Dielectric relaxation measurements of 12 kbp plasmid DNA

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Abstract

The dielectric properties of 12 kbp plasmid DNA have been measured as a function of temperature in the range 5°C to 40°C. Time domain reflectometry was used to obtain dielectric data over the frequency range from 200 kHz to 3 GHz. Values of the frequency dependent polarisability per DNA macromolecule have been determined from the measurements. Possible mechanisms that could account for the dielectric dispersion are also discussed, in particular the counterion fluctuation model of Manning–Mandel–Oosawa. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Over the past four decades there has been considerable interest in the dielectric properties of DNA suspended in solution [1–20]. There are a number of reasons for undertaking dielectric studies on DNA; in general, dielectric spectroscopy yields quantitative information about the response of a system of DNA macromolecules, and surrounding counterions, to alternating current (AC) electric fields. Although the precise reasons for dielectric dispersions are still being debated, the literature shows that DNA, similar to other polyelectrolytes [8], exhibits dispersions over a wide range of frequencies in the range from very low (3 Hz) to very high (1 GHz) frequencies [12].

Dielectric spectroscopy enables quantitative analysis of the frequency dependent polarisability of DNA macromolecules. Understanding the polarisability is essential for predicting how DNA can be manipulated using AC electric fields. Recent work by our group [21], and others [22–26], has demonstrated that DNA can be moved on micro-fabricated electrode arrays using a technique known as dielectrophoresis (DEP) [27]. Non-uniform electric fields are used to attract DNA to electrode edges through induced dipole forces. Using high frequency AC electric

fields, DEP avoids the problem of electrolysis, characteristic of electrophoresis, which occurs when DC potentials are applied to electrodes. Interest in using DEP to control the movement of biological polyelectrolytes, such as DNA, could enhance recent developments in ‘lab-on-a-chip’ technology. In this context, we have undertaken dielectric measurements to determine the dependence of polarisability on both AC electric field frequency and temperature of solution.

In this paper, we report measurements of a 12 kbp plasmid DNA suspended in low conductivity medium, for a range of temperatures, 5°C to 40°C. Time domain reflectometry (TDR), a technique well described in the literature [28], has been used over the frequency range 200 kHz to 3 GHz. Values of the frequency dependent polarisability per DNA macromolecule, a key parameter for DEP investigations, are determined from the TDR measurements. Possible mechanisms that could give rise to dielectric dispersion are also discussed, in particular with respect to the counterion fluctuation model of Manning–Mandel–Oosawa [1–7,16–20].

2. Materials and methods

2.1. DNA preparation

pTA250 is a 12 kbp plasmid that contains a single repeat of the 8.8 kbp ribosomal RNA coding unit from

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wheat (*Triticum aestivum* cv. Chinese Spring) [29], subcloned into the *EcoRI* site of pUC19.

The DNA was prepared according to the following method. *Escherichia coli* strain DH5 α containing pTA250 was grown in Luria Bertani broth containing 20 $\mu\text{g/ml}$ ampicillin and harvested according to standard protocols [30,31]. Plasmid DNA purification was achieved using a Qiagen-tip 2500 (Qiagen Ltd, Crawley, England) according to the manufacturer's instructions. Desalting was achieved by isopropanol precipitation. 10 μl volume stock samples were prepared by diluting purified DNA in double distilled water (ddH $_2\text{O}$) and stored at -20°C . The DNA concentration was estimated spectrophotometrically, assuming an extinction coefficient at 260 nm of 20 for 1 mg/ml [30]. From the A_{260} , the stock DNA concentration was calculated to be 2.7 $\mu\text{g}/\mu\text{l}$. Plasmid DNA was analysed by electrophoresis on non-denaturing agarose gels and visualised under UV illumination following ethidium bromide staining [30].

To achieve a low DC conductivity for TDR measurements, 20 separate 10 μl samples were thawed and dialysed against reverse osmosis (RO) water using membrane flotation filters (Millipore VSWP, 25 nm pore size) for 2 h. During dialysis the total volume of aqueous DNA increased from 200 μl to 600 μl , hence the final concentration of DNA used for the measurements was $C_{\text{DNA}} = 0.9$ $\mu\text{g}/\mu\text{l}$.

2.2. Dielectric spectroscopic measurements

The dielectric properties of the DNA were determined by a commercial time domain dielectric spectrometer (TDS-2.1), manufactured by Dipole TDS, Jerusalem [32]. This spectrometer determines dielectric properties of materials by measuring the response of a sample to a voltage step with a fast rise time. In the framework of the lumped capacitance approximation, the complex dielectric permittivity is written as follows:

$$(\epsilon^*)(\omega) = \frac{1}{i\omega C_0} \cdot \frac{L[I(t)]}{L[V(t)]} \quad (1)$$

where $I(t)$ is the current flowing through the sample, $V(t)$ is the voltage applied to the sample, L is the operator of the Laplace transform and C_0 is the capacitance of the empty sample holder terminated to the end of the coaxial line ($C_0 = 0.2$ pF).

