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MECHANISM OF INTERACTION OF DEOXYRIBONUCLEIC ACID (DNA) WITH 5 Hz TO 10 MHz RADIOFREQUENCY RADIATION

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NOTICES

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The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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Measurements have been made of the relative permittivity and conductivity of aqueous solutions of DNA in the 5 Hz-10 MHz frequency range. The types of DNA studied were plasmid and calf thymus. No evidence of resonance absorption or improved absorption was exhibited for either type of DNA in any part of the frequency range.

The plasmid was studied at 20 °C only, but measurements on the calf thymus DNA were extended down to -50 °C. These measurements revealed the existence of a novel and hitherto unexpected dispersion region, the origin of which is due probably to the motion of ions through the water matrix adjacent to the DNA molecule.

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MECHANISM OF INTERACTION OF DEOXYRIBONUCLEIC ACID (DNA) WITH 5 Hz TO 10 MHz RADIOFREQUENCY RADIATION

INTRODUCTION

The objective of this 4-year research program was to gain an understanding of how radiowaves and microwaves interact with aqueous solutions of deoxyribonucleic acid (DNA). The final report on the initial 3 years of the project was presented last year where it was shown that there was no resonance absorption, or enhanced absorption of any kind, observed in any of the forms of DNA studied at frequencies in excess of 1 GHz. The main emphasis was on plasmid DNA, but other forms, including human, xenopus, and calf thymus DNA, were also investigated.

In the second part of the program the aim was to investigate how lower frequency electromagnetic (EM) radiation interacts with plasmid and other forms of DNA. The frequency range covered was 5 Hz - 10 MHz for plasmid DNA and the dielectric measurements were made at room temperature. The dielectric behavior of calf thymus DNA was also investigated over a more limited frequency range (100 Hz - 10 MHz), but over a wide temperature range (-50 ^{o}C to 20 ^{o}C)(-58 ^{o}F to 68 ^{o}F). Measurements on frozen material are particularly valuable in that the magnitude of any polarization arising from Maxwell-Wagner and counterion effects is much reduced.

BACKGROUND

A considerable amount of experimental work on the dielectric properties of aqueous solutions of DNA at radiofrequencies has been carried out over the past 2 decades (1-5). It has been shown from these studies that DNA does not possess a permanent dipole moment, but that a large induced dipole moment arises as a result of the flow of counterions. Thus, for high molecular weight salmon sperm DNA (molecular length around 1,000 nm) the dielectric increment for a solution of concentration as low as 0.01% was observed (3) to be 3,600 and the relaxation time 1 ms. These low frequency measurements were carried out at 25 ^{o}C (77 ^{o}F). In later work with calf thymus DNA further dispersion regions were discovered at much higher frequencies corresponding to relaxation frequencies of around 10 MHz (2) and 100 MHz (5) at 20 ^{o}C (68 ^{o}F).

All these studies had been carried out on linear DNA and at, or near, room temperature. In view of the considerable interest in circular (plasmid) DNA arising because of the report of resonance

absorption in the microwave region (6), it was clear that our previous microwave measurements (7,8) should be extended down to the lowest attainable frequencies. Apart from checking the possibility of resonance, or other nonclassical behavior, the dielectric properties of circular DNA are of interest in their own right.

The low temperature work on linear calf thymus DNA was pursued to attempt to characterize the behavior of the bound (unfreezable) water molecules which are immediately adjacent to the DNA macromolecule. Since the structural integrity of the DNA molecules depends upon the properties of this associated water (9,10), it is important to be able to characterize these properties as accurately as possible, and a dielectric study is one means of achieving this objective.

MATERIALS AND METHODS

Three forms of plasmid DNA were studied: pUC8.c1 (supercoiled), pUC8.cl (relaxed and linearized) and pUC8.c2 (supercoiled). Plasmid DNA was obtained from *E.Coli* strain HB 101 grown in tryptone yeast broth supplemented with glycerol overnight at 37 °C (68.6 °F). Harvested cells were lysed by incubation in 50 mM Tris, 50 mM ethylenediaminetetra-acetic acid (EDTA), 25% (w/v) sucrose pH 8.0 containing lysozyme (2 mg/ml) at room temperature for 10 min followed by adding 1 vol 0.3% triton X-100 in 187.5 mM EDTA, 150 mM Tris pH 8.0. After centrifugation at 70,000 g for 60 min, the supernatant was diluted 1:2 and digested with RNAase A (0.6 mg/ml) for 10 min at room temperature. The sample was extracted 3 times with 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (25:1). Plasmid DNA was precipitated by adding 0.1 volume of 3M sodium acetate pH 6.5 and 2 volumes cold ethanol. After 12 h at -20 °C (-4 °F), DNA was recovered by centrifugation for 15 min at 12,000 g. The pellet was redissolved in 50 mM Tris, 10 mM EDTA, 0.5 M NaCl pH 7.5 and loaded on a column (1 x 25 cm) of Sepharose 4B that was washed with the same buffer. We collected 1 ml fractions and monitored absorbance at 2,260 nm. The first peak, corresponding to plasmid DNA, was retained. Plasmid DNA was extracted with phenol/chloroform/isoamyl alcohol, then with chloroform/isoamyl alcohol, and reprecipitated with ethanol.

Residual ethanol was removed under vacuum, and the pellet was resuspended in storage buffer (10 mM Tris, 10 mM NaCl, 1 mM EDTA pH 7.5). Only DNA samples with a ratio A_{260}/A_{280} superior to 1.9 were used. The purity of the plasmid form was checked by agarose gel electrophoresis. A concentration of pUC8.c2 is a dimer of pUC8.cl consisting of 2 molecules of pUC8.cl linked to form a circular double-stranded molecule of 5.4 kb. Isolated by the method described for pUC8.cl in the superhelical form, it was converted to the relaxed form by incubation with topoisomerase I, an enzyme that cuts 1 DNA strand causing the plasmid to unwind, lose its

superhelical twists, and adopt an open circular or relaxed structure. The change in form from supercoiled to relaxed is detectable by a change in electrophoretic mobility.

Topoisomerase I (from Bethesda Research Laboratories) was used according to the suppliers' instructions. After incubation the solution was extracted twice with phenol chloroform/ isoamyl alcohol, and DNA was reprecipitated with ethanol. The homogeneity of the relaxed form was checked by agarose gel electrophoresis. Linearization of the pUC8.c1 molecule was achieved by the action of the enzyme Hind iii. The calf thymus DNA (sodium salt) was obtained from the Sigma Chemical Company and was further purified by removing the proteins and histones.

The dielectric measurements over the frequency range 5 Hz - 50 kHz were carried out using a 4 electrode bridge to be able to correct effectively for electrode polarization. These measurements were performed in the laboratory of Professor Mandel at the University of Leiden. The experimental determinations between 100 Hz - 10 MHz were made at King's College using a Hewlett Packard 4192A Impedance Analyzer. This equipment, which is computer controlled, has a logarithmic sweep from 10 Hz - 10 MHz incorporating 20 frequencies/decade. The experimental cell is a parallel plate condenser and is based on a design which has been described in full in a previous article (11). To minimize electrode polarization the electrodes were constructed of platinum which had been roughened to increase the effective surface area. Platinum black was then deposited on to the electrodes to reduce further the effects of electrode polarization. By adopting these procedures, it was possible to measure the liquid DNA solutions down to frequencies of 10 kHz and the frozen samples down to 100 Hz. Experimental errors were typically ± 1% in ε' and ± 2% in ε''.

