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ELECTRIC PERMITTIVITY AND DIELECTRIC DISPERSION OF LOW-MOLECULAR WEIGHT DNA AT LOW IONIC STRENGTH *

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The dielectric properties of sonicated calf-thymus DNA ($M_W \approx 3 \times 10^5$ g mol^{-1}) have been investigated in a frequency range between a few kHz and 100 MHz. Two samples, sonicated in a different way were used after proper characterization including light-scattering, viscometry and contour length distribution by electron microscopy. Dielectric measurements were performed at several concentrations between 10^{-4} and 3×10^{-3} monomol l^{-1} and 22°C. Under all circumstances two separated dispersion regions were observed, the corresponding specific increments of which decreased with increasing concentration. The same was observed with the mean relaxation time of the high frequency dispersion. Both the frequency and concentration dependence was largely analogous to what is observed with other polyelectrolytes. Values of the dielectric parameters extrapolated to infinite dilution could also be interpreted in the same manner as for more simple, charged macromolecules and no specific effects had to be taken into account.

1. Introduction

Dielectric properties of aqueous solutions of charged macromolecules have been studied extensively in this laboratory in recent years [1–4]. Special methods have been developed to measure these properties in the frequency range between a few kHz and 100 MHz insuring adequate correction for parasitic electrode effects in the lower frequency region. In most cases simple, synthetic polyelectrolytes were investigated. We have now extended our study to DNA as conflicting mechanisms have been proposed so far in the literature to explain its dielectric behaviour and no systematic study has been performed covering the two dispersion regions observed separately by different investigators: one in the kHz-region or below, the other in the MHz region.

Preliminary measurements [5] have shown that commercially available calf-thymus DNA exhibits the two dispersions mentioned but with a lower critical frequency falling outside the frequency range of the experimental equipment available. Therefore sonicated DNA of lower molar mass (approximately 3×10^5 g mol^{-1}) was used in the present investigation. From the study of synthetic polyelectrolytes it has been found that the mean relaxation time of the low-frequency dispersion increases with molecular weight. It was therefore inferred that the sonicated DNA would be more suitable for investigation in the frequency range covered, as was found indeed.

We shall present the results obtained with two different samples of sonicated calf-thymus DNA which were properly characterized by light-scattering, viscometry and electron microscopy. We shall demonstrate that the dielectric behaviour of this low-molecular weight DNA is substantially analogous to that of other polyelectrolytes studied and that it can be interpreted in the same way without making use of effects specific for the biopolymer.

* Dedicated to Professor E. Havinga at the occasion of his retirement from the chair of organic chemistry at the University of Leiden.

2. Materials and methods

2.1. Preparation of DNA solutions

Calf-thymus DNA (sodium salt) from Worthinton Biochemical Co. was used without further purification.

The protein content, determined according to Lowry's method [6] was less than 0.9%. Stock solutions were prepared by allowing a few hundred milligrams of DNA to swell in 20–40 cm^3 of deionized water for about 24 hours, followed by dilution to a volume of a few hundred cm^3 under stirring until complete clarification and exhaustive dialysis against deionized water. Phosphor analysis according to standard methods [7,8] and extinction measurements yielded an extinction coefficient of 6440 ± 70 monomol l^{-1} at 269 nm in fair agreement with the literature value [9–11] of 6500. The concentration of the standard solutions were never less than 10^{-3} monomol l^{-1}. At this concentration no significant change of the extinction coefficient was found if NaCl was added up to a concentration of 0.2 M indicating absence of denaturation at 10^{-3} monomol l^{-1} or higher in water.

Solutions of smaller concentrations were always prepared by volumetric dilution. Stock solutions were kept frozen (–20°C); solutions prepared therefrom were stored at 4°C but never longer than a few days.

The molar mass of the original samples were determined viscosimetrically using the Mark-Houwink relation given by Crothers and Zimm [12]; it varied from sample to sample with an average value of $(5 \pm 1) \times 10^6$ g mol^{-1}.

Samples of smaller molar mass were prepared by sonication with a MEL ultrasonic process equipment with a titanium probe at a frequency of 21 kHz. In each run 40 cm^3 solution of 10^{-3} monomol l^{-1} Na-DNA were treated. Sonication was performed during approximately 9 minutes in two different solvents, viz. H_2O (DNA I) and in 0.2 M NaCl, 0.002 M EDTA, 0.002 M Na_2HPO_4 buffer (DNA II). The degradation was found from viscosity measurements to reach a stationary state around this time interval. No fractionation of the sonicated samples was performed; the polydispersity was checked by electron microscopy.

2.2. Other materials

All chemicals used were of analytical reagent grade. Deionized water was prepared using a mixed bed ion-exchanger (Amberlite MB-I); its specific conductivity was always smaller than 10^{-4} Ω^{-1} m^{-1}.

2.3. Light scattering

Conventional light scattering was performed at 25°C with a FICA 50 instrument and cylindrical cells at 436 nm with unpolarized light. DNA solutions in the same buffer as used in the preparation of DNA II were measured at several angles between 30 and 150 degrees; the DNA concentrations varied between 10^{-4} and 1.5×10^{-3} monomol l^{-1}. For all measurements the solution of the highest concentration was exhaustively dialized against the buffer; dilutions were performed with the same. All DNA solutions were filtered through Millipore GSTF filters (0.22 μm) immediately before measurements; concentrations were determined afterwards by extinction measurements at 260 nm.