A small sample (about 150 μl) of DNA solution was injected into the sample holder, and the time domain response of the sample was determined from the accumulation of 25 600 individual scans. Non-uniform sampling of the time window (5 μs) for each pulse enables the generation of spectra in the frequency range from 200 kHz up to 3 GHz. Measurements were taken as a function of increasing temperature from 5°C to 40°C , at intervals of 5°C . The measurement accuracy of the dielectric permittivity and loss was better than 5% [32]. The accuracy of

fitting (i.e. the mean square deviation) was less than 10^{-3} . Further details of the TDS technique can be found in other publications [32–34].

3. Results and analysis

DNA samples gave an absorbance ratio $A_{260}/A_{280} = 1.8$, indicating the DNA was substantially free from protein and other contaminants. Analysis using gel electrophoresis showed that the native pAT250 DNA migrated as a series of high molecular weight bands with mobilities consistent with a mixture of concatenated covalently closed supercoiled circles; bands with mobilities expected for relaxed circular or linear isoforms were not detectable. Following digestion with *HindIII* (which cuts at a single site [29] in pAT250) the DNA migrated as a single species with the expected molecular weight of ca. 10–12 kbp. By comparison with human mitochondrial DNA, we estimate that pTA250 should have an approximate contour size of 4 μm .

Time domain reflectometry (TDR) measurements were performed, under both heating and cooling cycles, for temperatures in the range 5 – 40°C . The data treatment was carried out directly in the time domain in terms of the macroscopic dipole correlation function $\Gamma(t)$ (DCF):

$$\psi(t) \approx \Gamma(t) = \frac{\langle \mathbf{M}(0) \cdot \mathbf{M}(t) \rangle}{\langle \mathbf{M}(0) \cdot \mathbf{M}(0) \rangle} \quad (2)$$

where $\mathbf{M}(t)$ is the macroscopic fluctuation dipole moment of the sample volume unit, which is equal to the vector sum of all the molecular dipoles; the symbol $\langle \rangle$ denotes averaging of the ensemble. The velocity and laws governing the decay function $\Gamma(t)$ are directly related to the structural and kinetic properties of the sample and characterise the macroscopic properties of the system under investigation. The least square fitting procedure based on the simplex method was used. Within the limit of experimental

Fig. 1. Signals recorded in a typical TDS experiment. Signal (1) is the reference signal (air); (2) the sample (DNA solution at 15°C); (3) the short circuit.

Fig. 2. The variation in dielectric decrement for each relaxation process as a function of temperature for pTA250 12 kbp plasmid DNA. Measurements were made with a DNA concentration of $C_{\text{DNA}} = 0.9 \mu\text{g}/\mu\text{l}$.

errors the DCF may be described by the sum of three exponential components corresponding to several dielectric relaxation processes. The optimal number of the exponents was obtained by the routine of sequential subtraction of relaxation components from the DCF [35–37]. Fig. 1 shows a graph of voltage signals recorded in a typical TDS experiment. The plot shows three signals: (1) that of the reference sample (air); (2) the DNA solution (at 15°C); and (3) the short circuit. Signal differences are proportional to the current following through the sample $I(t)$ and the voltage applied to the sample $V(t)$.

The analysis identified and resolved three temperature dependent dielectric dispersions. Results of the temperature dependent dielectric decrement $\Delta\epsilon$ and relaxation time τ , for each of the three identifiable dispersions are shown in Figs. 2 and 3, respectively. The figures show the heating cycle; the results for the cooling cycle were essentially the same, but not as comprehensive. The first dispersion, labelled with subscript 1 occurs at a frequency $f_1 = 137 \text{ kHz}$ at 25°C . Similarly, the second and third dispersions are at $f_2 = 2.01 \text{ MHz}$ and $f_3 = 11.6 \text{ MHz}$ at 25°C .

In Fig. 2 the major dielectric decrement (the first dispersion, $\Delta\epsilon_1$) shows a marked increase with temperature, with gradient $\partial(\Delta\epsilon_1)/\partial T \approx 0.25/^\circ\text{C}$. By contrast, the second and third (minor) dispersions exhibit decrements that decrease with temperature, with gradients $-0.01/^\circ\text{C}$ and $-0.02/^\circ\text{C}$, respectively. The relaxation times, shown in Fig. 3, all decrease with increasing temperature, a trend that agrees with the literature for solutions of low salt concentration [15,18]. The measured DNA solution conductivity was $\sigma = 8.5 \text{ mS/m}$ at 25°C .

