectric constant. The Cole–Cole plot of DNA solution is shown in Figure 1. The low and high dielectric constant as obtained from this plot are 620 and 80, respectively. The horizontal line in Figure 2 is the low-frequency dielectric constant obtained by the Cole–Cole plot. The value thus obtained seems to be in reasonable agreement with the value obtained by the extrapolation of the dispersion curve. The dispersion of conductivity is shown in Figure 2. The increment of the conductivity is about 0.08 μmho. This indicates that the measurement of conductivity dispersion is much more difficult than that of dielectric constant. The distribution parameter is calculated from the angle OAB ($\alpha\pi/2$). Here α is the Cole–Cole distribution parameter. It was observed that the dielectric increment depended on the concentration of DNA.[4] Schwarz and Eigen[15] also observed, in the study of orienting field effect, that the polarizability and the orientational relaxation time of DNA were dependent on the solute concentration. Figure 3 shows the dependence of specific increment and the dielectric relaxation time on concentration. One observes a considerable dependence of dielectric increment on concentration in the high concentration region; however, it becomes rather constant below 0.01%. Relaxation time also changes with concentration, particularly below 0.002%. From this figure, it is obvious that 0.01% seems to be the best concentration to carry out the dielectric measurement. The subsequent measurements were carried out at 0.01% unless otherwise stated.

Figure 4 illustrates the dispersion curves of salmon sperm DNA with various lengths. It is found that the dielectric increment as well as the relaxation time decrease with the decrease of the length. Particularly, the decrease in the dielectric increment is pronounced. Curve 1 in Figure 5 shows the relationship between the length of the molecule and the dielectric increment.

(15) M. Eigen and G. Schwarz, "Electrolytes," B. Pesce, Ed., Pergamon Press Ltd., London, 1962.