Calibration was performed with benzene of analytical grade dried with molecular sieve (0.4 nm) and also filtered over Millipore filters. For the Rayleigh ratio of benzene at 90 degrees, Coumou's value [13] $R_{B,90} = 4.56 \times 10^{-5}$ cm^{-1} was used although it is given at 23°C. According to Kratohvil et al. [13] the temperature coefficient of R_{90} is approximately 0.5%/°C only and the total change of $R_{B,90}$ from 23 to 25°C falls within the uncertainty about the absolute value of this ratio [13].

The refractive index increments at 436 nm and 25°C were determined with a Brice-Phoenix differential refractometer using for each concentration of DNA a solution exhaustively dialyzed against the buffer. Equilibrium was reached only after several days. Concentrations of DNA were determined by extinction measurements after dialysis. In order to obtain (dn/dC) with sufficient accuracy, higher concentrations than used in the scattering experiments were necessary (up to 1.5×10^{-2} monomol l^{-1}). The refractive index increment was found to be (0.166 ± 0.003) ml g^{-1}, the same value as found recently by Godfrey and Eisenberg [14].

From the light scattering measurements at 25°C and 436 nm the weight average molar mass $\bar{M}^w$ and the z-average mean square radius of gyration $\langle S^2 \rangle^z$ was found by the usual procedure. The scattering function $P^{-1}(\theta)$ appeared to be linear against $\sin^2(\theta/2)$ up to 120°. The differences between the molar masses obtained by the two extrapolations in the Zimm procedure (using a unweighted least-squares fit) were in all cases smaller than the standard

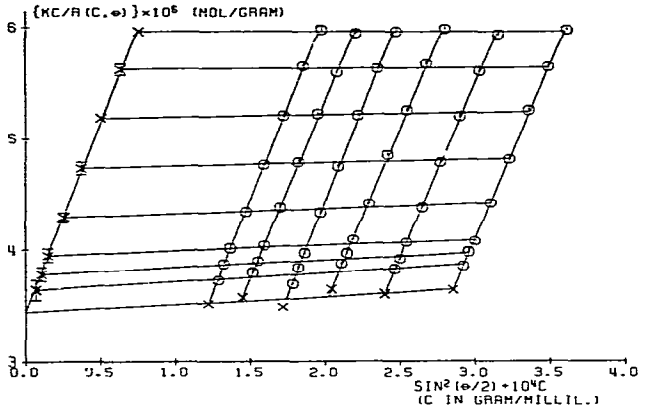

Fig. 1. A typical Zimm-diagram for DNA II in 0.2 M NaCl, 0.002 M EDTA, 0.002 M Na_2HPO_4; o: experimental values, X: extrapolated values.

deviation in each of them. A typical Zimm-plot is shown in fig. 1. For each sample of DNA at least four independent scattering measurements were performed; the molecular parameters were found to agree within their standard deviations so that average values could easily be calculated. These are quoted in table 1.

2.4. Viscometry

The specific viscosities η_{sp} of both DNA I and DNA II were measured at five concentrations between 8×10^{-4} and 3×10^{-3} monomol l^{-1} in 0.2 M NaCl, 0.002 M EDTA, 0.002 M Na_2HPO_4 buffer at 25°C with a Beckmann low-shear rotating inner cylinder viscometer, originally designed by Zimm and Crothers. The shear rate in water in this viscometer is 0.13 s^{-1} and the average shear stress in the solution 7×10^{-2} N m^{-2}, independent of the viscosity. The intrinsic viscosities were obtained by linear extrapolation of the η_{sp}/C using the method of unweighted least squares. A value of $\bar{M}^w$ was calculated from the $[\eta] - \bar{M}^w$ relation given by Godfrey [11]

$$[\eta] + 1.2 = (0.515 \pm 0.013) \times 10^{-4} (\bar{M}^w)^{0.867},$$

where $[\eta]$ is expressed in dl g^{-1}.

2.5. Electron microscopy

Films for electron microscopy have been prepared

Table 1
Molecular parameters of DNA as determined by different techniques at 0.2 M NaCl

		DNA I	DNA II
Light scattering			
$\bar{M}^w \times 10^{-5}$	(g mol^{-1})	3.53 ± 0.08	3.1 ± 0.2
$[\langle S^2 \rangle^z]^{1/2}$	(nm)	47 ± 1	46 ± 2
Viscosimetry			
$[\eta]$	(monomol^{-1} l)	73 ± 4	55 ± 2
$\bar{M}^w \times 10^{-5}$	(g mol^{-1})	3.6 ± 0.2	3.0 ± 0.1
Electron microscopy			
$\bar{L}_c^w$	(nm)	194	167
M_L	(g mol^{-1} nm^{-1})	1820 ± 40	1850 ± 120
Calculated values for wormlike chain [a]			
$[\langle S^2 \rangle^z]^{1/2}$	(nm)	51 ± 3	42 ± 2
$[\langle S^2 \rangle^w]^{1/2}$	(nm)	44 ± 2	38 ± 2
$[\eta]$ (d = 1.25 nm)	(monomol^{-1} l)	78 ± 7	62 ± 6
$[\eta]$ (d = 2.50 nm)	(monomol^{-1} l)	95 ± 9	76 ± 7

a) Calculated for a persistence length a = 55 ± 10 nm and a distribution of molecular weights as determined by electron microscopy (see text).

by the method of spontaneous adsorption as described by Lang and Mitani [15] using a solution of DNA concentration of 0.1 μg ml^{-1} in 0.5 M ammonium acetate, 0.001 EDTA, 0.07 formaldehyde and 2 μg ml^{-1} cytochrome C. DNA films, transferred to grids and stained with uranyl acetate were photographed in a Philips EM 2000 electron microscope (at the Biochemical Laboratory, University of Leiden) with a magnification of approximately 5000. The negatives were enlarged about 20 times by projection on paper and the images copied. The total magnification was determined with a calibration grid with a known number of lines per cm, photographed and projected under the same conditions as the films. The length of DNA molecules was determined from the projected images using a curvimeter with a millimeter division. The accuracy of the length determination of these projections was estimated to be 1 mm corresponding to an accuracy of approximately 10 nm in the length of the molecules.