3.1. Volume fraction and concentration of DNA monomers

The volume fraction v is the ratio of volume V occupied by DNA macromolecules with respect to the total volume, $v = V/V_{\text{Total}}$. A simple estimation of v , in terms of DNA solution concentration C_{DNA} ($\mu\text{g}/\mu\text{l}$) and molecular weight

[38], can be found by assuming a cylindrical geometry for each DNA macromolecule, hence

$$v = \frac{\pi r^2 L_{\text{bp}} N_{\text{Av}} C_{\text{DNA}}}{\text{MW}_{\text{bp}} 10^{-3}} \quad (3)$$

where the DNA macromolecule ‘effective cross-section’ radius $r = 1.5 \text{ nm}$, DNA contour length per base pair $L_{\text{bp}} = 3.4 \text{ \AA}$, molecular weight $\text{MW}_{\text{bp}} = 649 \text{ Da/base pair}$, N_{Av} is Avogadro’s number and 10^{-3} is g/kg conversion factor. Using $C_{\text{DNA}} = 0.9 \mu\text{g}/\mu\text{l}$, yields $v \approx 0.2\%$ which is close to values adopted in [17] for approximately the same DNA concentration. The calculation is in essence independent of the size of the macromolecule, thus enabling a comparison with volume fractions in other dielectric spectroscopy studies. A simple calculation also reveals that each 12 kbp DNA plasmid, if packed into a tight sphere, would have a radius of approximately 19 nm. This concurs with an estimate of 30 nm in [24], for λ -phage DNA that is four times longer.

Another parameter for determining polyelectrolyte polarisability is the molar concentration of monomers, or for DNA, the concentration of phosphate groups C_p . It can be expressed in terms of the average distance between charged sites b ,

$$C_p = \frac{L_{\text{bp}} C_{\text{DNA}}}{b \text{MW}_{\text{bp}} 10^{-3}} = \frac{2 C_{\text{DNA}}}{\text{MW}_{\text{bp}} 10^{-3}} \quad (4)$$

where for a B-DNA double helix, $b = 1.7 \text{ \AA}$. Using $C_{\text{DNA}} = 0.9 \mu\text{g}/\mu\text{l}$ in Eq. 4, then $C_p = 2.77 \text{ mol/m}^3$ or 2.77 mM/l .

3.2. Dielectric relaxation times and activation enthalpy

The relaxation time τ varies with temperature T according to the Arrhenius expression [12,28],

$$\tau = \tau_0 \exp\left(-\frac{\Delta H}{RT}\right) \quad (5)$$

where τ_0 is the nominal relaxation time, ΔH is the activa-

Fig. 3. The variation in the relaxation times of the three dispersions as a function of temperature.

Table 1

DNA *pTA250* dielectric decrement, relaxation frequency, activation energy and polarisabilities, determined from experimental data, for each of the three dispersions

Dispersion (at 25°C)			Activation enthalpy ΔH (kCal mol ⁻¹)	Polarisability per macromolecule α_m (F m ²) at 25°C	Polarisability for 30 nm subunit α_s (F m ²) at 25°C
No.	$\Delta\epsilon'$	f (MHz)			
1	20.3	0.137	1.4	7.88×10^{-30}	5.70×10^{-32}
2	4.82	2.01	3.8	1.87×10^{-30}	1.35×10^{-32}
3	0.507	11.7	4.0	1.97×10^{-31}	1.42×10^{-33}

tion enthalpy (J mol⁻¹), and the R molar gas constant. Rearranging Eq. 5, and differentiating with respect to $1/T$, it can easily be shown that a plot of $\ln(\tau)$ vs. $1/T$ yields a straight line with gradient $\Delta H/R$. The temperature dependent relaxation times shown in Fig. 3 for each of the three dispersions were computed as Arrhenius plots and the resulting activation enthalpy, ΔH for each dispersion, is listed in Table 1. All three ΔH values agree with published values in the range 1–4 kCal/mol [15], where the dispersions are attributed to counterion fluctuation.

3.3. Polarisability

An important reason for undertaking measurements of the dielectric properties was to estimate values for the frequency dependent polarisability, α (F m²). The polarisability is related to the dielectric decrement $\Delta\epsilon'$ [39,40], and can be expressed in several ways. The polarisability per macromolecule α_m is

$$\alpha_m = 3\epsilon_0\Delta\epsilon'/C_m \quad (6)$$

where C_m (m⁻³) is the number density of DNA macromolecules and ϵ_0 is the permittivity of free space. The molecular weight (MW) for this 12 kbp DNA (assuming MW = 649/bp) is 7.8×10^6 Da (7.8 MDa), hence $C_m = 6.85 \times 10^{19}$ macromolecules/m³. Values of α_m , calculated using Eq. 6, for each of the three $\Delta\epsilon'$ values are also listed in Table 1. Another way of expressing the polarisability is in terms of macromolecular subunit length L_s , an important parameter where counterion fluctuation models are used to describe the polarisability. In this case the polarisability α_s , per subunit length, is determined from measurements of the decrement $\Delta\epsilon'$ via the number density of subunits C_s [16,17]

$$\alpha_s = \frac{3\epsilon_0\Delta\epsilon'}{C_s} = \frac{3\epsilon_0\Delta\epsilon'}{N_{Av}C_p(b/L_s)} \quad (7)$$