RESULTS AND DISCUSSION

Dielectric Data on Plasmid DNA

Initially the plasmid DNA solutions were prepared in the form of a mixture of the pUC8.c1 and pUC8.c2 forms. Four different concentrations were used. The results are shown in Figure 1 and analyzed in Table 1. The analysis shows the presence of 2 Cole-Cole dispersion regions, with a moderate spread of relaxation times as shown by the respective values of α_1 and α_2. Obviously, each dispersion corresponds to 1 of the 2 forms of DNA present in the mixture. To check these data, solutions of pure pUC8.c2 DNA of the same concentration were prepared and measured. The dielectric data are shown in Figure 2, and the analysis is shown in Table 2. The values of the dielectric relaxation time in Table 2 agree with τ_1 in Table 1, thus verifying the dielectric behavior of the mixture is equivalent to the sum of the 2 different species acting separately. The degree of agreement between the measurements obtained at King's and in Leiden is shown in Figure 3 where the

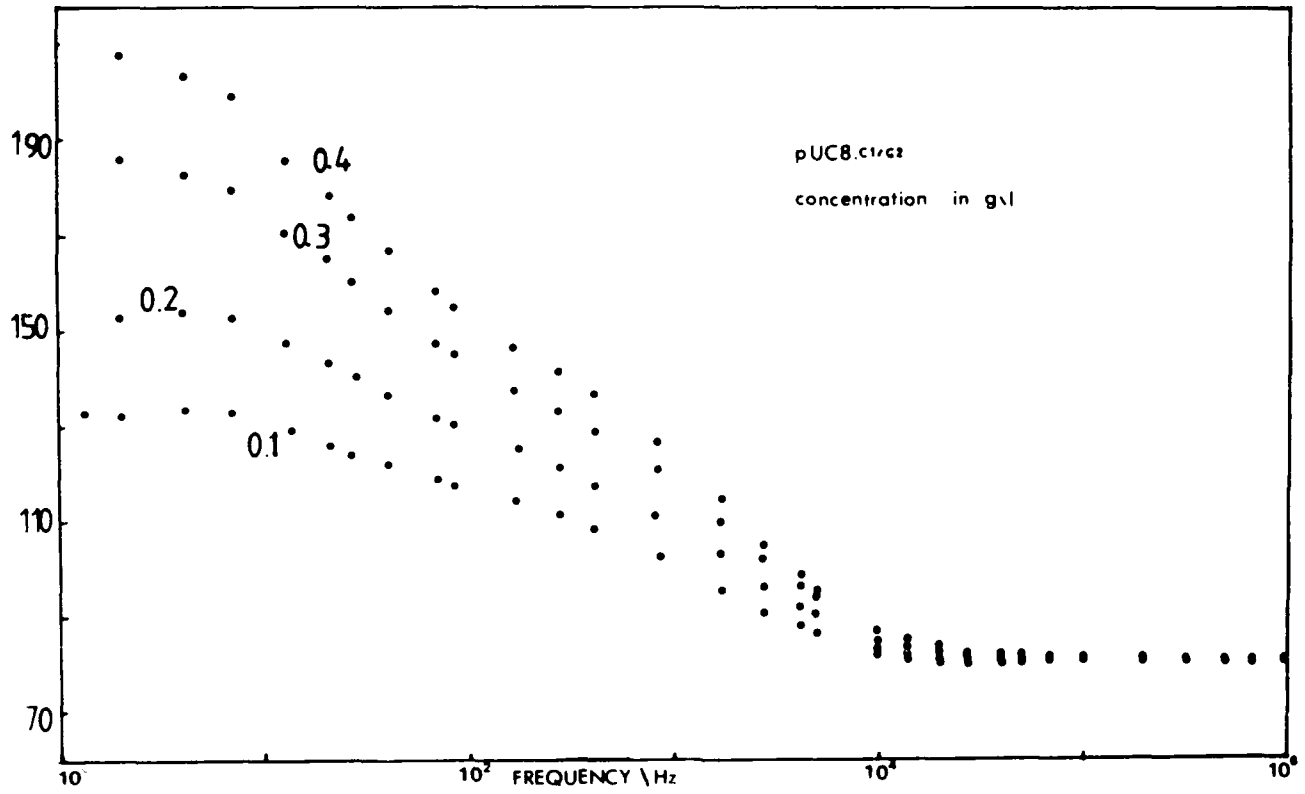

Figure 1. Dielectric behavior of plasmid pUC8.c1 and pUC8.c2 DNA mixture in aqueous solution at 20 °C.

TABLE 1. DIELECTRIC PARAMETERS OF PLASMID pUC8.c1 AND pUC8.c2 MIXTURES

Conc. (%)	ε_S	$\Delta\varepsilon_1$	τ_1 (μs)	α_1	$\Delta\varepsilon_2$	τ_2 (μs)	α_2
0.04	211 (7)	68.5 (6.0)	684 (59)	0.26 (0.03)	63.8 (3.8)	8.9(0.9)	0.23 (0.02)
0.03	179 (3)	49.9 (3.3)	600 (31)	0.22 (0.02)	53.6 (2.2)	8.9 (0.5)	0.24 (0.02)
0.02	157 (3)	29.0 (4.0)	593 (51)	0.13 (0.03)	47.5 (2.4)	11 (1.2)	0.27 (0.02)
0.01	134 (3)	17.5 (3.2)	606 (83)	0.15 (0.06)	36.6 (2.3)	14 (1.4)	0.22 (0.02)
0.005	122 (6)	16.5 (4.7)	480 (150)	0.24 (0.1)	25.8 (3.6)	19 (2.8)	0.12 (0.3)

NOTE: Values in brackets are parameter uncertainties.
All data are at 20 °C.

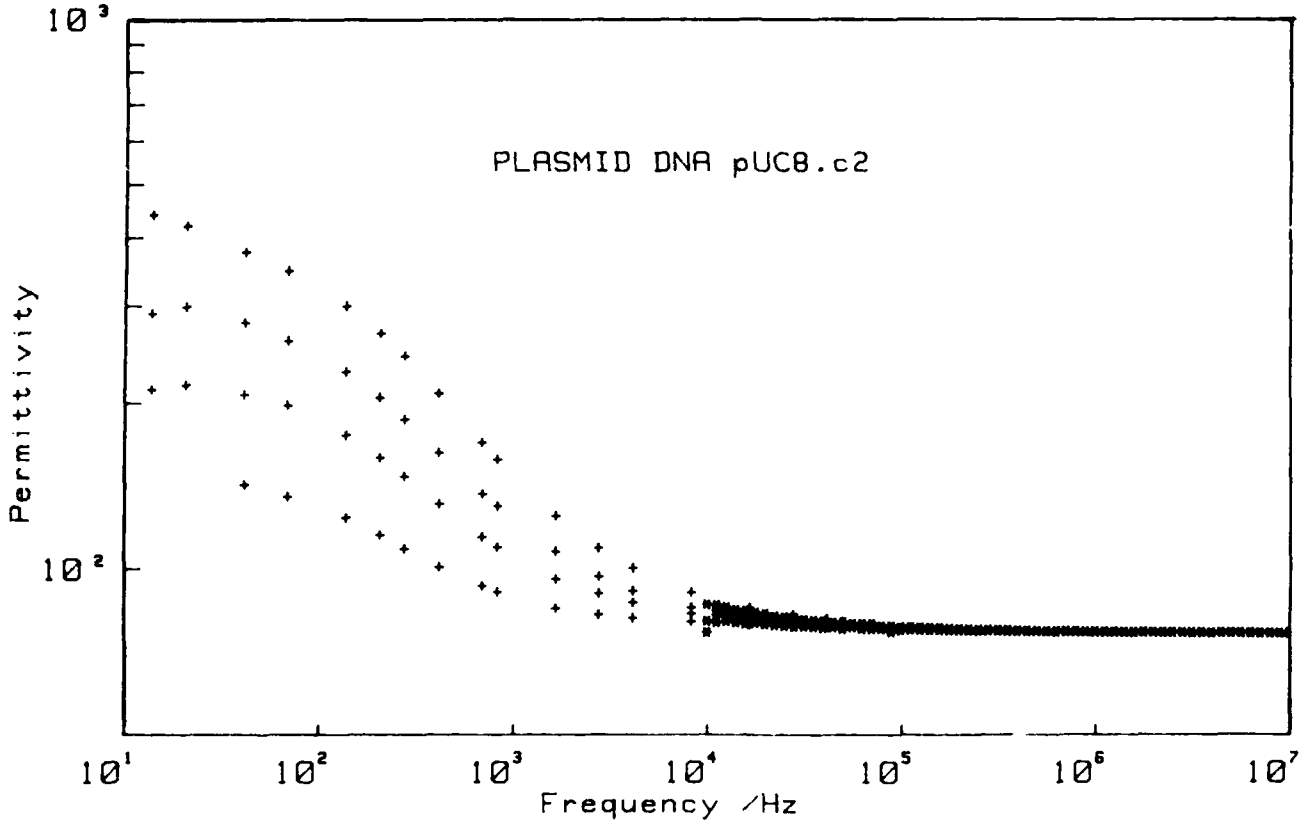

Figure 2. Dielectric behavior of plasmid pUC8.c2 DNA in aqueous solution at 20 °C (concentrations as in Fig. 1).