2.6. Dielectric measurements

For the determination of electric permittivities two measuring devices were used, one for the frequency range, 2.5–500 kHz and the other for the frequency range 1–100 MHz. In the low-frequency range a Schering type bridge originally described by Mandel and Jung [16] but later modified to improve the sensitivity [17] was used in connection with a Rhode and Schwarz RC generator (type SRB) and a Rhode und Schwarz tunable amplifier (type UBM) used as a detector. The measuring cell especially designed for the determination of electric permittivities ϵ' of highly conducting solutions in that Hz region has been described by Van der Touw et al. [18–20]; it contains two parallel, circular bright platinum electrodes of variable spacing d. The value of d is determined with respect to standards of calibrated thickness (of an accuracy better than one micron). For all electric permittivity determinations at a given frequency ten fixed distances are used. At frequencies above 10 kHz where the contribution to the measured capacitance due to electrode effects is relatively small the electric permittivity could be obtained with an accuracy of 0.2–0.3. At lower frequencies where the electrode effects become more important the inaccuracy on ϵ' increases depending on the specific conductivity. Values of the permittivity were considered unreliable if the inaccuracy was found to be larger than 1.5. Such values were not used thus limiting the frequency range at the lower end to different frequency values according to the specific conductivity.

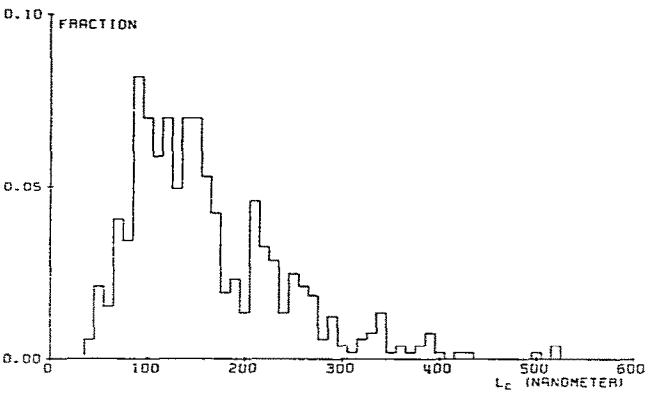

Fig. 2. Histogram for the distribution of the contour lengths L_c in DNA I as determined from electron microscopy.

In the megahertz region the Boonton Radio Company RX meter (type 250-A) has been used. Its application in the case of conducting solutions has been extensively investigated by Van Beek et al. [21]. It was used with a coaxial cell with variable height of liquid also described in the same paper. Heights smaller than 5 mm were used at 0.5 mm intervals. Measurements using standard KCl solutions with concentrations between 5×10^{-4} and 2×10^{-3} M yielded a constant value of ϵ' over the whole frequency range ensuring that correction for electrode effects may be neglected. With this meter and cell electric permittivities could be determined with an accuracy of 0.3 between 2 and 100 MHz and an accuracy of 0.5 between 1 and 2 MHz for the solutions considered.

Both methods also yield the specific conductance κ of the solutions investigated as a function of frequency. In the low frequency range up to 500 kHz, no significant increase with respect to the dc-component κ_0 could be detected so that the imaginary part ϵ'' of the complex electric permittivity could not be determined. In the high frequency region κ was found to increase with frequency and ϵ'' could be determined according to the equation

$$\epsilon'' = (\kappa - \kappa_0)/\omega, \qquad (2)$$

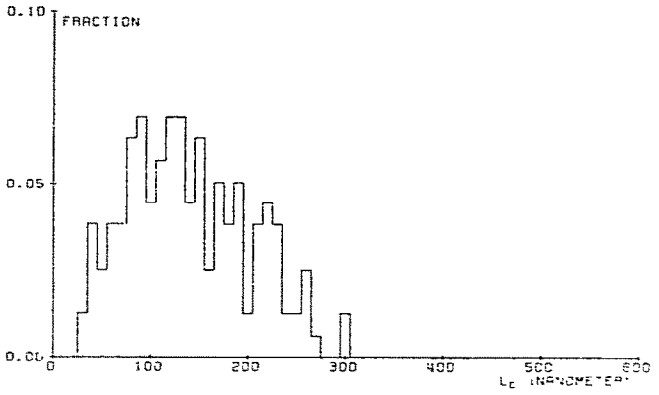

Fig. 3. Histogram for the distribution of the contour lengths L_c in DNA II as determined from electron microscopy.

where ω is the circular frequency.

During measurements on a given solution at 22°C, temperature fluctuations in both cells used could be kept within 0.03°C.