This definition enables α_s to be compared with other dielectric studies of DNA with different molecular weights. Using for example a subunit length $L_s = 30$ nm, reveals agreement with polarisability values measured by Saif et al. [17] for comparable values of $\Delta\epsilon'$. Their study of calf thymus DNA resolved three dielectric dispersions with characteristic frequencies higher than our measurements, but with an average decrement (for their dialysed solution)

of $\Delta\epsilon' = 1.94$ (normalised to 1 g/l) giving¹ $\alpha_s = 5.3 \times 10^{-33}$ (F m²). Our values also compare favourably with those calculated by Suzuki et al. [41] using measurements of fluorescence anisotropy of DNA in AC electric fields. They obtained $\alpha = 1 \times 10^{-32}$ (F m²) for pUC18 (2.7 kbp) which is 4.5 times smaller than our *pTA250* DNA, and therefore their polarisability should be increased by the same amount for a comparison with our values. However, the origin of the polarisability has not been resolved, and the differences in experimental methodologies between Suzuki et al. and ours, prevent an exact comparison.

3.4. Concentration of ions surrounding the DNA

The low frequency conductivity of the suspension $\sigma = 8.5$ mS/m (at 25°C) and is significantly larger than the conductivity of the (RO) water which was used for dialysis (0.2 mS/m). It is, however, comparable with the low frequency (100 Hz) conductivity measured by Saif et al. [17] who reported $\sigma = 5$ mS/m at 25°C, after their DNA solution had been dialysed for a week to eliminate bulk ions from the sample. Following their argument, it is possible to attribute the low frequency σ to the diffuse ionic phase surrounding the DNA, rather than the bulk ion phase (as described further in the next section). However, in the absence of conclusive evidence, it will suffice to designate C_i (mol/m³) as the combined concentration of bulk and diffuse phase ions surrounding the DNA macromolecules. Using the relation,

$$C_i = \frac{\sigma}{q\mu N_{Av}} \quad (8)$$

where typically (for potassium ions) the ion mobility $\mu = 8 \times 10^{-8}$ (m² V⁻¹ s⁻¹), q is the charge and other symbols are as previously stated. Ignoring the effect of the electrophoresis of DNA on the DC conductivity, C_i is calculated to be 1.10 mol/m³.

¹ In [17] the value 5.3×10^{33} is assumed to have a typographical error and we have used the value of 5.3×10^{-33} (F m²).

4. Discussion

Previous dielectric spectroscopic investigations of polyelectrolyte solutions, including DNA, have proposed a number of mechanisms for the dispersions. These include Maxwell–Wagner interfacial polarisation [12,17], rotation of bound water molecules [14] and polar groups [11] associated with the DNA, and fluctuations of counterions along the longitudinal [1–7] and transverse [10] axes of the DNA. Although it is difficult to draw a definitive conclusion about the mechanism(s), the evidence tends to favour counterion fluctuation along the DNA axis, in particular concerning the low frequency relaxation around 140 kHz.

4.1. Rotation of bound water molecules and polar groups

The characteristics of water molecule clusters bound along and across DNA grooves were shown by Mashimo et al. [14] to have an activation energy of 17 kcal/mol accompanied with a relaxation time $\tau = 1.4$ ns (relaxation frequency $f = 113$ MHz). These data were obtained for B-DNA below the melting point. Reports of dispersions around 100 MHz, for example, by Takashima et al. [11] have also attributed this high frequency dispersion to the motion of polar groups inside the DNA macromolecule. The dispersions exhibited by our DNA sample have considerably lower activation energies (1.4–4 kcal/mol) and relaxation frequencies (lower by an order of magnitude), values which do not agree with the τ and ΔH values expected for this type of mechanism. A similar conclusion can be drawn for the motion of DNA sugar phosphate groups. The analysis of [18] showed that for DNA concentrations and dielectric increments comparable to our investigation, the dipole moment per base pair exceeded expected values by more than two orders of magnitude.