TABLE. 2. DIELECTRIC PARAMETERS OF PLASMID pUC8.c2 DNA IN AQUEOUS SOLUTION

Concentration (%)	$\Delta\varepsilon$	ε_∞	τ (μs)	α
0.034	365 (3)	80.3 (0.3)	641 (10)	0.29 (0.005)
0.023	246 (2)	80.0 (0.3)	656 (13)	0.28 (0.005)
0.015	160 (2)	81.4 (0.2)	689 (17)	0.26 (0.005)
0.010	79 (2)	80.6 (0.3)	739 (55)	0.72 (0.01)

NOTE: Values in brackets are parameter uncertainties. All data are at 20 °C.

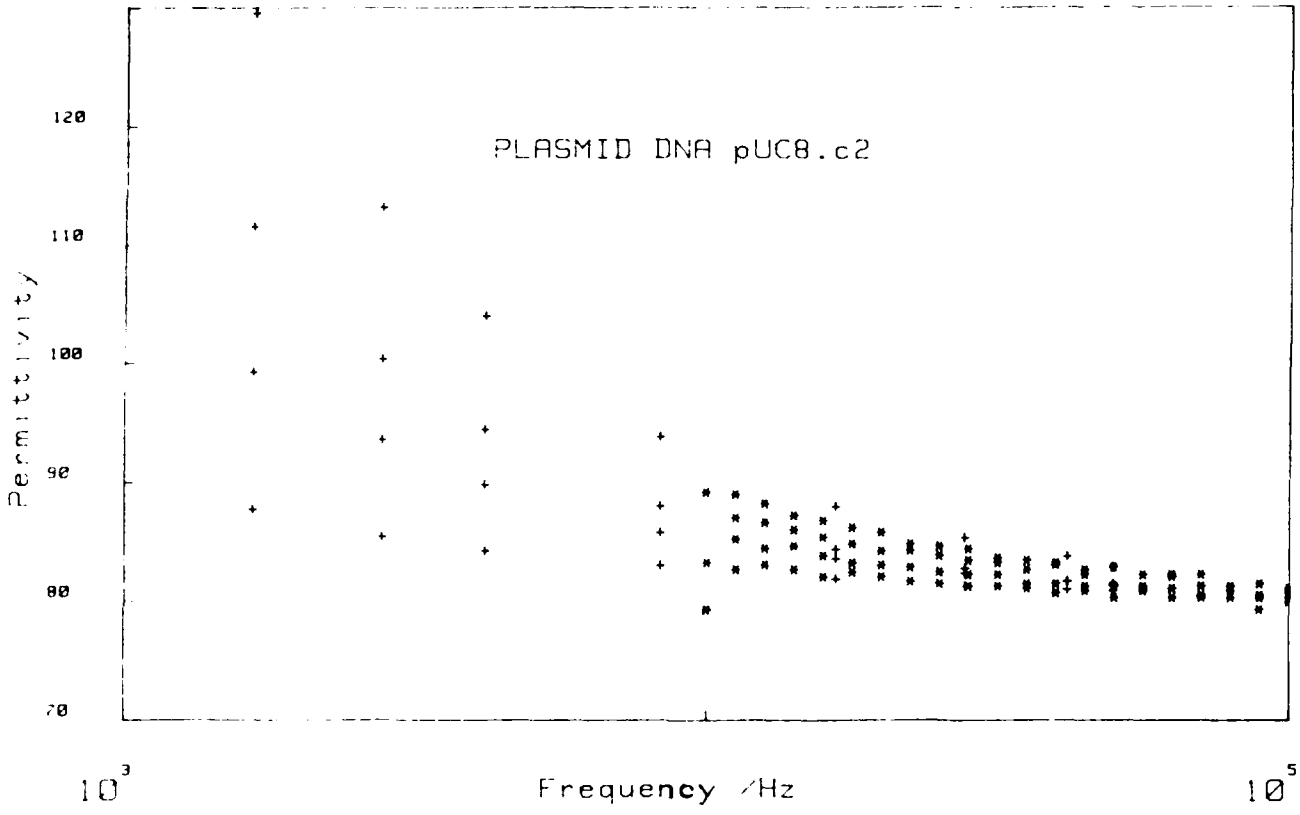

Figure 3. Permittivity measurements on plasmid pUC8.c2 DNA solution between 10^3 - 10^5 Hz.

overlap region between 10 - 50 kHz is highlighted. Figure 2 shows, for all 4 concentrations, the convergence of the values of the relative permittivity to the pure water value at frequencies in excess of 100 kHz.

The principal conclusions which may be drawn from Figures 1 - 3 are as follows. The first point to be noted is that there is no evidence of resonance absorption nor of any other sort of nonclassical dielectric behavior. In fact, there would be no reason from existing theories (12,13) to expect resonance absorption at frequencies as low as those used in the present work, but it is nevertheless of value to verify their nonexistence experimentally. The second point to be noted is that the values of dielectric increment given in Table 2 are much lower than those reported previously by Takashima (3) and others for linear DNA. This finding is compatible with the proposition that the dispersion is due to counterion relaxation, where the value of the dielectric increment is proportional to the square of the length of the molecule (3,14). The data in Table 2 can be used to perform a rough calculation as follows. If the shape of the molecule can be assumed to be an ellipsoid of revolution, then the relationship between the dielectric increment $\Delta\varepsilon$ and various molecular parameters is (14)

$$\Delta\varepsilon = \frac{9pe^2\sigma a^2}{8(1+p)^2kTb} \qquad (1)$$

In this equation a and b are the semimajor and semiminor axes respectively, σ is the charge density on the surface of the molecule, p is the volume fraction occupied by the solute molecule and e is the charge on the electron. Equation (1) is in its originally stated form in cgs system of units but may be changed to the SI version by the insertion of appropriate constants.

By assuming the supercoiled structure to have a semimajor axis of length 25 x 10^{-8} m, a semiminor axis of 35 x 10^{-10} m, and a surface charge density of 1.9 x 10^{18} m^{-2}, the calculated value of $\Delta\varepsilon$ for a 0.015% solution is 220. The experimental value from Table 2 is 160, which is satisfactory agreement within the limitations of the model and the assumptions made. The value of $\Delta\varepsilon$ measured by Takashima (3) for high molecular weight linear DNA (length ~ 100 x 10^{-8} m) was nearly 4,000, i.e., the present and past values of $\Delta\varepsilon$ comply with the square law relationship. We, therefore, conclude that the model for the circular, supercoiled DNA molecule acting like a linear rodlike structure is consistent with the experimental data. The observed dielectric behavior is due to the flow of counterions producing an induced polarization. Further evidence to support these conclusions comes from Figure 4. The lower curve shows the relative permittivity of 0.04% pUC8.c1 DNA in the supercoiled state. The molecule was first relaxed by the addition of topoisomerase and then linearized by the action of Hind iii. The increase in the length of the molecule and its effect on the dielectric behavior is seen in the upper curve of the figure. To refine further our understanding between the dielectric increment and the molecular dimensions, it will be necessary to take the measurements down to low frequencies. This part of the work is still in progress (May 1989) and will be completed shortly.