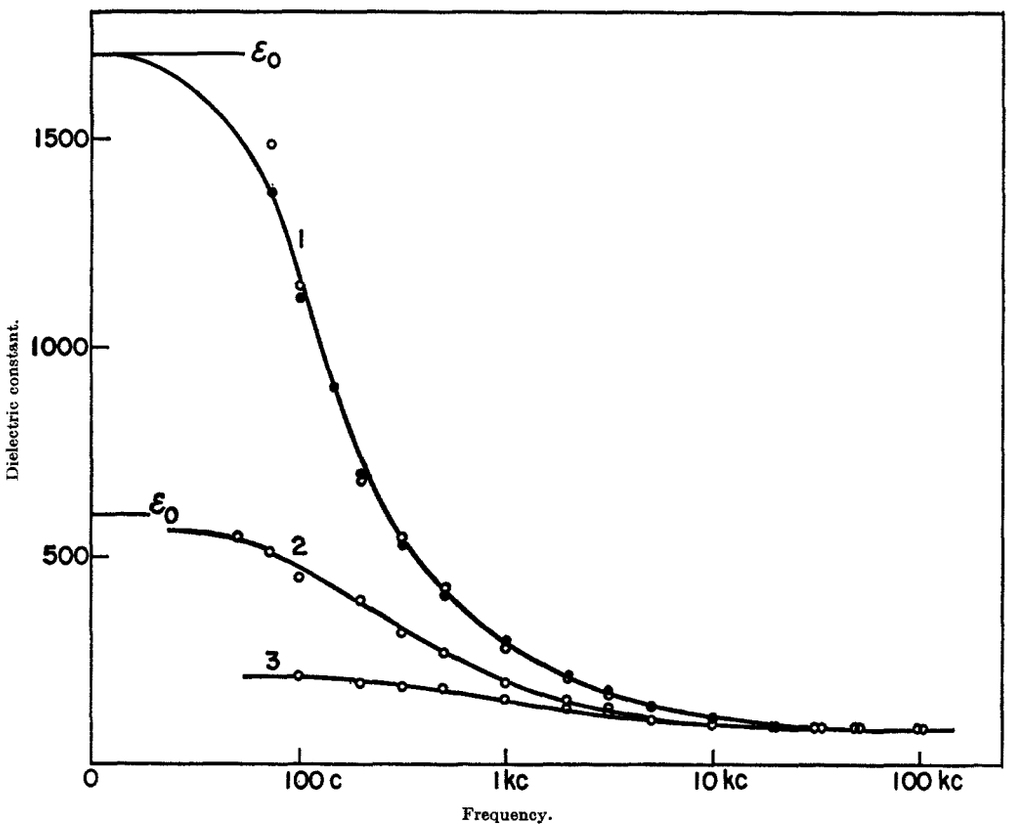

Figure 4. Dielectric dispersion of salmon sperm DNA. The lengths of DNA samples are 7400 (curve 1), 5600 (curve 2), and 1300 A (curve 3). The concentration is 0.01%. Curve 1 shows the result of two measurements.

The following relationship between the length and the dielectric increment is obtained

$$\Delta\epsilon = 0.29 \times 10^{-5} \times L^2 = AL^2 \qquad (8)$$

The relaxation time is calculated by using

$$\tau = {}^1/_2\pi f_c \qquad (9)$$

where τ is the relaxation time and f_c is the critical frequency. The relationship between the relaxation time and the length is shown in Figure 6. The relationship is expressed by the empirical formula

$$\tau = 1.6 \times 10^{-5} \times L^2 = BL^2 \qquad (10)$$

Curve 2 in Figure 5 illustrates the relationship between the dielectric increment of calf thymus DNA and its length. Curve 2 in Figure 6 illustrates the size dependence of relaxation time of calf thymus DNA. The relationships are very much the same as that of salmon sperm DNA. The dielectric increment and the relaxation time of this DNA can be expressed by using eq 8 and 10.

(*b*) *Denatured DNA.* Figure 7 illustrates the dielectric dispersion of heat-denatured DNA. It is known that DNA undergoes a transition from a double helical configuration to a single-strand random-coil configuration. The transition is caused by heating or by an extreme pH. The dispersion curve shown here represents a dielectric dispersion of heat-denatured coil-form DNA. The denaturation is confirmed by the decrease of viscosity and by the disappearance of flow birefringence. It must be noted that the scale of the ordinate in Figure 7 is greatly enlarged. Although the magnitude of dielectric increment is much smaller than that of helical DNA, one can still observe a considerable increment and its dependency on frequency. Undoubtedly, the dielectric dispersion of the coil form exists in a higher frequency region than that of the helical form.

(*c*) *Comparison between the Rotary and Dielectric Relaxation Time.* The conclusion drawn by Allgen and Jungner was based on the fact that the dielectric relaxation time they observed was widely different from the rotary relaxation time. As was mentioned, the dielectric relaxation time observed in this experiment is considerably larger than those observed by Allgen and Jungner. A comparison of dielectric relaxa-