4.2. Maxwell–Wagner interfacial polarisation

An accepted cross-sectional view of rod-like DNA in solution is that of an insulating core surrounded by a highly conducting layer, or ‘sheath’, immersed in an electrolyte. The insulator (2 nm in diameter) corresponds to the amino acid, sugar phosphate, double helix that constitutes DNA and the high conductivity layer represents the bound counterions on the negatively charged sugar phosphate backbone. The low frequency polarisation of this charge had been identified with the classical α dispersion with a higher frequency relaxation corresponding to an interfacial polarisation (Maxwell–Wagner) mechanism. Grosse [13] modelled Maxwell–Wagner dispersion for DNA, and derived an expression for the dielectric decrement

$$\Delta\epsilon' = 8\epsilon_r v/3 \quad (9)$$

where ϵ_r is the relative permittivity of the bulk medium,

and v is the volume fraction occupied by DNA in solution. Similarly, the high frequency relaxation [13], can be expressed in terms of the electrolyte conductivity σ and fraction of condensed counterions, ϕ

$$f_R = \sigma\phi/(4\pi\epsilon_0\epsilon_r v) \quad (10)$$

where $\sigma = 8.5 \times 10^{-3}$ S/m at 25°C. Assuming values $v = 0.22\%$, $\phi = 0.24$ and $\epsilon_r = 79$, Eqs. 9 and 10 yield $\Delta\epsilon' \approx 0.46$ and $f_R \approx 106$ MHz. Although the decrement is close to the value resolved in our third dispersion, the relaxation frequency is an order of magnitude higher. It should be noted that Grosse’s model assumes the thickness of the counterion layer to be much smaller than the radius of the DNA macromolecule. To accommodate a more realistic diffuse ionic layer thickness of 7 Å, Saif et al. [17] modified Eq. 9 reducing our predicted decrement to $\Delta\epsilon' \approx 0.07$. Furthermore, as an alternative to Eq. 10, their relaxation frequency was based on a reported concentration of 1.2 M Na⁺ in the counterion layer [6] leading to an increase in our predicted relaxation frequency $f_R \approx 1.2$ GHz. It appears then that the predictions of Grosse’s ‘modified’ Maxwell–Wagner model deviate further from the original predictions of Eqs. 9 and 10. Consequently, Maxwell–Wagner interfacial polarisation is unlikely to be the cause of our observed dispersions. However, it cannot be entirely ruled out, particularly regarding the third, high frequency, relaxation around 12 MHz.

4.3. Counterion fluctuation

The third possible cause of dispersions is attributed to counterion polarisation along segments of the DNA longitudinal axis. The length L_s of each subunit is deemed to be the average macromolecular conformation between ‘breaks’, or potential barriers, resulting from perturbations in the equipotentials. Counterions move freely along these subunits in response to the component of the external electric field parallel to the subunit. However, they can cross over from one subunit to a neighbouring one only by overcoming these potential barriers [4]. None of the polyelectrolytes studied in [4] include DNA, however the dispersion characteristics are not dissimilar to those measured for DNA. There are several variations of this scheme, and in particular Manning [2,6,7] proposed that a proportion of the counterions are so strongly attracted to the polyelectrolyte that they are said to ‘condense’ onto the DNA backbone. Essentially there are three distinct phases [17]:

1. *Condensed* counterions, characterised by delocalised binding to phosphate groups of the DNA and thereby neutralising a fraction of the DNA charge.
2. *Diffuse* counterions which are responsible for neutralising the remainder of the DNA charge, with a density which decreases exponentially with distance from the axis.

3. Bulk ions or ‘added salt’ ordinary aqueous solution ions.

A feature of the condensed state is that the local concentration of counterions around the DNA does not tend to zero when the bulk electrolyte concentration does. The fraction of condensed counterions ϕ is expressed in terms of the charge density parameter ξ and ion valency z ,

$$\phi = 1 - |z|^{-1} |\xi|^{-1} = 1 - 4\pi\epsilon_r\epsilon_0 kTb/q^2 |z| \quad (11)$$

where q the electronic charge and kT is the Boltzmann temperature. Using $\epsilon_r \cong 79$ and $z=1$, the charge density parameter at 25°C is evaluated to be $\xi=4.17$. The polarisability α_s is dependent on the number of condensed counterions n

$$\alpha_s = \frac{z^2 q^2 L_s^2 n A}{12kT} = \frac{z^2 q^2 L_s^2 \{\phi L_s/(zb)\} A}{12kT} \quad (12)$$

A is the stability factor of the ionic phase and includes mutual repulsion between fixed charges on the backbone and the effect of Debye screening,

$$A = [1 - 2(|z|\xi - 1)\ln(\kappa_s b)]^{-1} \quad (13)$$

where κ_s^{-1} is the Debye screening length

$$\kappa_s = \left[\left(\frac{N_A v q^2}{\epsilon_r \epsilon_0 kT} \right) \left(\sum_i C_i z^2 + \frac{C_p}{\xi} \right) \right]^{0.5} \quad (14)$$

Combining Eqs. 7 and 11–14 leads to a prediction for L_s in terms of $\Delta\epsilon'$

$$L_s = \sqrt{\frac{9\Delta\epsilon'}{\pi\epsilon_r(|z|\xi - 1)AN_A v C_p b}} \quad (15)$$

Substituting $C_p = 2.77 \text{ mol/m}^3$, $C_i = 1.1 \text{ mol/m}^3$ and listed values for all other parameters, Eqs. 13 and 14 yield as an intermediate step, $\kappa_s^{-1} \cong 10.3 \text{ nm}$, and $A \cong 0.037$ at 25°C. Hence, for the first dispersion with $\Delta\epsilon'_1 = 20.3$, Eq. 15 gives $L_s = 149 \text{ nm}$. Values of L_s calculated from the other two dispersions are listed in Table 2.

