Dielectric behavior of aqueous solutions of plasmid DNA at microwave frequencies

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ABSTRACT The relative permittivity and dielectric loss of aqueous solutions of plasmid (pUC8.c1 and pUC8.c2) DNA have been measured at 20°C over the frequency range 100 MHz–10 GHz. The solutions had a concentration of 0.1% DNA, and were studied both in the relaxed and the supercoiled form. The dielectric measurements were made using a variety of techniques including frequency domain and time domain methods of operation. No evidence of any resonance absorption, nor of any other kind of enhanced absorption, was observed.

INTRODUCTION

A considerable amount of experimental work on the dielectric properties of aqueous solutions of DNA has been carried out over the past decade (1–8). Much of this research has concluded (1–6) that the dielectric behavior of DNA in water at radiowave and microwave frequencies can be described by classical dielectric theory based on the Debye equations or by some small deviation therefrom such as the Cole-Cole function. An example of how these equations can be used for this purpose has been described by us previously (4) in this journal.

In contrast, other workers have claimed (7, 8) on the basis of their measurements that the dielectric behavior of DNA in aqueous solution exhibits an unusually high attenuation coefficient over a wide frequency range which cannot be accounted for by any of the traditional dielectric theories. Moreover for one particular type of DNA (plasmid), resonance absorption behavior has been reported (8, 9) as occuring at frequencies of a few gigahertz, an observation which is quite unprecedented for experimental studies of the dielectric behavior of biological molecules in solution. This experimental work, by Edwards et al. (8), has been supported by theoretical calculations of Prohofsky, Van Zandt, and their colleagues (10, 11), who developed a model of electromagnetic energy coupling through acoustical vibrations along the axis of helical polymers. An alternative approach which would also account for the experimental DNA data is that of Scott (12) who applied a nonlinear soliton approach by assuming the excited acoustic wave to be anharmonic.

The conclusions drawn by Edwards et al. (8) from their experimental observations have not, however, been universally accepted. Therefore, one of the purposes of the present work was to measure independently the dielectric properties (relative permittivity ϵ' and dielectric loss ϵ'') of aqueous solutions of DNA prepared by exactly the same procedure as that adopted by Edwards et al. (8). The measurements were made in two laboratories (London and Uppsala), and three different types of experimental equipment were employed. An abridged publication of part of the work has appeared previously (13).

MATERIALS AND METHODS

Sample preparation

Three forms of plasmid DNA were studied: pUC8.c1 (supercoiled), pUC8.c2 (supercoiled), and pUC8.c2 (relaxed). Plasmid pUC8.c1 is a small covalently closed circular 2.7-kb double-stranded DNA molecule. Most of the plasmid DNA isolated for *E. coli*, the host organism, is in the form of supercoiled molecules that have superhelical twists.

Plasmid DNA was obtained from *E. coli* strain HB 101 grown in tryptone yeast broth supplemented with glycerol overnight at 37°C. Harvested cells were lysed by incubation in 50 mM Tris, 50 mM EDTA, 25% (wt/vol) sucrose pH 8.0 containing lysozyme (2 mg/ml) at room temperature for 10 min followed by addition of 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 three times with 1 vol of phenol/chloroform/isoamyl alcohol (25:24:1) and once with choloroform/isoamyl alcohol (25:1). Plasmid DNA was precipitated by addition of 0.1 vol of 3 M sodium acetate pH 6.5 and 2 vol cold ethanol. After 12 h at −20°C, 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 × 25 cm) of Sepharose 4B that was washed with the same buffer. 1-ml fractions were collected, and the absorbance at 260 nm was monitored. 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.

pUC8.c2 is a dimer of pUC8.c1 consisting of two molecules of

0006-3495/89/01/29/06 $2.00

pUC8.c1 linked to form a circular double-stranded molecule of 5.4 kb. Isolated by the method described for pUC8.c1 in the superhelical form, it was converted to the relaxed form by incubation with topoisomerase I, an enzyme that cuts one 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 (Bethesda Research Laboratories, Gaithersburg, MD) 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.

These methods of preparation of the DNA solutions followed in detail those adopted by Edwards et al.(8).