3. Characterization of the DNA samples

In table 1 we have collected the results obtained from light scattering measurements, viscometry and electron microscopy for DNA I and DNA II respectively. $\bar{M}^w$ from viscosimetry was obtained with the help of (1). The value of the weight average contour length $\bar{L}_c^w$ was obtained from the histograms representing the distribution of contour lengths from electron microscopy photographs (figs. 2 and 3). From such a distribution different average values of a quantity X which depends on the contour length or molar mass can be calculated from

$$\bar{X}^\gamma = \sum_{(i)} p_i L_{c,i}^{\gamma-1} X_i \Big/ \sum_{(i)} p_i L_{c,i}^{\gamma-1}, \qquad (3)$$

where p_i is the fraction of molecules with contour length $L_{c,i}$ and $\gamma = 1,2,3$ corresponds to the number (n), weight (w) or z-average values respectively. For $X_i = L_{c,i}$ and $\gamma = 2$, $\bar{L}_c^w$ is obtained from (3). It can be seen that for both samples the values for $\bar{M}^w$ obtained from light scattering and viscometry are in good agreement. Combination of $\bar{L}_c^w$ and $\bar{M}^w$ gives the value of mass per unit length M_L. In 0.2 M sodium salt the value of 1950 g mol^{-1} nm^{-1}, found for the crystalline B-form, is commonly accepted and has recently been found indeed by Yamakawa and Fujii from their analysis of sedimentation and viscosity data from the literature using theoretical expressions for a wormlike chain [22,23]. The values of M_L found presently (see table 1) are only slightly smaller than 1950 g mol^{-1} nm^{-1}. The difference might be due to the handling of the sample in the electron microscopy experiments. Cohen and Eisenberg [24] and Godfrey and Eisenberg [14] also found some discrepancies in their estimates of M_L for sonicated DNA with respect to the accepted value, although their values turned out to be somewhat larger.

Since the wormlike chain model is believed to be the best representation of a DNA molecule [14], the mean square radius of gyration $\langle S^2 \rangle$ can be calculated according to the equation of Benoit and Doty [25].

$$\langle S^2 \rangle = \frac{L_c^2}{x^2}\left[\frac{x}{3} - 1 + \frac{2}{x} - \frac{2}{x^2}(1 - e^{-x})\right], \qquad (4)$$

where $x = L_c/a$ and a represents the persistence length. Values of $\langle S^2 \rangle^z$ have been calculated for DNA I and DNA II for the distribution of contour lengths as found from electron microscopy with (3) and (4) using a persistence length $a = (55 \pm 10)$ nm according to recent literature data. These values are found to be in fair agreement with the radius of gyration data from light scattering (see table 1).

We have also compared our intrinsic viscosities with theoretical values of $[\eta]$ (in unit volume per monomol) for wormlike chains according to Yamakawa and Fujii [23]

$$[\eta] = L_c^{3/2} P^{-1} \Phi(L_\tau, d_\tau). \qquad (5)$$

Here P is the degree of polymerization and $\Phi(L_\tau, d_\tau)$ (expressed in macromol^{-1}) represents the viscosity function for a wormlike chain as function of the relative contour length $|L_\tau| = L_c(2a)^{-1}$ and relative diameter $d_\tau = d(2a)^{-1}$ with respect to Kuhn's statistical segment length $2a$, for which Yamakawa and Fujii gave tabulated values and approximate analytical expressions. In principle with the help of (5) the intrinsic viscosity may be calculated taking into account the distribution of contour lengths

$$\overline{[\eta]} = \lim_{C \to 0} \frac{\Sigma_{(i)}[\eta]_{sp,i}}{C} = \frac{\Sigma_{(i)} C_i [\eta]_i}{\Sigma_{(i)} C_i} = \frac{\Sigma_{(i)} P_i L_{c,i} [\eta]_i}{\Sigma_{(i)} P_i L_{c,i}} \qquad (6)$$

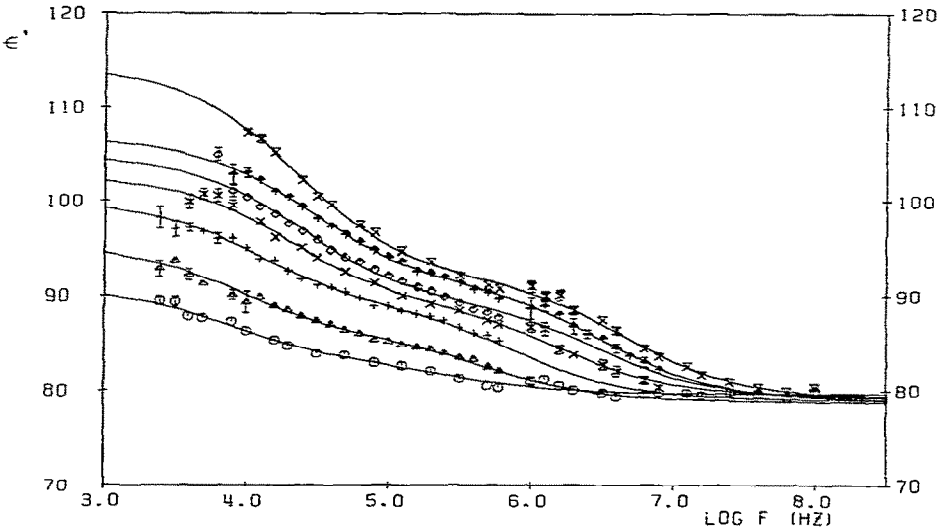

Fig. 4. Frequency dependence of the electric permittivity of DNA I in H_2O. The concentrations are 1.0×10^{-4} (○), 2.5×10^{-4} (△), 5×10^{-4} (+), 7.5×10^{-4} (×), 1.5×10^{-4} (◇), 2.00×10^{-3} (✢) and 2.88×10^{-3} (✕) monomol l^{-1}. For clarity many high-frequency data and duplicate measurements have been omitted.