An alternative method of estimating L_s uses an expression developed by Mandel [1] for the relaxation time,

$$\tau = \frac{L_s^2 q}{\pi^2 kT \mu} \quad (16)$$

where all other symbols are as previously defined. Rearranging Eq. 16 enables prediction of subunit length L_s for

each of the three resolved relaxation times. The values are listed in Table 2. It can be seen that they agree with those determined by the dielectric decrement and are within the range quoted in the literature.

The relatively high values of L_s , particularly for the *first* dispersion, are consistent with conditions of low ionic strength where very little electrostatic screening of the negative phosphate charges occurs. Each DNA macromolecule can be conceived of as a ‘worm-like chain’ consisting of freely jointed, relatively straight segments, or ‘links’ [42]. Each link has a length equal to the Kuhn length L_k (twice the persistence length), a statistical measure of DNA rigidity with respect to thermal energy that tends to randomise its macromolecular shape [43]. In very low salt solutions (e.g. 1 mM Na^+), the persistence length has been measured in most studies to be 80–100 nm, though some have been as low as 60 nm. This gives typical values of the Kuhn length of $L_k \approx 160 \text{ nm}$ [44]. In this respect the subunit length $\sim 150 \text{ nm}$, calculated for the first (major) dispersion (from $\Delta\epsilon'_1$ and τ_1) is remarkably close to the Kuhn length, i.e. $L_{s1} \approx L_k$. This gives a physical interpretation to the most significant dispersion exhibited by the plasmid DNA suspended in the low salt electrolyte. Counterions are envisaged to move unhindered along each subunit, in the direction of the AC electric field, until they reach a potential barrier. These barriers result from ‘breaks’ in the average conformational shape [4], and it is reasonable to attribute these to structural features of the macromolecule, such as kinks or bends.

One aspect that questions the relationship of the counterion model L_{s1} to the structural parameter L_k is the temperature dependence of L_{s1} determined from the dielectric decrement as shown in Fig. 2. In the following discussion we shall denote this subunit length as L_{se1} where the ϵ subscript indicates that the dispersion is calculated from $\Delta\epsilon'_1$. Since $\Delta\epsilon'_1$ increases with temperature, and Eq. 15 shows $L_s \propto \sqrt{\Delta\epsilon'}$, then L_{se1} also increases with temperature. However, it is known L_k should decrease as the temperature increases since the macromolecule becomes more flexible [43,44]. At this stage, it is not clear whether this ‘opposite’ temperature dependence for L_{se1} is a second order effect, with respect to L_k , that is not accounted for in the counterion model. However over the measured temperature range ($5 \leq T \leq 40^\circ\text{C}$) the variation in L_{se1} ($126 \leq L_{se1} \leq 156 \text{ nm}$) lies within the range of values listed for L_k ($120 \leq L_k \leq 200 \text{ nm}$). The temperature dependence of subunit length, predicted from the relaxa-

Table 2
Subunit lengths determined from measured dielectric decrements and relaxation times (all at 25°C)

Dispersion (at 25°C)			Subunit length L_s (nm) determined from $\Delta\epsilon'$	Subunit length L_s (nm) determined from τ
No.	$\Delta\epsilon'$	τ (ns)		
1	20.3	1165	149	154
2	4.82	79.1	72.4	40.1
3	0.507	13.7	23.5	16.7

tion time τ_1 , is less clear due to the uncertainty in the temperature dependence of condensed counterion mobility μ . Assuming, to a first approximation that μ remains constant, then over the same temperature range, Eq. 16 predicts a subunit length ($171 \leq L_{st1} \leq 145$ nm), so L_{st1} follows the same trend as L_k , and lies within the same range of values.

Both subunit lengths calculated from the second and third dispersions (L_{se2} and L_{se3}) tend to decrease with increasing temperature. Although this trend is expected if the subunit length corresponds to macromolecular structural features, such as the Kuhn length, the values are smaller than the published L_k values at this very low salt concentration. Another interpretation of the subunit length may be attributed to DNA strands crossing over each other from plasmid supercoiling, concatenation, or from neighbouring macromolecules where strong electrostatic interactions between chains impose some form of order as discussed in [19,45,46]. In this case, the subunit length is equal to the mean correlation length L_c between contact points, that is, $L_s = L_c$.