Dielectric measurements

The various dielectric investigations that were carried out are summarized in Table 1, where the variety of the experimental techniques adopted is illustrated. For pUC8.c2 six independent time domain spectroscopy (TDS) methods spread over the two laboratories were used, and a frequency domain (FD) technique was also employed in the Uppsala laboratory. The measurements of pUC8.c1 were fewer in nature and were all carried out in the London laboratory. In addition to the determinations of the supercoiled DNA, some measurements were made on the relaxed form of the pUC8.c2 molecule (Table 1).

A common and most important feature in the experimental procedure is the use of a reference sample to normalize the measured reflection and transmission coefficient. This will minimize systematic artifacts which may arise, for example, from slight impedance mismatches within the

TABLE 1 Details of experimental techniques

Technique	Material	Configuration	Reference
TDS (*1*): Reflection from a coaxial sensor	pUC8.c1 pUC8.c2 pUC8.c2	Supercoiled Supercoiled Relaxed	Gabriel et al., 1986 (14)
TDS (*2*): Total reflections from a 0.9-mm sample in a matched coaxial line	pUC8.c1 pUC8.c2 pUC8.c2	Supercoiled Supercoiled Relaxed	Dawkins et al., 1981 (15)
TDS (*3*): Total transmission from a 5-mm sample in a matched coaxial line	pUC8.c2	Supercoiled	Gestblom and Elmgren, 1982 (18)
TDS (*4*): Total transmission from a 10-mm sample in a matched coaxial line	pUC8.c2	Supercoiled	Gestblom and Elmgren, 1982 (18)
TDS (*5*): Single reflection from a sample in a matched coaxial line	pUC8.c2	Supercoiled	Gestblom and Noreland, 1984 (16)
TDS (*6*): Single reflection from a sample in a matched coaxial line	pUC8.c2	Supercoiled	Gabriel et al., 1984 (17)
FD (*7*): Total transmission from a sample in a matched coaxial line	pUC8.c2	Supercoiled	Gestblom, 1982 (19)

All measurements were made at 20°C.

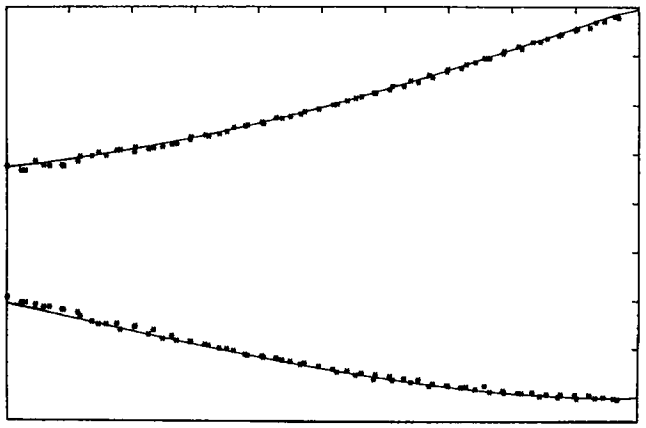

FIGURE 1 Relative permittivity (ϵ') and loss factor (ϵ'') of 0.1% plasmid (supercoiled) DNA solution at 20°C. Measurements taken at King's College London using technique *1*. ——, Literature values for pure water.

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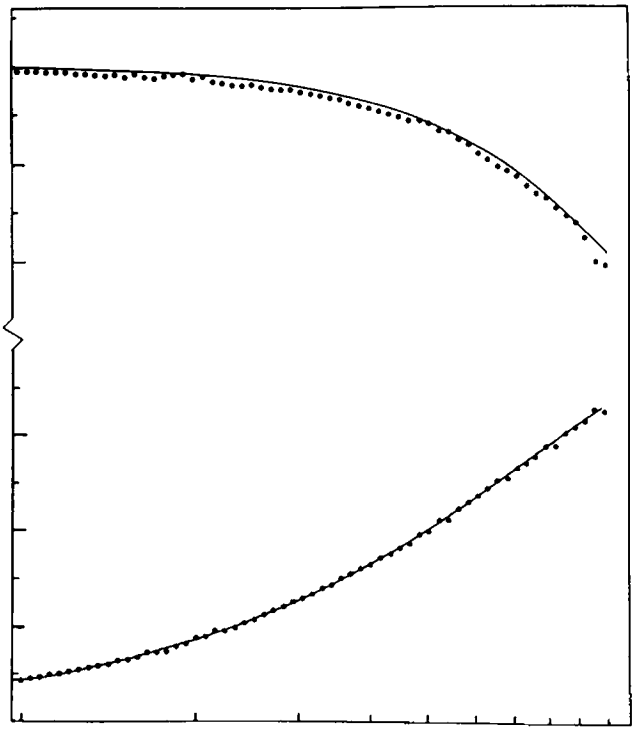