One of the problems of working with plasmid DNA is that it is very expensive and difficult to prepare in large quantities. This high cost precludes the possibility of preparing solutions of concentration sufficiently high to study the structure and behavior of the water adjacent to the macromolecule. Previous work by us (15,16) and others (17,18) had shown that this bound water, or water of hydration, is of considerable significance for protein and other biological molecules. Extension of the work to DNA was, therefore, undertaken and forms the concluding section of this 4-year research program. Calf thymus DNA was chosen for this purpose.

Dielectric Behavior of Calf Thymus DNA at Low Frequencies and Subzero Temperatures (Celsius)

Previous dielectric work by us (5) on calf thymus DNA had been carried out using measuring equipment spanning a frequency range 0.1 MHz - 70 GHz. Aqueous solutions of concentration around 1% were measured at 20 °C (68 °F). Besides confirming the

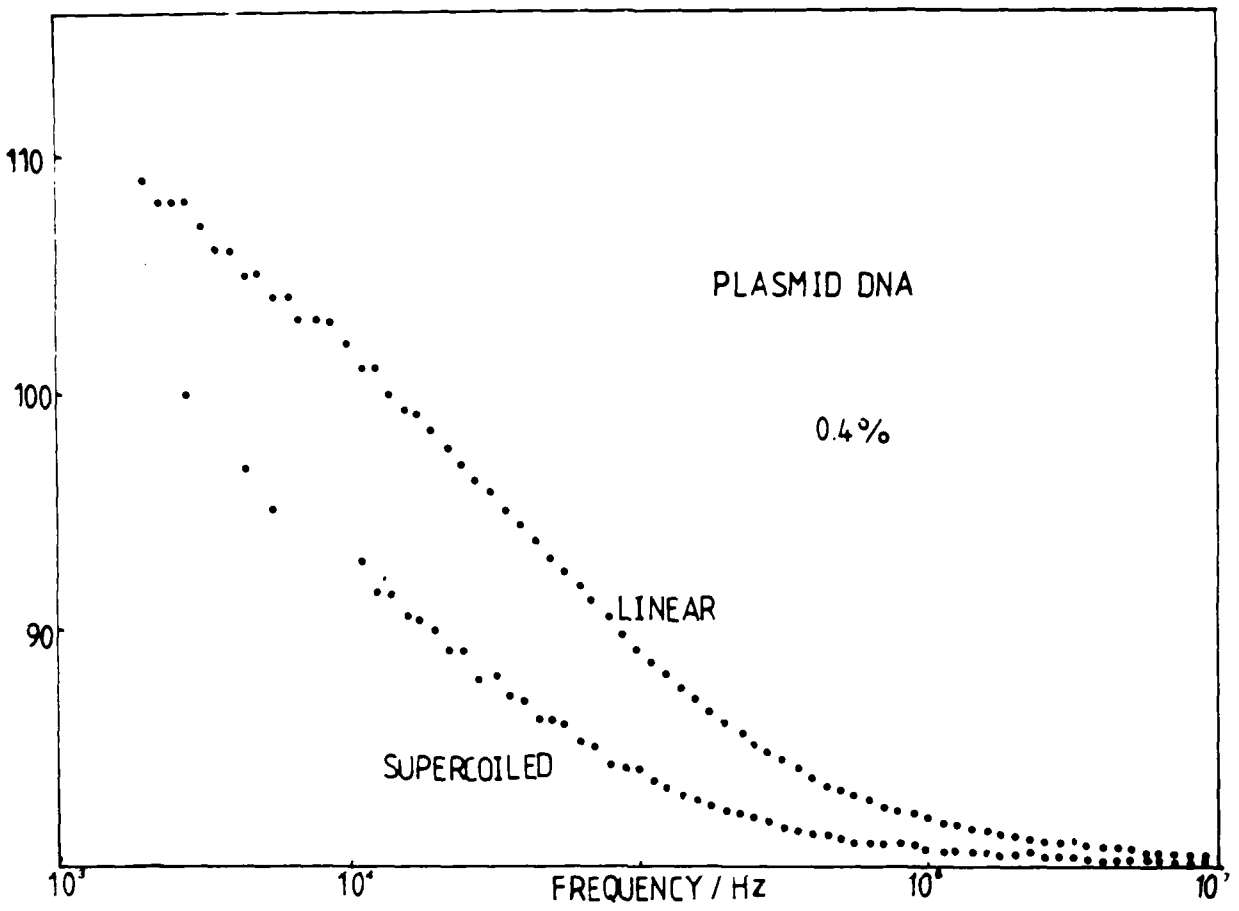

Figure 4. Dielectric behavior of linear and supercoiled plasmid pUC8.c1 DNA.

presence of the high polarization, low frequency, dispersion regions reported by Takashima (3) and Mandel (2), a small dispersion region of total increment around 10 and relaxation frequency 80 MHz was observed. The relative permittivity was found to fall from 89 to 79 and the dielectric behavior characterized approximately by a single relaxation time. The origin of the dispersion was not established, but clearly it could not be normal irrotationally bound water of the form that exists with protein molecules because the relative permittivity at the low frequency end of the dispersion [89] is significantly higher than the static permittivity of pure water [81]. If this dispersion were due to the small quantity of bound water present in the solution, it would need to have virtually ferroelectric properties.

One problem with interpreting unambiguously these kinds of data is that the small dispersion tends to become overshadowed by the Maxwell-Wagner and counterion dispersion regions occurring at lower frequencies. One way of dealing with this problem, as we have shown for ocular tissues (19), is to freeze the material and

thus reduce significantly the conductivity. This method then causes considerable reduction in the amplitudes of the low frequency dispersions arising from the properties of the macromolecule itself and allows any subsidiary dispersion to be resolved much more clearly. The position of the small dispersion in the frequency spectrum for the DNA solution referred to above suggests that its origin is related to the bound water, although not due to an orientation polarization mechanism.

In the present work DNA solutions of concentration 1.4%, 1.0%, 0.36% and 0.095% were studied from 100 Hz - 10 MHz and over a temperature range -10 ^{o}C to -50 ^{o}C (14 ^{o}F to -58 ^{o}F). Some measurements were also carried out at room temperature to verify previous work.

To check the performance of the equipment for low temperature measurements, the dielectric properties of pure ice were measured at various temperatures between zero and -40 ^{o}C (-40 ^{o}F). The values of relative permittivity and total dielectric loss at -12 ^{o}C (10.4 ^{o}F) are shown in Figure 5. Even with pure ice there is a small residual ionic conductivity and this is shown in the upturn of the ε'' values at lower frequencies. When corrected for conductivity the values of complex permittivity were in excellent agreement with those obtained previously by other workers (20). The data were fitted to the Cole-Cole dispersion equation

$$\varepsilon = \varepsilon' - j\varepsilon'' = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (j\omega\tau)^{1-\alpha}} \qquad (2)$$

where τ is the dielectric relaxation time and α is the distribution parameter. The high frequency and low frequency values of relative permittivity are designated by ε_∞ and ε_S respectively and ω is the angular frequency. Alternatively $\omega\tau$ may be written as f/f_R where f_R is the relaxation freqency. The fit to the data are shown in Table 3 where it can be seen that for ice α tends to zero, i.e., the dielectric behavior accords to the Debye equations. The expected fall of ε_S with temperature is indicated, although the precise variation is obscured by increased uncertainties in the very low frequency measurements. The value of ε_∞, on the other hand, is very sharply defined and agrees well with measurements obtained directly in the microwave and infrared regions (21). The absence of any temperature variation in respect of ε_∞ over this temperature range is convincingly demonstrated and indicates strongly that no orientation polarization is involved. However, the value of ε_∞ is significantly greater than the square of the optical refractive index and shows that some form of atomic polarization, presumably bond stretching, occurs. The relaxation times were plotted logarithmically against the reciprocal of the absolute temperature (Fig. 6). The straight line relationship indicates that the dielectric behavior may be represented as a rate process (22).