Here it has been assumed that no interaction between different particles occurs. In order to calculate $[\eta]_i$ according to (5) the value of the hydrodynamic diameter has to be known. Yamakawa and Fujii [23] have estimated $d = 2.5$ nm whereas Godfrey and Eisenberg [14] proposed $d = 1.2$ nm. We have calculated $[\eta]$ with (5) and (6) using two different values of d, viz. 1.25 nm and 2.50 nm. The values calculated

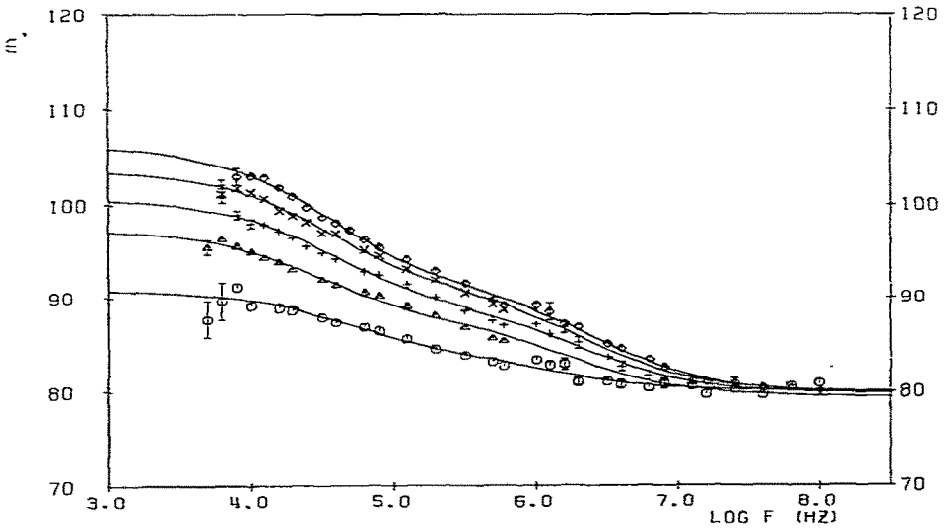

Fig. 5. Frequency dependence of the electric permittivity of DNA II in 3×10^{-4} M NaCl. The DNA concentrations are 3.0×10^{-4} (○), 6.0×10^{-4} (△), 9.0×10^{-4} (+), 1.20×10^{-3} (×) and 1.50×10^{-3} (◇) monomol l^{-1}. For clarity many high-frequency data have been omitted.

Table 2
Dielectric parameters for DNA as obtained by fit of experimental data to the Cole-Cole equation (6)

$C \times 10^3$ (monomol l^{-1})	ϵ_∞	$\Delta\epsilon_s$	$\tau_1 \times 10^6$ (s)	$\Delta\epsilon_2$	$\tau_2 \times 10^6$ (s)
DNA I/H_2O					
0.10	79.2 ± 0.1	5.4 ± 0.8	17 ± 9	2.5 ± 0.2	0.25 ± 0.7
0.10	79.7 ± 0.2	11 ± 2	20 ± 10	3.3 ± 0.8	0.5 ± 0.3
0.25	78.8 ± 0.1	17 ± 2	17 ± 6	6.8 ± 0.4	0.31 ± 0.06
0.50	79.0 ± 0.2	21 ± 2	13 ± 3	9.9 ± 0.3	0.19 ± 0.02
0.50	79.2 ± 0.2	26 ± 1	16 ± 3	9.4 ± 0.4	0.16 ± 0.02
0.75	79.4 ± 0.3	23 ± 2	8 ± 2	9.8 ± 0.4	0.10 ± 0.01
1.00	79.5 ± 0.3	33 ± 3	15 ± 3	9.1 ± 0.4	0.10 ± 0.01
1.00	79.3 ± 0.2	26 ± 1	9 ± 2	11.0 ± 0.3	0.08 ± 0.01
1.50	79.1 ± 0.3	26 ± 2	7 ± 1	10.3 ± 0.3	0.0051 ± 0.005
1.50	79.1 ± 0.3	32 ± 2	10 ± 2	10.9 ± 0.3	0.0055 ± 0.007
2.00	78.9 ± 0.2	27.9 ± 0.9	3.7 ± 0.6	12.3 ± 0.2	0.047 ± 0.002
2.88	79.1 ± 0.4	35 ± 3	7 ± 2	12.6 ± 0.4	0.033 ± 0.004
DNA II/3 × 10^{-4} M NaCl					
0.3	80.0 ± 0.3	11 ± 1	3 ± 1	3.7 ± 0.8	0.12 ± 0.006
0.6	79.4 ± 0.3	18 ± 1	6 ± 2	8.1 ± 0.6	0.09 ± 0.02
0.9	79.7 ± 0.3	21 ± 1	5 ± 1	9.1 ± 0.6	0.07 ± 0.01
1.2	79.8 ± 0.3	24.0 ± 0.9	4.6 ± 0.7	10.9 ± 0.4	0.071 ± 0.008
1.5	79.7 ± 0.3	27 ± 1	4.3 ± 0.6	11.1 ± 0.4	0.058 ± 0.006

with the former are in very good agreement with our experimental data (see table 1) whereas the latter yields intrinsic viscosities which are appreciably higher.

The consistency between the different data obtained for both samples thus shows that these samples have been properly characterized and that our sonicated DNA at infinite dilution in the buffer can be described as a wormlike chain. The difference in the molecular parameters between DNA I and DNA II can be completely attributed to the difference in the distribution of contour lengths or molar mass, the former exhibiting a broader distribution than the latter.