FIGURE 2 Relative permittivity (ϵ') and loss factor (ϵ'') of 0.1% plasmid (supercoiled) DNA solution at 20°C. Measurements taken in Uppsala using technique *3*. ——, Literature values for pure water.

system. The reference liquid was pure water, the dielectric parameters of which are well known. In one case, as futher discussed below, the reference chosen was an aqueous electrolyte solution.

Below are given some details about the experimental measurements as listed in Table 1; further information about the experimental set-ups can be found in the appropriate references cited.

In TDS, the measurement is based on the study of the influence of the dielectric sample of a pulse propagating in a coaxial line. In the reflection methods the pulse reflected from the sample $r(t)$ is compared with the pulse reflected from the reference liquid $r_{ref}(t)$. Fourier transformation $F(\omega) - f(t) \exp(-i\omega t)\, dt$ of both pulse shapes gives a reflection coefficient ratio $R(\omega)/R_{ref}(\omega)$ at a chosen frequency. From transmission line theory this ratio can be expressed as a function of permittivity, and solution of the corresponding equation will give the permittivity spectrum. In the transmission methods the pulse transmitted through the dielectric samples is instead studied, the permittivity spectrum then being worked out from the transmission coefficient ratio.

An important feature of the total transmission and total reflection TDS methods is that the time domain data will immediately give the dc conductivity σ of the sample. Thus σ can be deduced from the ratio of the final levels of the incoming and reflected/transmitted pulses, $v(t \to \infty)$ and $r(t \to \infty)$, respectively. This makes it possible to correct directly the measured ϵ''_{tot} values for the conductivity contribution $\sigma/\omega\epsilon_0$ to obtain the dipolar contribution ϵ''.

In measurement *1*, a probe consisting of an open-ended coaxial line is placed in contact with the sample. The signal reflected from the probe–sample interface is compared with that reflected from the probe–reference interface. The probe, which is 650-mm long, is made of 3.6-mm diam cable, the functional end being gold plated and fitted with a thermocouple. The probe incorporates its own delay line chosen to eliminate the reflection of its connector from the observational time window. Specimen holders with the DNA samples, buffer, and water are placed in a temperature-controlled unit. The samples and buffer were measured alternately using the same water reference. The measurements of the buffer served to ensure that the technique is free from systemmatic experimental artifacts. The results obtained by this method for supercoiled plasmid are shown in Fig. 1, and the results for relaxed plasmid are included in the data in Fig. 4.