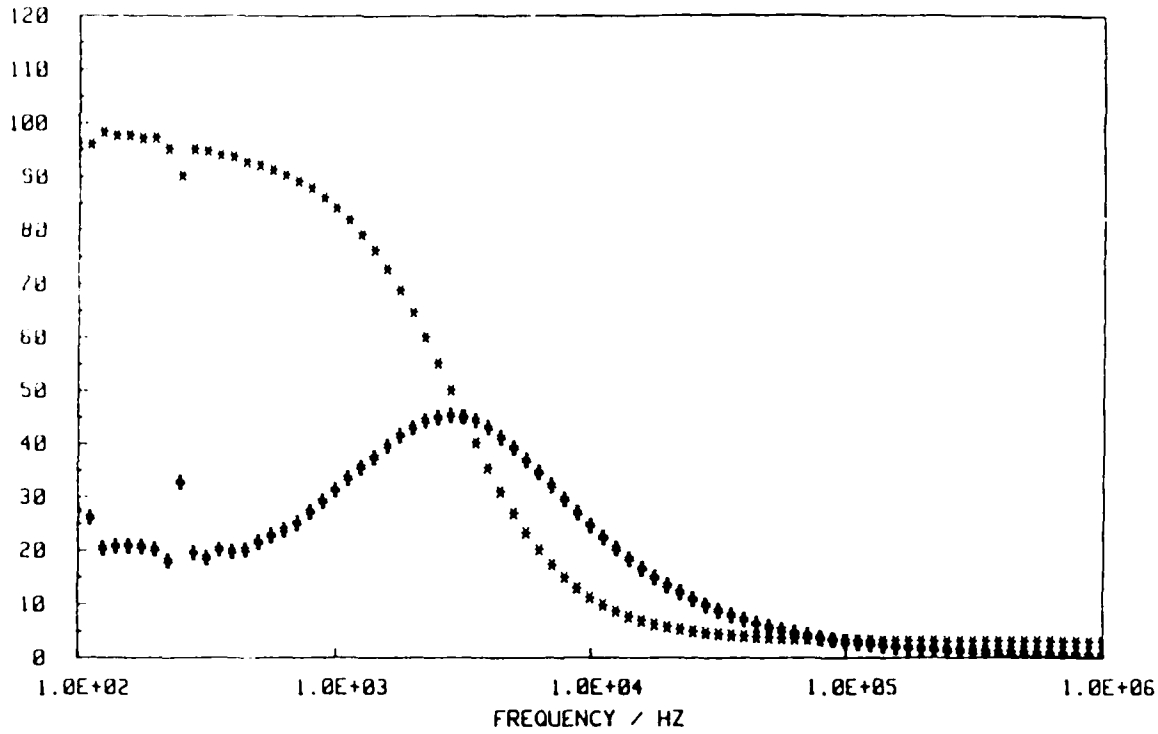

Figure 5. Relative permittivity and dielectric loss of pure ice at - 12 °C.

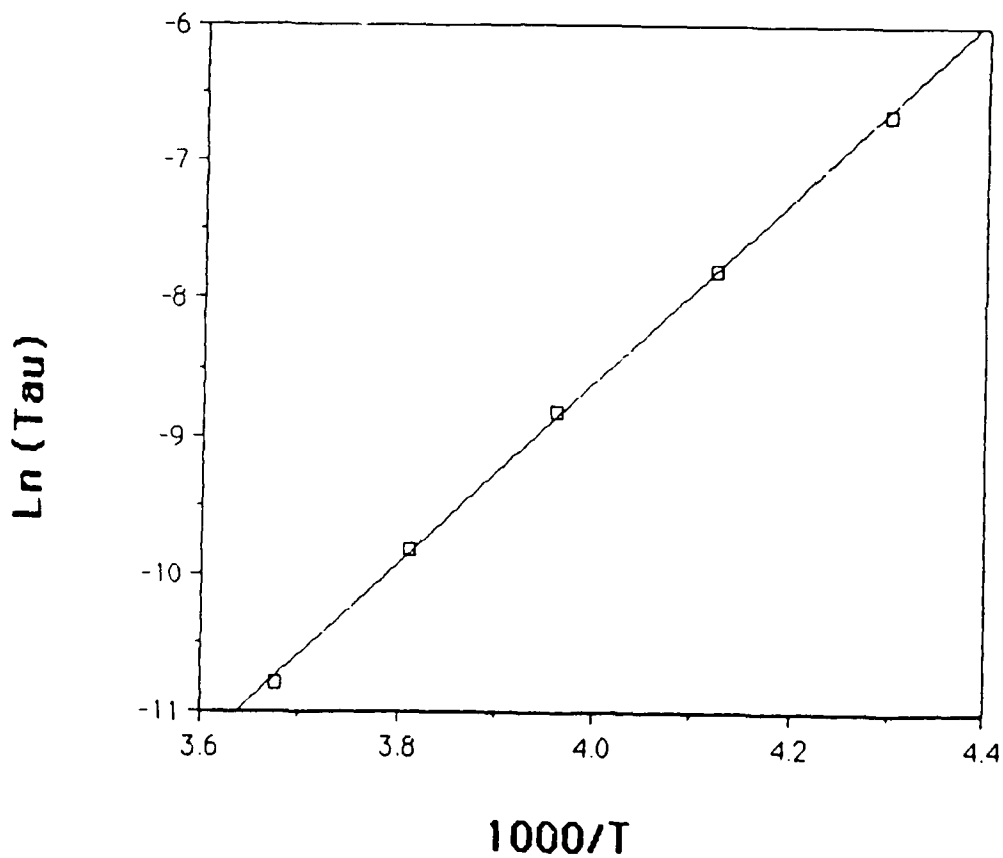

Figure 6. Activation enthalpy plot for pure ice.

$$\tau = A \exp (\Delta H/RT) \quad (3)$$

where R is the Gas Constant, A is another constant, and ΔH is the activation enthalpy which is related to the height of the potential barrier which has to be surmounted during the dielectric relaxation process. The equation of the line describing the fitted data appears at the top of the figure, and the value of ΔH obtained from the slope is 55 ± 3 kJ/mole which accords well with the Bjerrum defect theory (21). However, this value would also correspond to the energy required to break 3 hydrogen bonds, a fact which may point towards another possible mechanism to account for dielectric relaxation in ice. The majority of molecules in ice are 4-bonded, and the breaking of 3 bonds would produce a molecule with the required rotational freedom to rotate as a dipole.

After having established the satisfactory functioning of the equipment through measurements on pure ice, the DNA solutions were investigated at the 4 stated concentrations and at temperatures of -10, -15, -20, -25, -30, -40 and -50 ^{o}C (14, 5, -4, -13, -22, -40, and -58 ^{o}F). Typical data for complex permittivity $\varepsilon = \varepsilon' - j\varepsilon''$ are shown in Figures 7 - 13. The values of permittivity ε' and loss factor (ε'') shown in Figure 7 indicate that there are 2 dielectric dispersion regions present, centered respectively at around 3 kHz and 2 MHz respectively. The total loss factor ε''_T, which is also shown, is given by the expression

$$\varepsilon''_T = \varepsilon''_D + \frac{\sigma_i}{\omega \varepsilon_o} \quad (4)$$

where σ_i is the ionic (nondispersing) conductivity. The value of this parameter is shown in the final column of Table 3. The complete dispersion behavior can be accounted for by the sum of 2 Cole-Cole functions (Equation 2), and the characteristics of the 2 dispersion regions are shown in Tables 3 and 4 which give mean-value parameters prior to a full error analysis.

Before discussing these parameters it will be profitable to show some of the other data in graphical form. Figure 8 indicates how the low frequency dispersion moves to the left as the temperature is lowered. The complementary data for the dielectric loss (Fig. 9) are an alternative means of showing the same behavior. Both of these figures have a frequency range 100 Hz - 100 kHz; i.e., they exclude the part of the spectrum where the small (high frequency) dispersion is present. This dispersion is indicated in Figures 10 and 11, where the frequency range has now been restricted to the higher end of the region. The activation energy plot (Equation 3) for the low frequency dispersion region is shown in Figure 12 and that for the high frequency region in Figure 13.