4. Dielectric results

The electric permittivities ϵ' of DNA I in H_2O and DNA II in 3×10^{-4} M NaCl were measured as function of the frequency in the concentration range 10^{-4}–3×10^{-3} monomol l^{-1} and 3×10^{-4}–1.5×10^{-3} monomol l^{-1} respectively. All measurements were performed at 22°C in a frequency range with a lower limit between 2.5 and 10 kHz depending on the conductance of the solution and a upper limit of 100 MHz. The results are represented in figs. 4 and 5. In all cases, at the lowest frequency attainable no constant value of ϵ' was reached yet whereas at the highest frequency all values converged to the static electric permittivity of the pure solvent ($\epsilon_s^0 = 79.7$) thus indicating the absence of specific solvent effects.

From the variation of ϵ' with the frequency it appeared that in general two dispersion regions can be distinguished. For each concentration the frequency dependence could be fitted with a weighted least-squares method to a function representing a superposition of two Cole-Cole equations [26] (shown in figs. 4 and 5 by the drawn curves), in agreement with what is found generally for polyelectrolytes.

$$\epsilon' = \epsilon_\infty + \tfrac{1}{2} \sum_{k=1,2} \Delta\epsilon_k \left[1 - \frac{\sinh\beta_k (\ln f - \ln f_{c,k})}{\cosh\beta_k (\ln f - \ln f_{c,k}) + \cos\tfrac{1}{2}\beta_k} \right] \quad (6a)$$

$$\Delta\epsilon_1 = \epsilon_s - \epsilon_2, \qquad \Delta\epsilon_2 = \epsilon_2 - \epsilon_\infty. \quad (6b)$$

Here f is the frequency, ϵ_∞ the extrapolated value at

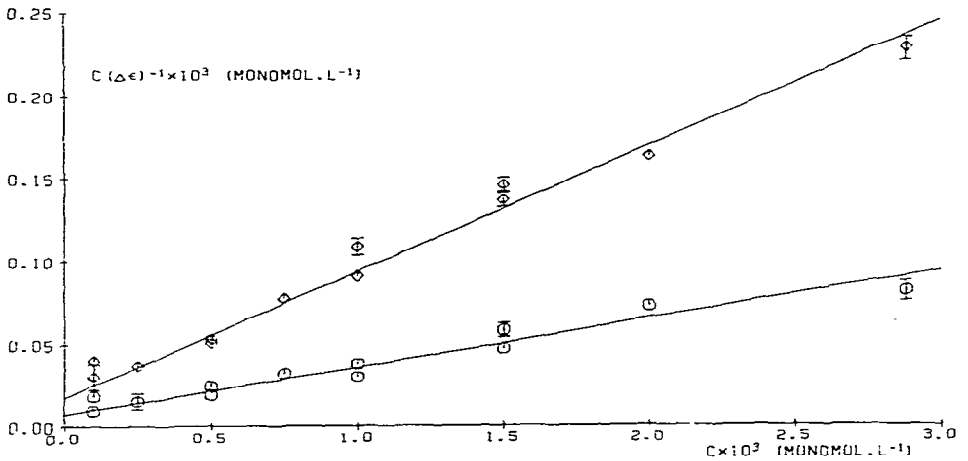

Fig. 6. Concentration dependence of the reciprocal specific increments of DNA I in H_2O; ○: $c(\Delta\epsilon_s)^{-1}$, ◇: $c(\Delta\epsilon_2)^{-1}$.

the high frequency end, $\Delta\epsilon_k$ the amplitude of the kth dispersion region, ϵ_s the extrapolated value at the low frequency end representing the static electric permittivity of the solution, $f_{c,k}$ the critical frequency of the kth dispersion region and β_k the Cole-Cole parameter of this region ($0 < \beta_k < 1$). Note that by equating ϵ_s to the static electric permittivity we assume that no third dispersion may occur at much lower frequencies than investigated here. Note also that $\Delta\epsilon_s = \epsilon_s - \epsilon_\infty = \Delta\epsilon_1 + \Delta\epsilon_2$.

From the least-squares fitting procedure it was found that no correlation was apparent between changes in β_k and variation of the concentration and the nature of the DNA system. The value of β_k seems to oscillate more or less randomly around 0.75 within its standard deviation. Therefore, in order to reduce the computer time a fitting procedure with five unknown parameters was used assuming $\beta_1 = \beta_2 = 0.75$. A six parameters fit with an undefined value of $\beta = \beta_1 = \beta_2$ was checked not to yield significantly better results.

In the fitting procedure weight factors were calculated from the standard deviations in ϵ' as obtained at each frequency. Occasionally for some points the standard deviation turned out to be rather small. As a consequency the corresponding weight factor for such a point could be exaggeratingly large as compared to the others. Therefore a lower limit on the standard deviation of ϵ' was used, estimated from a large number of measurements to be 0.25.

The values of the five parameters together with their standard deviation as obtained by the weighted fitting procedure have been collected in table 2 for the two Na-DNA samples at the different concentrations investigated. Here $\tau_k \equiv (2\pi f_{c,k})^{-1}$ represents the mean relaxation time of the kth dispersion region. Note that the reproducibility of the parameters referring to the low frequency dispersion is rather poor due to the lack of experimental points at the lower frequency end. However in all cases $f_{c,1}$ was found to ly within the frequency range explored.