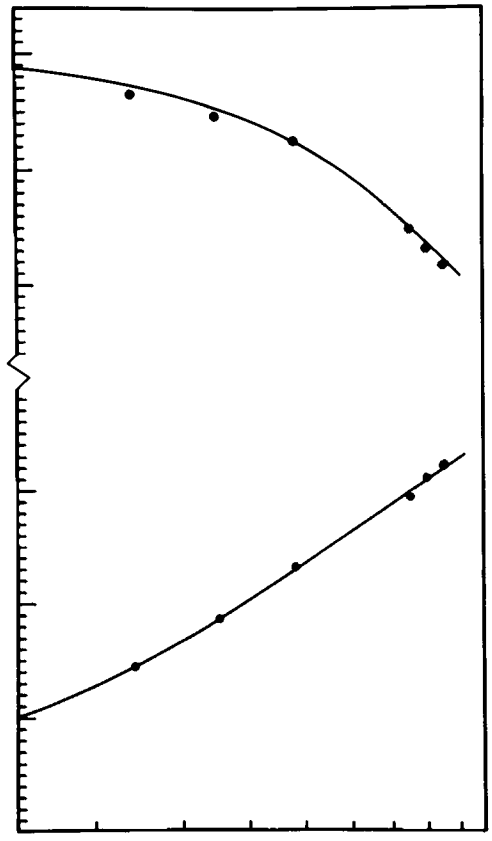

FIGURE 3 Relative permittivity (ϵ') and loss factor (ϵ'') of 0.1% plasmid (supercoiled) DNA solution at 20°C. Measurements taken in Uppsala using technique *7*. ——, Literature values for pure water.

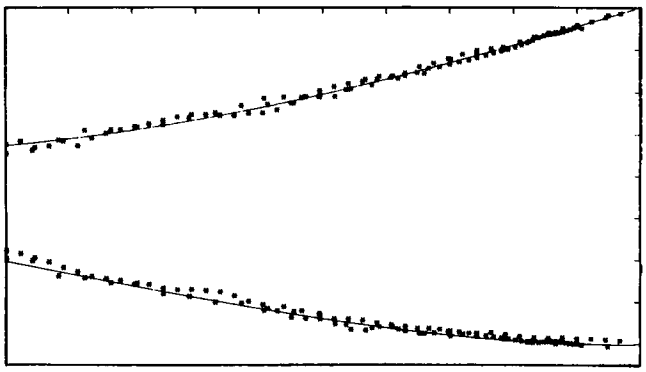

FIGURE 4 Relative permittivity (ϵ') and loss factor (ϵ'') of 0.05% plasmid (relaxed) DNA solution at 20°C. Measurements taken at King's College London using techniques *1* and *2*. ——, Literature values for pure water.

Measurement *2* consisted on an in-guide technique with a sample thickness of 0.9 mm. The specimen cell was loaded in turn with the DNA sample, buffer, and water reference. The results for relaxed plasmid using this technique are shown in Fig. 4 pooled with the data obtained from measurement *1*.

With measurement technique *3*, a 5-mm sample length was used. The observational time window was 10 ns. To check the measurement procedure a dioxane–water solution was prepared, and the cell was alternately filled with test solution, the DNA solution, and pure water. The average of 36 spectra was calculated for the two solutions. The spectrum of the DNA solution is shown in Fig. 2. The spectrum of the test solution is not included, but its excellent agreement with the expected behavior of "diluted water" confirmed the reliability of the measurement procedure.

In measurement *4*, the sample length was increased to 10 mm with a time window of 10 ns. Again 36 independent spectra were recorded by repeated fillings of the DNA solution, the reference water, and the dioxane–water. In this case the upper frequency limit of the spectrum lies around 7 GHz due to the strong signal absorption by the sample above this frequency.

In the single reflection method the pulse reflected from the first air–dielectric interface of a long dielectric sample is compared with the pulse reflected from a known dielectric. In contrast to the total reflection/transmission techniques, the conductivity of the sample cannot be obtained directly from the time domain data in this case. Instead σ has to be deduced by other methods, either independent measurements or from the $\sigma/\omega\epsilon_0$ behavior of ϵ''_{tot} at low frequencies.

In measurement *5*, an aqueous solution of potassium chloride was used as reference. The concentration was chosen to give a standard

solution with dc conductivity close to that of the DNA solution. The observational time window was 5 ns, sufficiently long to give accurate Fourier transforms above 1 GHz and at the same time sufficiently short to keep multiple reflected signals out of the time window. 18 independent spectra were recorded by repeated fillings of the DNA solution and the reference solution. The limited number of independent spectra in this case lead to larger uncertainties in the dielectric parameters than for the methods discussed above.

In measurement *6*, the sample length was 60 mm, the observational time window 2 ns. The DNA sample and buffer were measured using water as a reference. The sample was loaded only once, and the time domain data were recorded five times and subsequently normalized against five different spectra for water.

The total transmission method can also be employed to make measurements directly in the frequency domain. In this case the pulse generator is exchanged for an oscillator and the transmission coefficient measured at spot frequencies, the remaining analysis being the same as for the TDS method.