The dielectric parameters of the low frequency dispersion are shown in Table 3. For all concentrations studied, including pure ice itself, the values of α are barely distinguishable from zero, indicating that single relaxation time behavior is present. This

TABLE 3. DIELECTRIC PARAMETER OF ICE AND ICE - DNA COMPLEX

Temp. °C (°F)	Concentration (%)	ε_s	ε_∞	f_r (kHz)	α	Conductivity (mS/m)
-10 (14)	1.4	1490	36	3.15	0.002	8.80
	1.0	1340	31	2.98	0.006	5.06
	0.45	1040	18	2.89	0.06	0.72
	0	94	3.5	2.80	-	
-15 (5)	1.0	1270	30	2.00	-	2.14
	0.45	1070	15	1.67	0.06	0.29
-20 (-4)	1.4	1400	30	1.18	0.04	1.45
	1.0	1330	25	1.03	0.06	0.86
	0.45	954	10	0.99	0.08	0.12
	0	95	3.3	1.04	-	-
-25 (-13)	1.0	1330	26	0.61	0.06	0.33
-30 (-22)	1.4	1440	26	0.41	0.08	0.24
	1.0	1280	23	0.37	0.07	0.14
	0	91	3.5	0.37	-	-
-40 (-40)	1.4	1280	14	0.154	0.096	0.04
	0	104	3.5	0.12	-	-
-50 (-58)	1.4	919	7	0.078	0.13	0.007

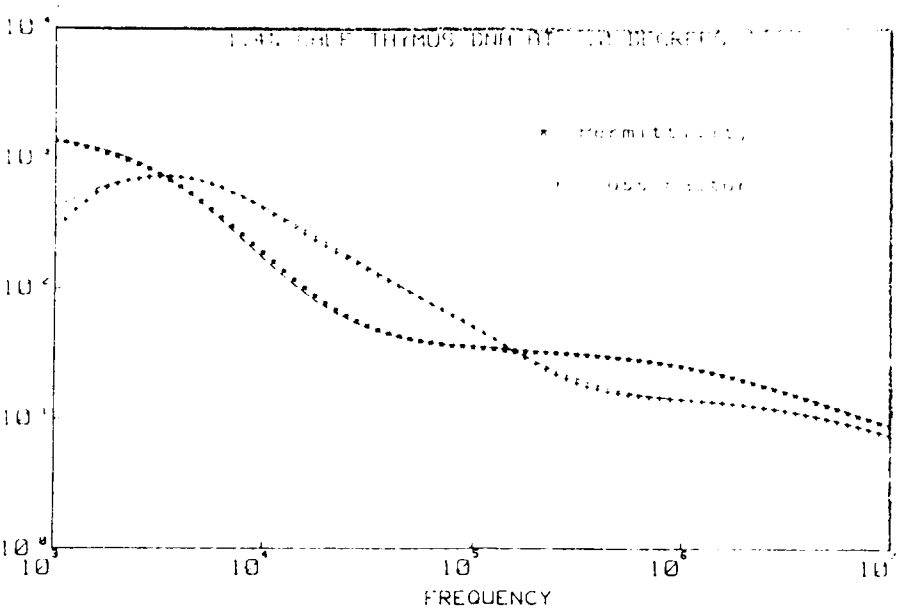

Figure 7. Dielectric behavior of frozen aqueous solution of calf thymus DNA between 10^3 - 10^7 Hz.

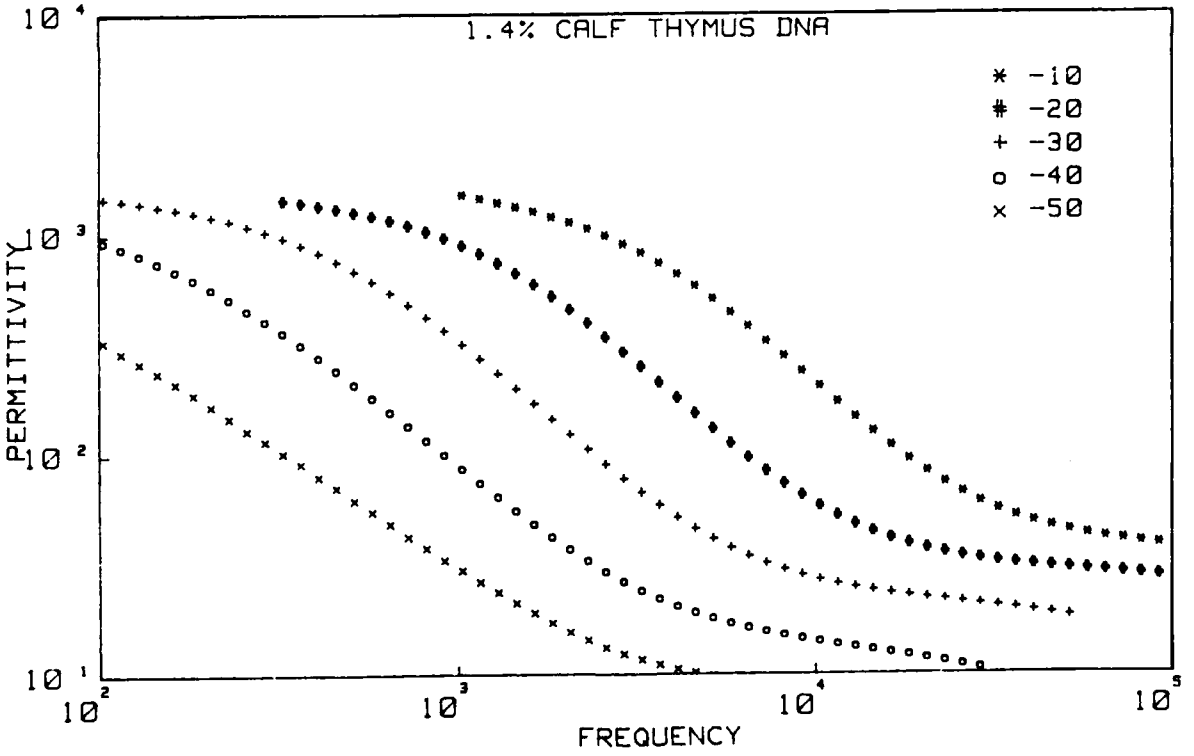

Figure 8. Principal dielectric dispersion region of frozen DNA solution between -10 °C and -50 °C.

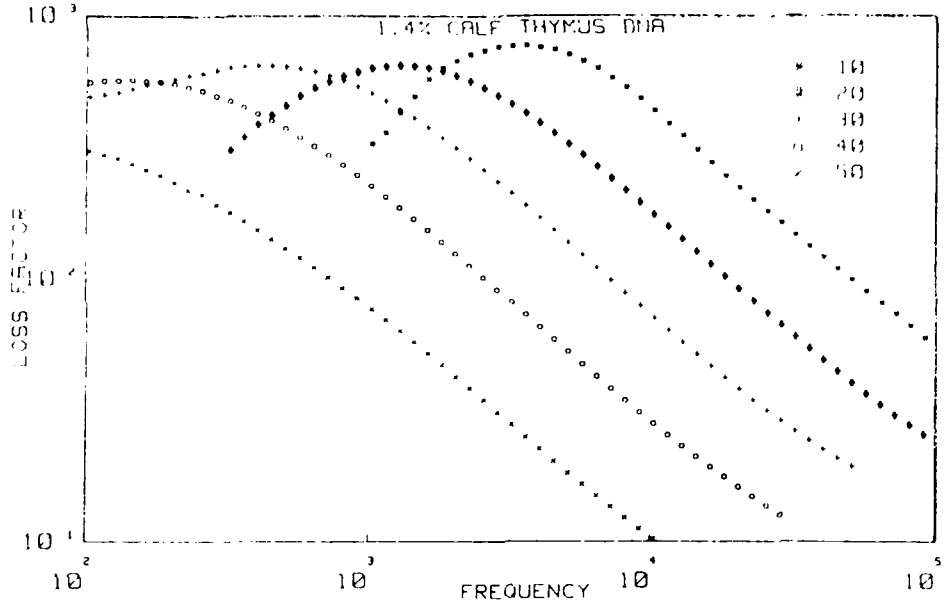

Figure 9. Dielectric loss data for principal dispersion region of frozen DNA solution -10 °C and -50 °C.