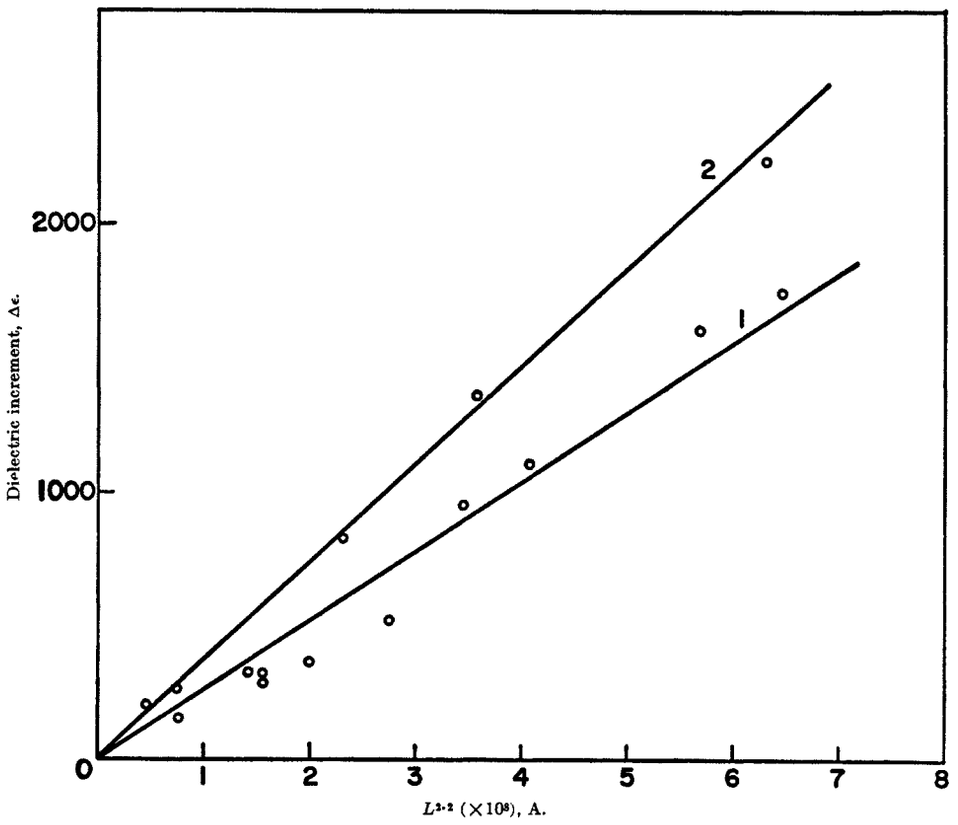

Figure 5. The plot of dielectric increment against the length of DNA: curve 1, salmon sperm DNA; curve 2, calf thymus DNA. The ordinate is dielectric increment as measured and the abscissa is the 2.2 power of the length in angstroms. Concentration is 0.01%.

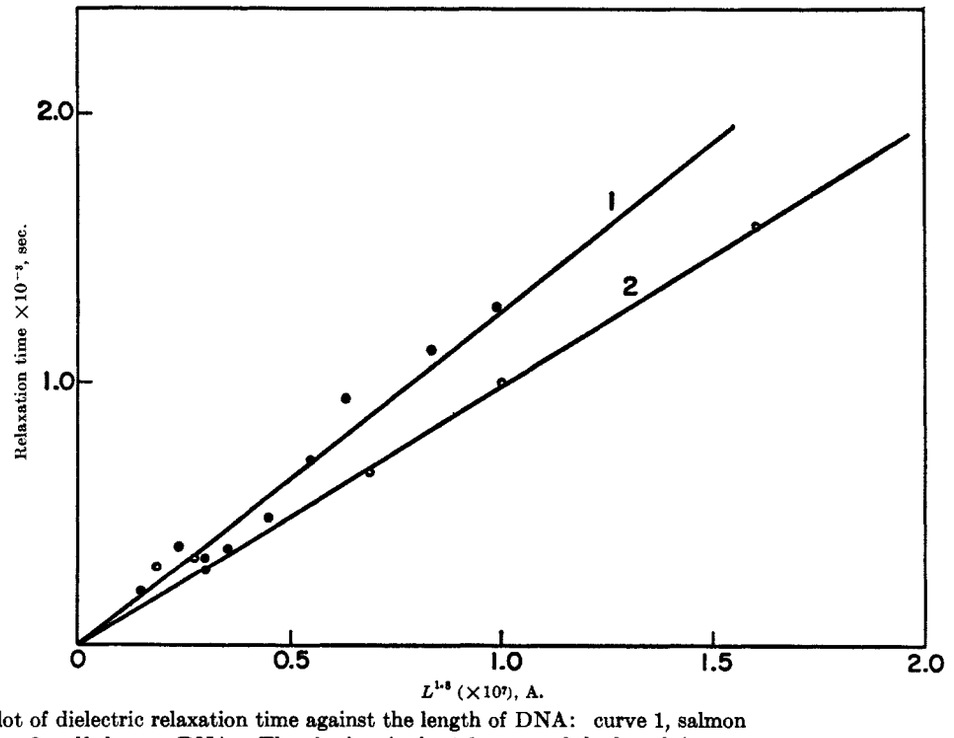

Figure 6. The plot of dielectric relaxation time against the length of DNA: curve 1, salmon sperm DNA; curve 2, calf thymus DNA. The abscissa is the 1.8 power of the length in angstroms.