In FD measurement *7*, a 2-mm sample was used at 3.4 and 4.5 GHz. The sample length was reduced to 5 mm at the higher frequencies to allow sufficient amplitude of the signal to be transmitted. As for the TDS measurements, pure water was used as reference. Dielectric data obtained by this method are shown in Fig. 3.

No significant differences were obtained in the measured dielectric parameters (examples of which are given in Figs. 1–4) for the DNA solutions, irrespective of which of the seven experimental techniques was used. The implication of the results will now be discussed.

RESULTS AND DISCUSSION

As noted above our reason for making dielectric measurements on dilute aqueous solutions of DNA at microwave frequencies was so that a direct comparison could be made with the results obtained by Edwards et al. (8). Otherwise there would be little scientific interest in determining the dielectric properties of an aqueous solution of concentration as low as 0.1% at frequencies in excess of 1 GHz. To obtain information of any academic value one would need to work at a much higher concentration or, alternatively, at considerably lower frequencies where polarization mechanisms arising from the properties of the solute molecule would come into play. We are currently carrying out such investigations for DNA in the radiofrequency region, and these will form the subject of a future publication.

Returning to the present measurements we found that none of the samples listed in Table 1 exhibited dielectric properties which could be distinguished from those of pure water once corrections for ionic conductivity had been made to the dielectric loss ϵ''. Typical results are shown in Figs. 1 and 2, where values of ϵ' and ϵ'' for supercoiled pUC8c2 are portrayed. In Fig. 1 are shown the data obtained at King's College where it is noticed that a linear scale has been chosen for frequency. This is in order to give a direct comparison with the work of Edwards et al. (8), where the values of ϵ' and ϵ'' were displayed in the same form. The results shown in Fig. 2 refer to the same samples of DNA as those in Fig. 1 but were obtained in the Uppsala laboratory. In this plot the more traditional logarithmic representation of frequency has been adopted. In both Figs. 1 and 2, a continuous line is drawn to illustrate the dielectric behavior of pure water. As would be expected in the absence of nonclassical behavior for a biological solution of concentration as low as 0.1%, there is no significant deviation from the pure water values. In both figures each data point is the average of up to 36 independent measurements on samples from four plasmid preparations. In Fig. 3 are shown values of ϵ' and ϵ'' for pUC8.c2 supercoiled DNA using a frequency domain method. There are fewer data points with this technique but nevertheless there is sufficient information in the plot to infer that the dielectric behavior is classical. The measurements on the relaxed DNA gave the same results as those on the supercoiled form, i.e., dielectric behavior indistinguishable from that of pure water. The experimental data are shown in Fig. 4.

The observation that dilute aqueous solutions of DNA might behave in a manner not consistent with the Debye or Cole-Cole dispersion equations was first made by Swicord and Davis (7) who reported an enhanced electric field attenuation coefficient (α) in the frequency region 8–12 GHz. The conclusions from this work were that there were no observed resonant peaks, the behavior exhibited being characterized by an increase in α over the pure water value of 40% at 8 GHz, diminishing monotonically to 11% at 12 GHz. In these studies sized fragments of *E. coli* chromosomal DNA were used, whereas pUC8 (circular) DNA was used in their subsequent investigations (8) and in our present work being reported in this paper. The value of α may be calculated from the dielectric data according to the equation

$$\alpha = \frac{\omega}{c}\left[\frac{(\epsilon'^2 + \epsilon''^2)^{1/2} - \epsilon'}{2}\right]^{1/2},$$

where ω is the angular frequency and c is the velocity of light in vacuo. In their measurements on supercoiled pUC8 DNA, the same authors (8) displayed their data somewhat differently. In this case, for a 0.053% DNA solution, the increase in the power attenuation coefficient over the buffer solution was calculated and plotted as a function of frequency (Fig. 5). This change in parameter by Edwards et al. may be considered to be confusing, particularly as in the different publications they used the same symbol (α) to represent both the electric field and the power attenuation coefficients. Moreover the units are also the same. It is obviously better not to mix the parameters but in any event different symbols should be used, e.g., the power coefficient might be designated α_p.