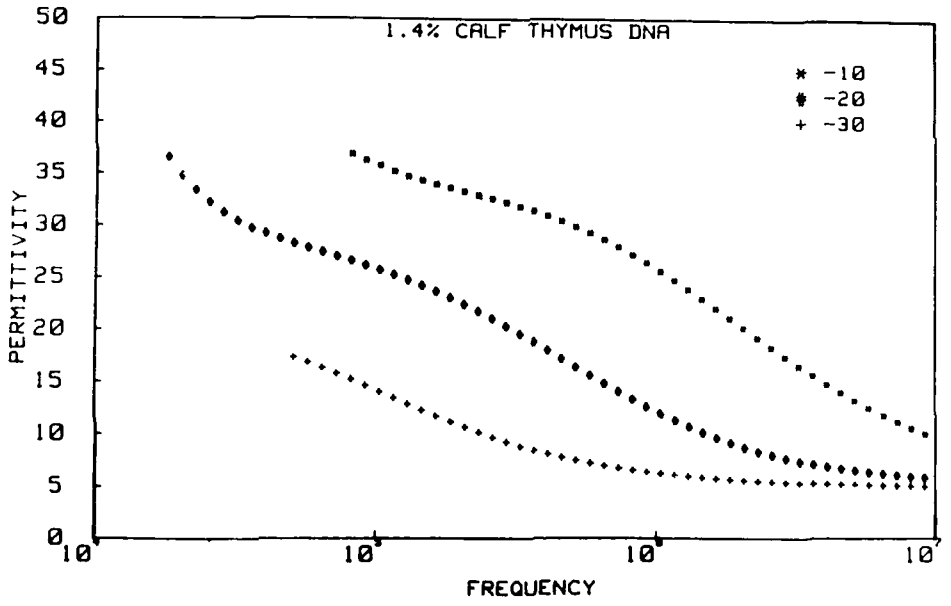

Figure 10. Subsidiary dielectric dispersion region of frozen DNA solution between -10 °C and -30 °C.

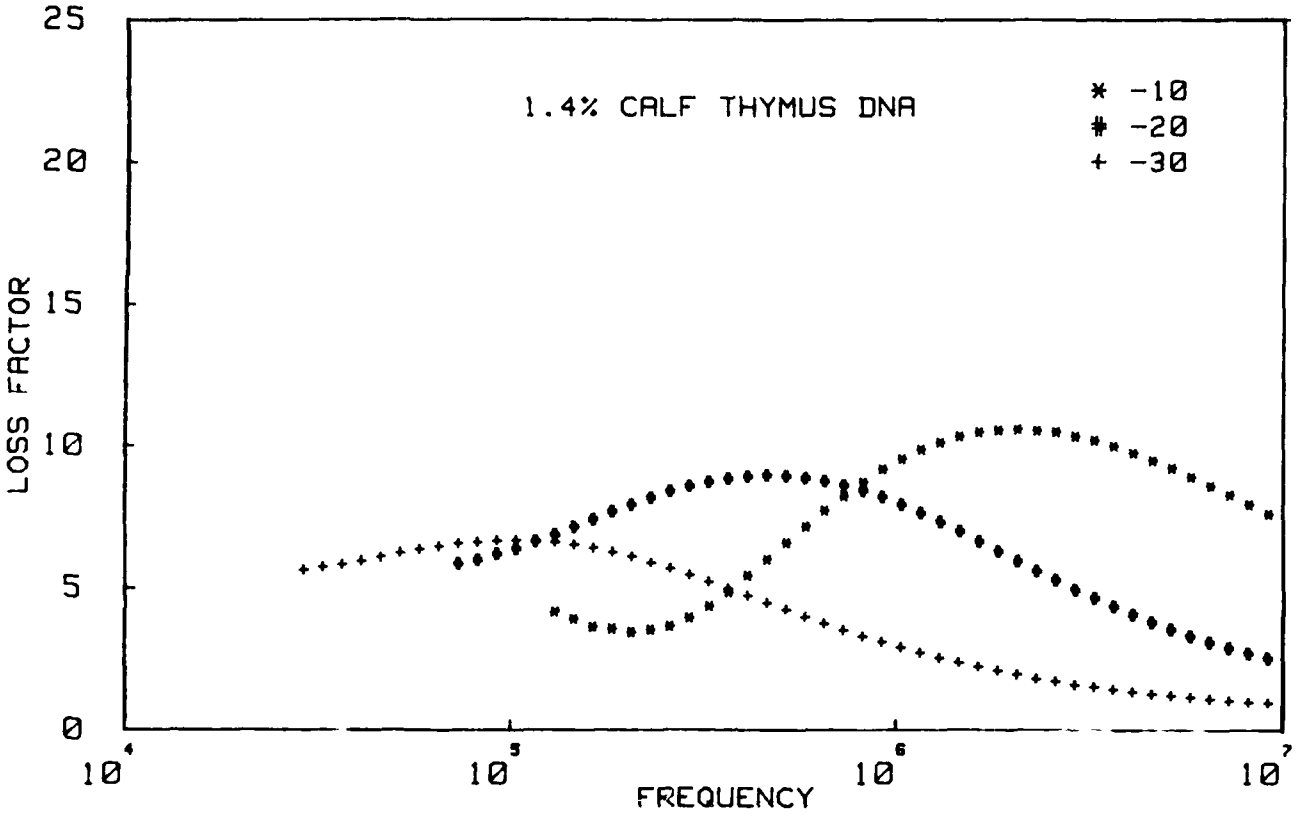

Figure 11. Dielectric loss data for subsidiary dispersion region of frozen DNA solution between -10 °C and -30 °C.

behavior is consistent with a defect mechanism being responsible for the dispersion. As the concentration of the DNA is increased so is the number of charge carriers, and this in consequence results in the higher static permittivity. The activation energy plot (Fig. 11) gives a value of ΔH equal to 52 ± 8 kJ/mole, which is indistinguishable from the value obtained for pure ice.

In contrast to the low frequency dispersion, whose characteristics were rather as expected, the small high frequency dispersion displayed some novel features (Table 4). First, the actual presence of the dispersion could not necessarily have been anticipated, whereas the existence of the dispersion due to pure ice is well established and the consequent modifications to it by the presence of the DNA would be expected. Despite its small magnitude the high frequency dispersion is nevertheless well resolved. Although the dielectric increment is between 1 and 2 orders of magnitude lower than that of the principal dispersion, the relaxation frequencies are separated by a factor of a thousand. This observed dielectric behavior was further confirmed by D. Anagnostopoulou-Konsta of the Physics Department at the National Technical University of Athens who also resolved 2 dispersions in the same samples of DNA using the method of thermally stimulated depolarization currents (TSDC). Two particularly interesting features of the small dispersion are the very high activation energy (ΔH = 86 ± 12 kJ/mole) and the fact that Figure 12 when extrapolated to T = 293K predicts a relaxation frequency of between 80 - 90 MHz, i.e., as observed in the previous work (5)

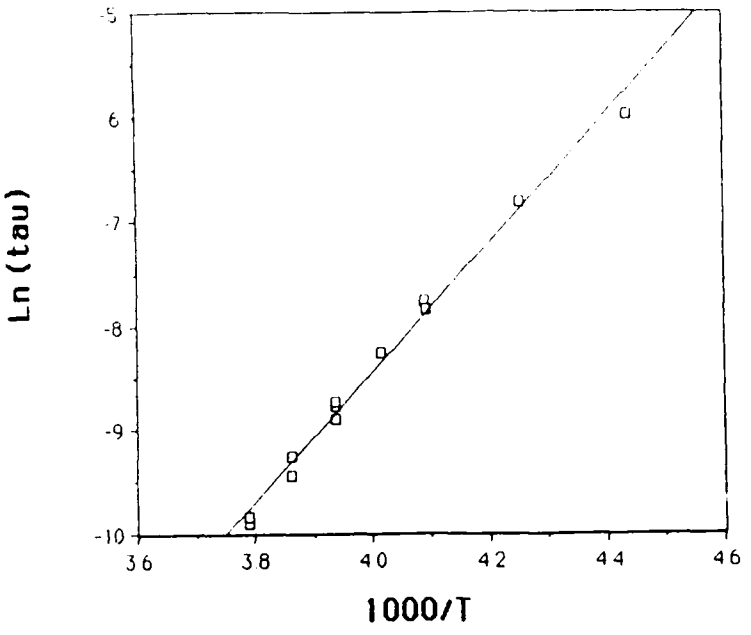

Figure 12. Activation enthalpy plot for principal dispersion in frozen DNA.