From table 2 it can be seen that the specific increments $\Delta\epsilon_s/C$ and $\Delta\epsilon_2/C$ as well as τ_2 appear to be strongly concentration dependent. As no theoretical expression for this concentration dependence has been presented so far, the empirical equation proposed by Van der Touw and Mandel [1] was applied to these data. For the specific increments this empirical relation found to hold for many polyelectrolytes [1,2,4] is given by

$$\Delta\epsilon_i/C = (\Delta\epsilon_i/C)_0/(1 + B_i C), \qquad i = s, 2 \qquad (7)$$

where $(\Delta\epsilon_i/C)_0$ is the ith increment extrapolated to infinite dilution, B_i is an interaction parameter and C the concentration of the polyelectrolyte (in monomol l^{-1}). For both DNA I and DNA II in H_2O and 3×10^{-4} M NaCl respectively eq. (7) seems to represent the data in a satisfactory way, as can be seen from figs. 6 and 7. The corresponding extrapolated specific increments and interaction parameters were calculated

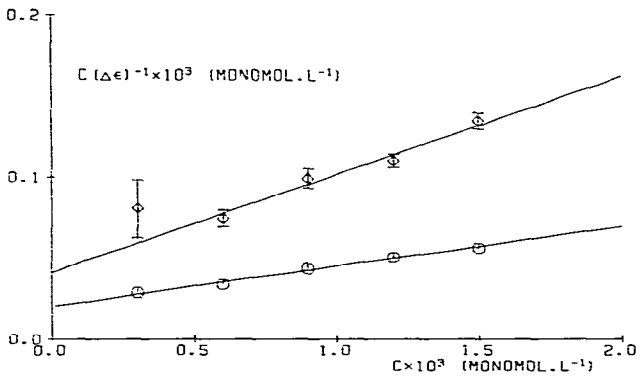

Fig. 7. Concentration dependence of the reciprocal specific increments of DNA II in 3×10^{-4} M NaCl; ○: $C(\Delta\epsilon_s)^{-1}$, ◇: $C(\Delta\epsilon_2)^{-1}$.

by weighted least-squares fitting of $C(\Delta\epsilon_i)^{-1}$ versus C, using weight factors determined from the standard deviations in $\Delta\epsilon_i$ as estimated from the least-squares fit of the dispersion curve with a lower limit of 1.5 for $\Delta\epsilon_s$ and 0.5 for $\Delta\epsilon_2$. They are presented in table 3.

The values of the interaction parameters in DNA I/H_2O are rather high compared to other systems [1]; this means that the concentration dependence of the specific increments is more pronounced. The origin of this is not clear yet. The decrease of B_i upon addition of a small amount of salt is probably due to screening of the polyion-polyion interactions.

It was found for DNA II in 3×10^{-4} M NaCl that no change in extinction coefficient occurred over the concentration range investigated. Therefore it can safely be assumed that no denaturation has taken place, the double helical structure being stabilized by the salt. For DNA I/H_2O an increase of the extinction coefficient was detected at the smallest concentrations (15% at 1×10^{-4}, 10% at 2.5×10^{-4} and 5% at 5.0×10^{-4} monomol l^{-1} at 260 nm). This indicates an offset of denaturation. No qualitatively different concentration dependence of ϵ' has been found for DNA I/H_2O as compared to DNA II/3×10^{-4} M NaCl however. Apparently a slight denaturation does not affect the dielectric properties in an appreciable way.

The concentration dependence of τ_2 for both DNA samples was found to obey the empirical equation [1]

$$\tau_2 = (\tau_2)_0/(1 + B'_2 C) \tag{8}$$

as can be seen from figs. 8 and 9. The extrapolated values $(\tau_2)_0$ and interaction parameters B'_2 are also collected in table 3. In as far as the mean relaxation time of the low frequency dispersion region is concerned, no definite conclusions can be reached about its concentration dependence. Although a slight decrease of τ_1 with concentration according to an equation analogous to (8), as found with other polyelectrolytes, cannot altogether be excluded, τ_1 may as well be assumed to remain constant for both samples within the concentration range investigated.

As stated above, it is only possible to determine ϵ'' in the high frequency dispersion region as at lower frequencies the specific conductance is determined entirely by its d.c.-value κ_0. The values of ϵ'' in the high frequency region should obey a Cole-Cole function given by

$$\epsilon'' = \Delta\epsilon_2 \frac{\frac{1}{2}\sin\frac{1}{2}\pi\beta_2}{\cosh\beta_2(\ln f - \ln f_{c,2}) + \cos\frac{1}{2}\pi\beta}. \tag{9}$$

However the values of ϵ'' derived from the specific conductance were too inaccurate to allow application of a fitting procedure analogous to the one used for ϵ'. Therefore in order to check the consistency between

Table 3
Dielectric parameters extrapolated to infinite dilution and interaction parameters according to (7) and (8) for Na-DNA

			DNA I/H_2O	DNA II/3×10^{-4} M NaCl
$(\Delta\epsilon_S/C)_0$	$\times 10^{-3}$	(monomol^{-1} l)	140 ± 30	51 ± 9
B_S	$\times 10^{-3}$	(monomol^{-1} l)	4 ± 1	1.3 ± 0.3
$(\Delta\epsilon_2/C)_0$	$\times 10^{-3}$	(monomol^{-1} l)	60 ± 10	25 ± 5
B_2	$\times 10^{-3}$	(monomol^{-1} l)	4.4 ± 0.9	1.5 ± 0.5
$(\tau_2)_0$	$\times 10^{-6}$	(s)	1.3 ± 0.9	0.15 ± 0.07
B'_2	$\times 10^{-3}$	(monomol^{-1} l)	14 ± 10	1.0 ± 0.9