The relaxed form of pUC8.c2 DNA is of particular interest in that recent theoretical work of Van Zandt (20) had predicted that resonance absorption in this form of

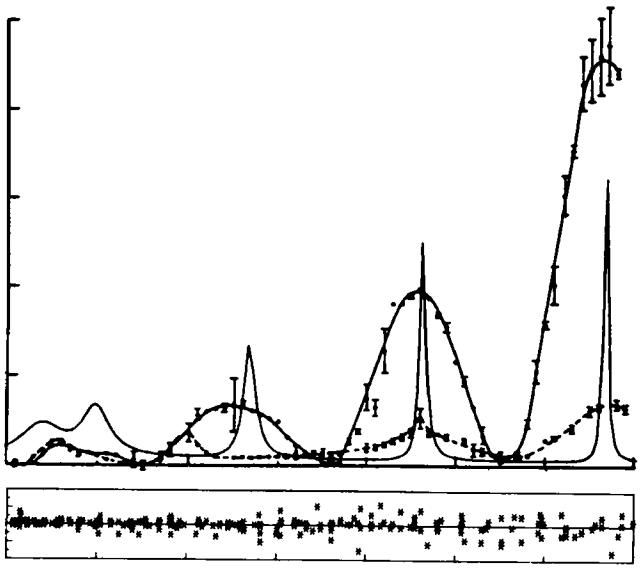

FIGURE 5 Incremental power attenuation coefficient $\Delta\alpha_p$ for plasmid DNA solution at 20°C. (*Upper figure*) ——, theoretical curve (20) based on a possible model for relaxed DNA; ●—●, previous experimental observations (20) for 0.01% relaxed DNA; ●-●-●-●-●-●, previous experimental observations (8) for 0.053% supercoiled DNA. (*Lower figure*) Present experimental values for 0.05% relaxed DNA and 0.1% supercoiled DNA. Pooled data for both types of DNA measured by the seven experimental techniques (see text).

the molecule should be characterized by peaks of much greater amplitude than for the supercoiled DNA. In this same publication (20) Van Zandt shows experimental data by Davis, Edwards, and their colleagues on a solution of relaxed DNA of concentration only 0.01%, which exhibit peaks in the power attenuation coefficient of magnitude greater than thirty times the value for pure water. These experimental results are shown in the upper curve of Fig. 5. Finally in the lowest part of this same figure are shown the results obtained in the present work. To effect a direct comparison with the data of Edwards et al., we have used the same form of representation, i.e., the incremental values ($\Delta\alpha_p$) of the power absorption coefficient. It should be pointed out that this method of displaying the data does not show how small a fraction of the background attenuation the amplitudes of the reported resonance peaks actually are. For example the value of α_p for water at 2.8 GHz is 0.83 cm^{-1} rising to 7.2 cm^{-1} at 9 GHz. Thus for the supercoiled DNA the background value of α_p is three times the magnitude of the resonance peak $\Delta\alpha_p$ at 2.8 GHz and 10 times the magnitude of that for the 9-GHz resonance.

All the results taken in the present work on pUC8.c1 and pUC8.c2 are included in Figs. 5, relaxed and supercoiled forms alike. Since it is clear from the figure that in no cases do the values of the power attentuation coefficient deviate from those of pure water, the present results and the previous resonance data are irreconcilable. Subsequent to the commencement of the present work it has been suggested by Foster et al. (21) that the apparently observed resonance (8) could have been caused by experimental artifacts.

CONCLUSIONS

Despite extensive measurements at microwave frequencies on three different forms of pUC8 DNA in dilute aqueous solution, no dielectric behavior has been observed which cannot be interpreted in terms of the relaxation of water dipoles. The use of seven different experimental techniques spread over two laboratories enables this conclusion to be made strongly, notwithstanding the existence in the literature of contrary claims.

We thank Professor J. B. Bateman for useful discussions throughout the research.

We are also grateful to the United States Air Force for supporting this work under contract AFOSR-85-0183.

Received for publication 13 May 1988 and in final form 28 September 1988.

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