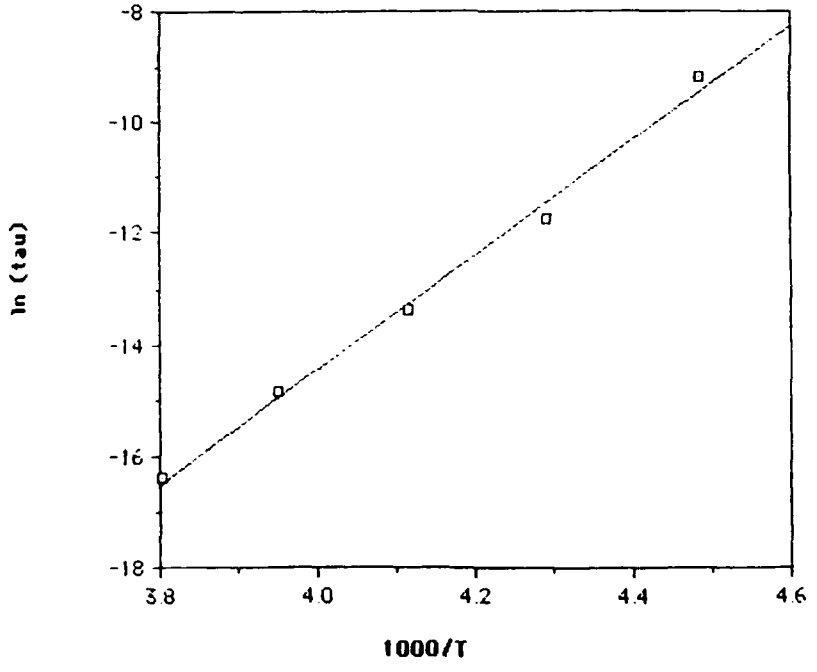

Figure 13. Activation enthalpy plot for subsidiary dispersion in frozen DNA.

TABLE 4. DIELECTRIC PARAMETERS OF THE HIGH FREQUENCY DISPERSION IN FROZEN DNA SOLUTION

Temperature °C (°F)	Concentration (%)	ε_s	ε_∞	f_r (kHz)	α	Conductivity (mS/m)
-10 (14)	1.4	35.7	3.4	2069	0.28	9.06
	1.0	32.7	3.3	2004	0.28	5.29
-15 (5)	1.0	29.6	3.4	838	0.29	2.28
-20 (-4)	1.4	29.3	3.9	442	0.24	1.54
	1.0	26.6	3.7	405	0.26	0.93
-25 (-13)	1.0	24.0	3.9	184	0.24	0.38
-30 (-22)	1.4	22.4	4.0	102	0.24	0.28
	1.0	21.2	3.9	92	0.24	0.16
-40 (-40)	1.4	17.7	3.9	19.97	0.32	0.055
-50 (-58)	1.4	21.7	3.8	1.50	0.44	0.011

carried out at 20 °C (68 °F). In other words, this dispersion preserves its characteristics through the change of state; freezing the substance does not apparently have any effect. The origin of the dispersion is not yet clear but various possibilities might be considered, and certain of them rejected. It has been explained earlier why irrotationally bound water could not be responsible, on the grounds that the values required for the low frequency permittivity of the bound water would need to be unreasonably high. This view is supported by the observed activation enthalpy of 86 kJ/mole which is 3 times greater than that reported previously for protein bound water (17). The high value of ΔH also rules out the Maxwell-Wagner mechanism, proton fluctuation, and tangential counterion flow around the surface of the DNA molecule but within the bulk liquid. However, since the observed dispersion is preserved intact during the process of freezing it is likely to be concerned with the layer of bound, or 'unfreezable' water

associated with the DNA macromolecule. The effective viscosity of this water of hydration will be considerably greater than that of bulk water, and a higher temperature coefficient would also be likely. This effect, in turn, would increase the temperature coefficient of the mobility of ions moving through this water lattice and, in consequence, any process involving ionic motion would exhibit a high activation enthalpy. Such a process of counterion flow, either radially or tangentially, could be the mechanism responsible for this small observed dispersion. In any subsequent program of work this hypothesis could be checked by varying the nature of the ions present.

CONCLUSIONS

Dielectric measurements have been made on aqueous solutions of plasmid DNA over the frequency range 1 Hz - 10 MHz. Three different forms of pUC8 DNA were used, and 4 concentrations were measured. No resonance absorption was observed and all the data could be interpreted in terms of the supercoiled molecule behaving like a linear rodlike structure of comparable dimension.

Measurements on frozen solutions of calf thymus DNA revealed the existence of a small, but well resolved, dispersion besides the expected dispersion arising from the ice lattice as modified by the DNA. This small dispersion, which occurs at frequencies 3 orders of magnitude higher than the principal one, may be ascribed provisionally to the motion of ions through the structured water adjacent to the DNA molecule.

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APPENDIX A

PERSONNEL INVOLVED IN THE DNA RESEARCH PROGRAM

KING'S COLLEGE LONDON

Physics Department (Dielectrics Research Group)

Professor E. H. Grant (Project Leader)
Dr. C. Gabriel
Mr. G. F. Evans
Mr. P. McArthur

Professor J. B. Bateman (Visiting Professor)

Biochemistry Department

Dr. P. R. Brown
Mrs. R. Tata

COLLABORATING RESEARCH GROUPS

University of Uppsala (Physics Department)

Dr. B. Gestblom
Dr. E. Noreland

University of Leiden (Department of Physical Chemistry, Gorlaeus Laboratories)

Professor M. Mandel
Mr. J. van der Ploeg

National Technical University of Athens (Physics Department)

Dr. A. Anagnostopoulou-Konsta

APPENDIX B

VISITS AND MEETINGS INVOLVING MEMBERS OF KING'S COLLEGE DIELECTRICS GROUP

(events prior to August 1988 covered in previous Report)

September 1988. Dielectric behavior of natural and synthetic biopolymers including DNA. Presentation to Courtauld's Limited, Coventry (EHG).

January/February 1989. Visits to Professor R. Pethig, Institute of Molecular and Biomolecular Electronics, School of Electrical Engineering, University College of North Wales; to discuss future collaboration in biomolecular research (EHG).

February 1989. Biological effects and industrial applications of electromagnetic fields. Presentation to the Maxwell Society, King's College, London (EHG).

March 1989. The biological effects of microwaves. Presentation to Institution of Electrical Engineers Meeting on the biological effects of non-ionising radiation (EHG).

March 1989. The dielectric properties of DNA. Presentation to Institute of Molecular and Biomolecular Electronics. University College of North Wales (CG).

March 1989. Mechanism of interaction of radiowaves and microwaves with biological material at frequencies greater than 1 GHz. Talk to the Institute of Physics London (CG).

April 1989. Visit to USAFSAM, Brooks Air Force Base, San Antonio, Texas. To discuss present and future research collaboration and to present a seminar on the Dielectric behavior of DNA at radiowave and microwave frequencies. (EHG).

March - May 1989. Appointment as Visiting Professor, Department of Biomedical Electronic Engineering, University of Pennsylvania, Philadelphia. Collaboration with Professor H.P. Schwan, S. Takashima and K. R. Foster. Presented seminars on dielectric behavior of DNA and related topics (EHG).

May 1989. The dielectric behavior of DNA. Presentation at Drexel University, Philadelphia (EHG).

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