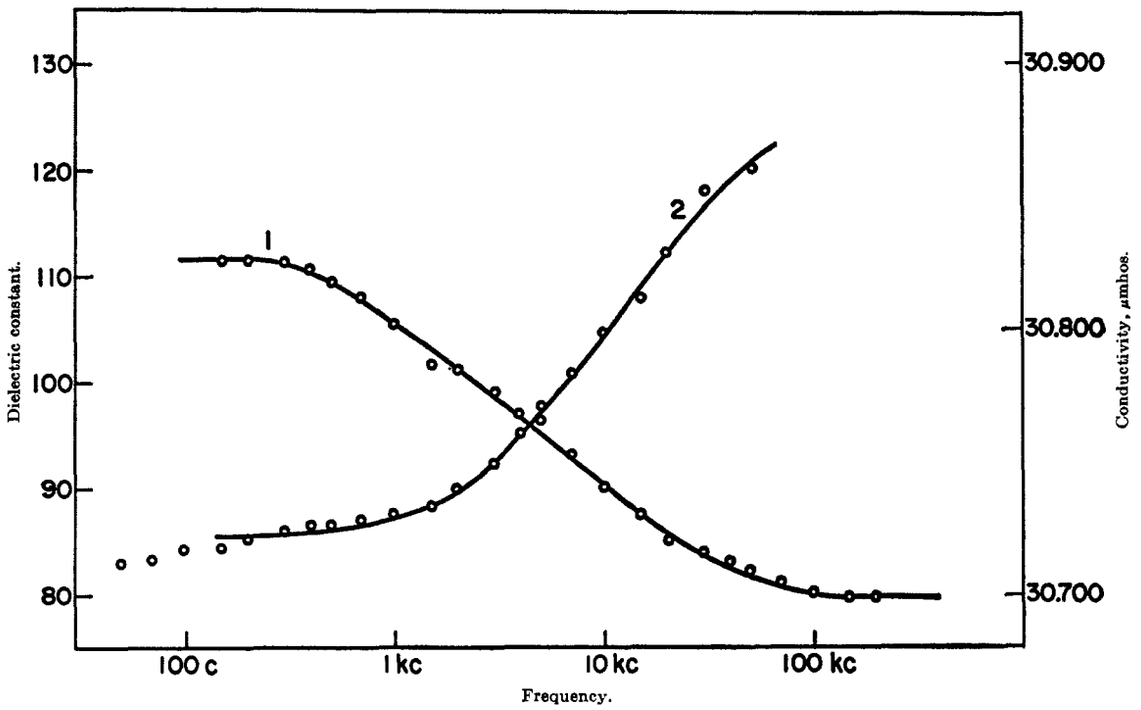

Figure 7. Dielectric dispersion of heat-denatured DNA: curve 1, dielectric constant; curve 2, conductivity in micromhos. Concentration is 0.03%.

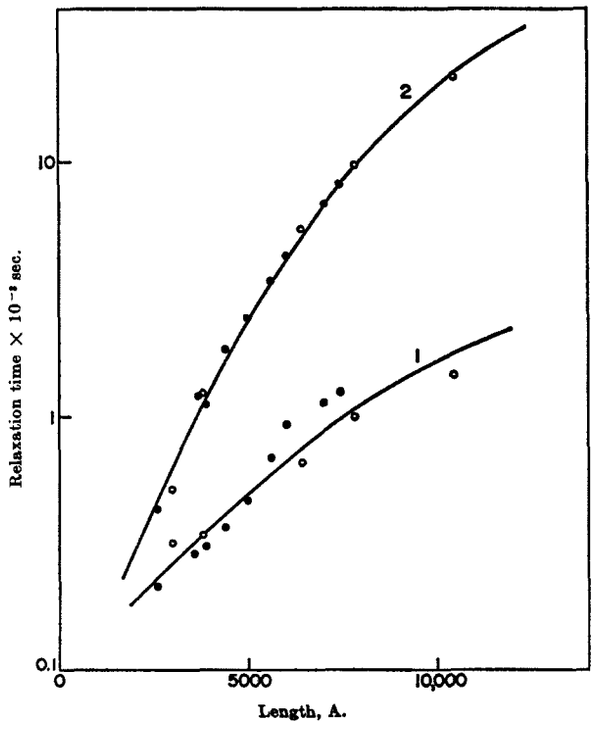

Figure 8. Dielectric relaxation time and rotary relaxation time of DNA plotted against the length of the DNA molecule; curve 1, dielectric relaxation time: curve 2, rotary relaxation time: ○, salmon sperm DNA; ●, calf thymus DNA. The ordinate is in logarithmic scale.