5. Conclusion

A dielectric spectroscopy study of 12 kbp plasmid DNA has resolved three dispersions at 140 kHz, 2 MHz and 12 MHz. The mechanism responsible for the polarisability is thought to be counterion fluctuation along subunits of the double-helical axis, although Maxwell–Wagner interfacial polarisation cannot be ruled out for the third dispersion. The subunit length for the first (major) dispersion concurs with the Kuhn length, although the temperature dependence is not as expected. An alternative interpretation of subunit length is attributed to DNA entanglements. This correlates with observations of DNA entanglements made by fluorescence microscopy. The measured polarisabilities for the first, second, and third dispersions are approximately 80, 20, and 2×10^{-31} (F m²) per DNA macromolecule.

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References

- [1] M. Mandel, The electric polarisation of rod-like, charged macromolecules, *Mol. Phys.* 4 (1961) 489–496.
- [2] G.S. Manning, Limiting laws and counterion condensation in polyelectrolyte solutions I. Colligative properties, *J. Chem. Phys.* 51 (1969) 924–933.
- [3] F. Oosawa, Counterion fluctuation and dielectric dispersion in linear polyelectrolytes, *Biopolymers* 9 (1970) 677–688.
- [4] F. Van der Touw, M. Mandel, Dielectric increment and dielectric dispersion of solutions containing simple charged linear macromolecules, *Biophys. Chem.* 2 (1974) 218–241.
- [5] M. Mandel, Dielectric properties of charged linear macromolecules with particular reference to DNA, *Ann. N.Y. Acad. Sci.* 303 (1977) 74–87.
- [6] G.S. Manning, The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides, *Q. Rev. Biophys.* 11 (1978) 179–246.
- [7] G.S. Manning, Limiting laws and counterion condensation in polyelectrolyte solutions V. Further development of the chemical model, *Biophys. Chem.* 9 (1978) 65–70.
- [8] C.F. Anderson, M.T. Record Jr., Polyelectrolyte theories and their applications to DNA, *Annu. Rev. Phys. Chem.* 33 (1982) 191–222.
- [9] M. Mandel, T. Odijk, Dielectric properties of polyelectrolyte solutions, *Annu. Rev. Phys. Chem.* 35 (1984) 75–108.
- [10] J.R. de Xammar Oro, J.R. Grigera, Dielectric properties of aqueous solutions of sonicated DNA above 40 MHz, *Biopolymers* 23 (1984) 1457–1463.
- [11] S. Takashima, C. Gabriel, R.J. Sheppard, E.H. Grant, Dielectric behaviour of DNA solution at radio and microwave frequencies (at 20°C), *Biophys. J.* 46 (1984) 29–34.
- [12] S. Takashima, *Electrical Properties of Biopolymers and Membranes*, Adam Hilger, Philadelphia, 1989.
- [13] C. Grosse, Microwave absorption of suspensions of DNA type particles in electrolyte solution, *Alta Freq.* 58 (1989) 365–368.
- [14] S. Mashimo, T. Umehara, S. Kuwabara, Dielectric study on dynamics and structure of water bound to DNA using a frequency range 10 MHz–10 GHz, *J. Phys. Chem.* 93 (1989) 4963–4967.
- [15] F. Pedone, A. Bonincontro, Temperature dependence of DNA dielectric dispersion at radio frequency, *Biochim. Biophys. Acta* 1073 (1991) 580–584.
- [16] L.M. Penafiel, T.A. Litovitz, High frequency dielectric dispersion of polyelectrolyte solutions and its relation to counterion condensation, *J. Chem. Phys.* 97 (1992) 559–567.
- [17] B. Saif, R.K. Mohr, C.J. Montrose, T.A. Litovitz, On the mechanism of dielectric relaxation in aqueous DNA solutions, *Biopolymers* 31 (1991) 1171–1180.
- [18] S. Bone, C.A. Small, Dielectric studies of ion fluctuation and chain bending in native DNA, *Biochim. Biophys. Acta* 1260 (1995) 85–93.
- [19] S. Bone, R.S. Lee, C.E. Hodgson, Dielectric studies of intermolecular interactions in native DNA, *Biochim. Biophys. Acta* 1306 (1996) 93–97.
- [20] U. Mohanty, Y. Zhao, Polarisation of counterions in polyelectrolytes, *Biopolymers* 38 (1996) 377–388.
- [21] D. Bakewell, J. Milner, H. Morgan, Characterisation of the dielectrophoretic movement of DNA in micro-fabricated structures, in: *Proc. Inst. Phys. 10th Int. Conf. Electrostatics*, Cambridge, 1999, pp. 73–76.
- [22] C.L. Asbury, G. van den Engh, Trapping of DNA in nonuniform electric fields, *Biophys. J.* 74 (1998) 1024–1030.
- [23] M. Washizu, O. Kurosawa, Electrostatic manipulation of DNA in microfabricated structures, *IEEE Trans. Ind. Appl.* 26 (1990) 1165–1172.
- [24] M. Washizu, S. Suzuki, O. Kurosawa, T. Nishizaka, T. Shinohara, Molecular dielectrophoresis of biopolymers, *IEEE Trans. Ind. Appl.* 30 (1994) 835–843.
- [25] M. Washizu, O. Kurosawa, A. Ichiro, S. Suzuki, N. Shimamoto, Applications of electrostatic stretch-and-positioning of DNA, *IEEE Trans. Ind. Appl.* 31 (1995) 447–456.
- [26] K. Morishima, T. Fukuda, F. Arai, H. Matsuura, K. Yoshikawa, Noncontact transportation of DNA molecule by dielectrophoretic

- force for micro DNA flow system, in: Proc. IEEE Int. Conf. on Robotics and Automation, Minnesota, April 1996, pp. 2214–2219.
- [27] H.A. Pohl, Dielectrophoresis, Cambridge University Press, Cambridge, 1978, Ch. 2.
- [28] E.H. Grant, R.J. Sheppard, G.P. South, Dielectric behaviour of biological molecules in solution, in: W.F. Harrington, A.R. Peacocke (Eds.), Monographs on Physical Biochemistry, OUP, 1978.
- [29] W.L. Gerlach, J.R. Bedbrook, Cloning and characterisation of ribosomal RNA genes from wheat and barley, *Nucleic Acids Res.* 7 (1979) 1869–1885.
- [30] J. Sambrook, E.F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
- [31] QIAGEN Plasmid purification handbook, January, 1997.
- [32] Yu.D. Feldman, A. Andrianov, E. Polygalov, I. Ermolina, G. Romanichev, Y. Zuev, B. Milgotin, Time domain dielectric spectroscopy: An advanced measuring system, *Rev. Sci. Instrum.* 67 (1996) 3208–3216.
- [33] Yu.D. Feldman, Yu.F. Zuev, E.A. Polygalov, V.D. Fedotov, Time domain dielectric spectroscopy. A new effective tool for physical chemistry investigation, *Colloid Polym. Sci.* 270 (1992) 768–780.
- [34] I.V. Ermolina, E.A. Polygalov, G.D. Romanichev, Yu.F. Zuev, Yu.D. Feldman, Time domain dielectric spectroscopy with non-uniform signal sampling, *Rev. Sci. Instrum.* 62 (1991) 2262–2265.
- [35] I.V. Ermolina, I.N. Ivoylov, V.D. Fedotov, Dielectric relaxation, molecular motion and inter-protein interactions in myoglobin solution, *J. Biomol. Struct. Dyn.* 15 (1997) 381–391.
- [36] A. Kamyshny, I. Ermolina, S. Magdassi, Yu. Feldman, Study of the dynamic structure of native and hydrophobized glucose oxidase by time-domain dielectric spectroscopy, *J. Phys. Chem. B* 104 (2000).
- [37] I.V. Ermolina, I.N. Ivoylov, V.D. Fedotov, Dielectric relaxation, molecular motion and interprotein interactions in myoglobin solution, *J. Biomol. Struct. Dyn.* 15 (1997) 381–391.
- [38] I.H. Segel, *Biochemical Calculations*, Wiley, London, 1976.
- [39] A.R. Von Hippel, *Dielectrics and Waves*, Wiley, London, 1954.
- [40] F. Van der Touw, J.W.H. Briede, M. Mandel, Electric permittivity of Alfalfa Mosaic virus in aqueous solutions, *Biopolymers* 12 (1973) 111–119.
- [41] S. Suzuki, T. Yamanashi, S. Tazawa, O. Kurosawa, M. Washizu, Quantitative analysis of DNA orientation in stationary AC electric field using fluorescence anisotropy, *IEEE Ind. Appl.* 2 (1995) 1374–1382.
- [42] S.B. Smith, A.J. Bendich, Electrophoretic charge density and persistence length of DNA as measured by fluorescence microscopy, *Biopolymers* 29 (1990) 1167–1173.
- [43] V.A. Bloomfield, D.M. Crothers, I. Tinoco, Jr., *Physical Chemistry of Nucleic Acids*, Harper and Row, New York, 1974.
- [44] P.J. Hagerman, Flexibility of DNA, *Annu. Rev. Biophys. Biophys. Chem.* 17 (1998) 265–286.
- [45] T. Odijk, Possible scaling relations for semidilute polyelectrolyte solutions, *Macromolecule* 12 (1979) 688–693.
- [46] R.S. Lee, S. Bone, Dielectric studies of chain melting and denaturation in native DNA, *Biochim. Biophys. Acta* 1397 (1998) 316–324.