tion time and the rotary relaxation time is attempted. The rotary and the dielectric relaxation time are measured simultaneously with the same DNA sample. The results obtained with calf thymus DNA are tabulated in Table I. To show the relationship more clearly, those relaxation times are plotted in Figure 8 for both DNA's. In both cases dielectric and rotary relaxation

Table I: Comparison between Rotary Diffusion and Dielectric Relaxation

Length, A	Rotary relaxation time, sec × 10^8	Dielectric relaxation time, sec × 10^8
10,400	21.2	1.57
7,800	9.6	1.0
6,400	5.36	0.66
3,800	1.21	0.33
3,000	0.52	0.31

times are close to each other in the low molecular weight region. This result is in contrast with the observation by Allgen and Jungner. They observed a 1000-fold difference between these relaxation times in the same molecular weight range. Undoubtedly, the discrepancy between the present results and theirs is

beyond the experimental error. The deviation of both relaxation times becomes more pronounced with increasing molecular weight. This is not really surprising because the rotary diffusion constant is proportional to the cube of the length and dielectric relaxation time is proportional to the square of the length. It was originally expected that the curves would be either far apart from each other or cross each other at some point. This expectation is because of the different size dependency of both relaxation times. Although the rotary and dielectric relaxation times are different by a factor of about 20 or more at the molecular weight of 5,000,000, this discrepancy is still much smaller than the difference observed previously by Allgen and Jungner.

Discussion

As has been mentioned, there are considerable discrepancies between our observations and those by previous workers. First of all, the magnitude of dipole moment and relaxation time is widely different. The difference is undoubtedly beyond the experimental error. Above all, it was observed[1] that the relaxation time of their DNA was around 10^{-7} sec and far smaller than the rotary relaxation time observed by Edsall, *et al.*[5] We observe that the dielectric relaxation time is about 1–2 msec and also that the dielectric relaxation time and the rotary relaxation are not drastically different for the DNA samples used in this experiment. Considering the facts mentioned above, we have good reasons to believe that there is a missing link between our measurements and theirs. Recently, O'Konski[16] reported that DNA had two dielectric dispersions, the one in a low-frequency region and the other in the megacycle region. The magnitude of the latter dispersion is smaller than the former. Apparently, Allgen and Jungner observed only the second dispersion and our conclusion is based on the observation on the low-frequency dispersion alone. According to the results presented above, the magnitude of the second dispersion is much smaller than the first one and seems to be less significant. The discussion concerning the second dispersion, therefore, will be presented elsewhere.

It is clear from Figure 8 that the dielectric relaxation time does not quite agree with the rotational relaxation time. Both quantities are similar only in the low molecular weight region, but the disparity is more and more pronounced as the molecular weight increases. Eigen and Schwarz[15] state that in an orienting field (field strength above 1000 v/cm), an induced dipole is created and the whole molecule orients itself in the direction of the field. They found that the relaxation time of DNA in an orienting field is approximately 10 msec. The rotational relaxation time in a flow was found to be about 10 msec with a slightly larger DNA in this experiment. Indeed, the value they found is of the same order of magnitude as that of rotary relaxation time in a mechanical flow. This strongly indicates that the orienting field effect is rather more similar to a rotary diffusion process in a mechanical flow than to dielectric relaxation (field strength about 0.5 v/cm). The dielectric relaxation time is smaller than the others and, moreover, is proportional to the square of the length. It is, therefore, unlikely that dielectric relaxation is similar to either mechanical orientation or orienting field effect. There is some indication that dielectric relaxation is similar to mechanical orientation in the low molecular weight region. However, the situation seems to be more complicated in a larger molecule.

Pollack[17] calculated the dielectric relaxation time on the basis of the Maxwell–Wagner theory[18] for an ellipsoid. He found that the relaxation time in the longitudinal direction is proportional to the square of the length, which is in qualitative agreement with the present results. His theory is, however, restricted to special cases because of the assumptions he used.

Recently, Pennock and Takashima[19] extended the counterion polarization theory by Schwarz[20] to cylindrical molecules with a parallel external field. They obtained the following equations for dielectric increment and relaxation time

$$\Delta\epsilon = {}^9/_4 \frac{p}{(1+p)} \frac{e^2\sigma}{kT} \frac{a^2}{b} \tag{11}$$

$$\tau = \frac{a^2 + b^2}{2ukT} \tag{12}$$

where p is the fraction of DNA, e is the electronic charge, σ is the charge density per square centimeter, a and b are the major and minor axes, and u in eq 12 is the mobility of counterions. According to eq 11, the increment is proportional to a^2/b. Since b is almost independent of the molecular weight, we can assume, without serious error, that b is a constant. Therefore, we can conclude that $\Delta\epsilon$ is proportional to a^2, the square

(16) N. C. Stellwagen, M. Shirai, and C. T. O'Konski, Abstracts, Annual Biophysical Society Meeting, San Francisco, Calif., Feb 24, 1965.

(17) M. Pollack, *J. Chem. Phys.*, **43**, 3, 908 (1965).

(18) K. W. Wagner, *Arch. Electrotech.*, **3**, 83 (1914).

(19) B. E. Pennock and S. Takashima, to be published.

(20) G. Schwarz, *J. Phys. Chem.*, **66**, 2636 (1962).

of the major axis. Likewise, the relaxation time is proportional to $a^2 + b^2$. Since b is negligibly small compared with a, we can conclude that the relaxation time also is proportional to the square of the major axis. Thus the counterion polarization theory seems to explain the dielectric relaxation of DNA. This theory will be discussed in a forthcoming paper.

Acknowledgment. The author is indebted to Professor H. P. Schwan for his continuing interest and valuable advice.

The Concept of Length in the Thermodynamics of Elastic Bodies

by Hans J. Kuhn

Institute of Physical Chemistry, University of Basel, Basel, Switzerland *(Received July 20, 1965)*

Several experimental findings related to the thermodynamic behavior of elastic bodies call for a new basic principle: the postulate of inaccessibility of states under isometric conditions. It follows from this postulate that functions of state of elastic bodies can only be calculated if changes of length are measured on a new scale. This physical scale of length is a universal function of the force only. Physical length changes, *i.e.*, length changes measured on the physical scale of length, are constructed in analogy to the construction of entropy by Carathéodory. Variables of state of an elastic body are physical length, temperature, mole numbers, and exchange numbers. Entropy is a function of state of these variables. Internal energy, however, is a mere definition and is not a function of state. It is shown that, as a consequence of the concept of physical length, neither chemical nor thermal energy can be completely converted into useful mechanical work.

1. Introduction

A state of a gas or a liquid is characterized by a homogeneous pressure within the sample, and the shape of the gas or the liquid does not influence the properties of such samples. Tensorial force components which depend on the shape of the solid determine the state of a solid which is described with reference to a standard state. The differences between gases or liquids and solids are an idealization which may be nullified in reality.

Ordinary thermodynamics is founded on gases or liquids.[1] The shape of a solid, especially the shape of the body in the state of reference, is a quality at random. One has to ask if this random shape of the reference state has consequences for the future states or if it affects only the reference state. In this paper is presented evidence that the present state and all future states of each elastic system are linked to the random shape of the initial state, a fact which cannot be circumvented by any approximation.

2. Mathematics

A system is defined by a set of variables: $x, y_1, \ldots, y_n$ or $z_1, \ldots, z_n$.

(i) A Pfaffian dR is integrable

$$dR = R_x dx + \sum_{j=1}^{n} R_j dy_j = \varphi(x, y_j) d\rho \qquad (2.1)$$

i.e., integrating denominators φ and perfect differentials ρ exist, if R_x and R_j are finite and continuous functions of the variables and if in any vicinity of a point of the system there are other points which are not accessible

(1) C. Carathéodory, *Math. Ann.*, 67, 355 (1909).