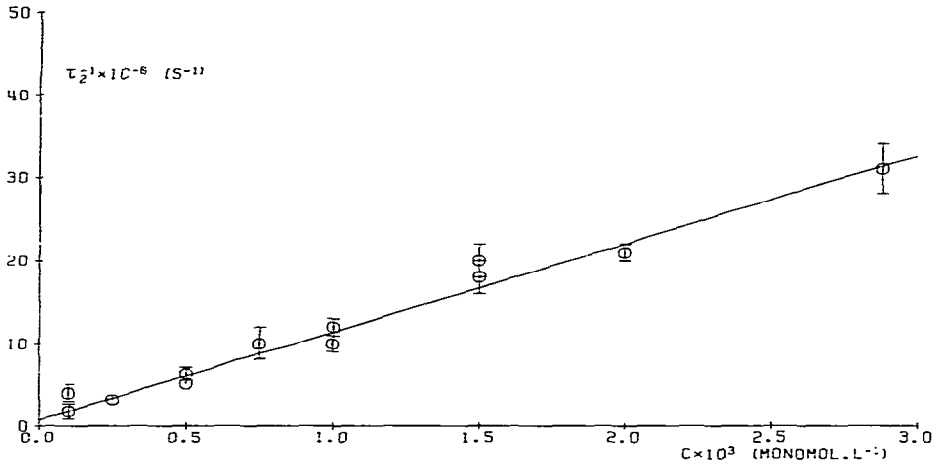

Fig. 8. Concentration dependence of τ_2^{-1} of DNA I in H_2O.

ϵ' and κ values, the latter were calculated according to the following equation obtained from (2) and (9)

$$\kappa(\omega) = \kappa_0 + \tfrac{1}{2}\frac{\Delta\epsilon_2}{\tau_2} \times \frac{\exp(\ln f - \ln f_{c,2}) \sin \tfrac{1}{2}\pi\beta_c}{\cosh \beta_2(\ln f - \ln f_{c,2}) + \cos \tfrac{1}{2}\pi\beta_2}, \qquad (10)$$

using the experimental values of κ_0 and the parameters found from electric permittivity for the high frequency dispersion. They yielded for the two DNA samples investigated the drawn curves in figs. 10 and 11 which compare satisfactorily with the experimental specific conductance data.

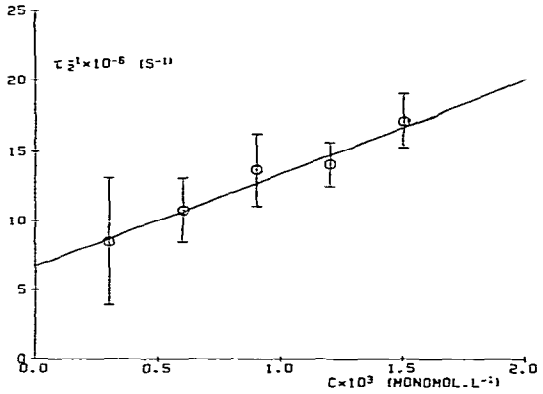

Fig. 9. Concentration dependence of τ_2^{-1} of DNA II in 3×10^{-4} M NaCl.

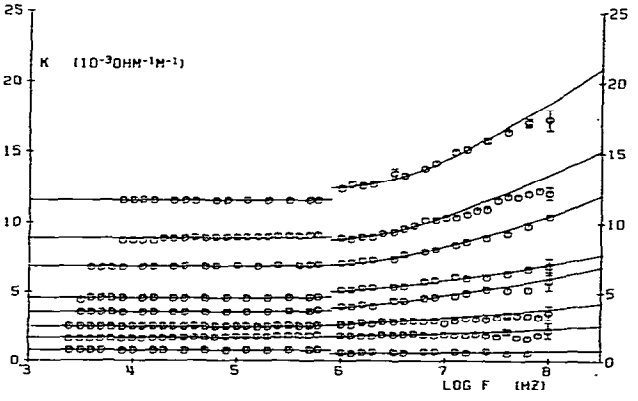

Fig. 10. Frequency dependence of the specific conductance κ of DNA I in H_2O. The concentrations are from bottom to top: 1.0×10^{-4}, 2.5×10^{-4}, 5×10^{-4}, 7.5×10^{-4}, 1.00×10^{-3}, 1.50×10^{-3}, 2.00×10^{-3} and 2.88×10^{-3} monomol l^{-1}. The points represent experimental values, the drawn curves are calculated from (10) (see text).

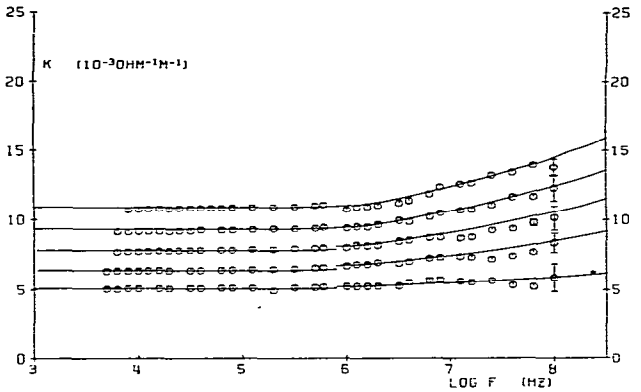

Fig. 11. Frequency dependence of the specific conductance κ of DNA II in 3×10^{-4} M NaCl. The concentrations are from bottom to top: 3.0×10^{-4}, 6.0×10^{-4}, 9.0×10^{-4}, 1.20×10^{-3} and 1.50×10^{-3} monomol^{-1} l. The points represent experimental data, the drawn curves are calculated from (10) (see text).

5. Discussion of the dielectric results

The electric permittivity results for DNA show the same qualitative behaviour as found for other polyelectrolytes in aqueous solution [1,2,4,5]. Two dispersion regions are observed and as shown previously only the parameters of the low frequency dispersion are molecular weight dependent. Both $\Delta\epsilon_s$ and $\Delta\epsilon_2$ as well as τ_2 exhibit the same concentration dependence as other polyelectrolytes; only for τ_1 this seems to be somewhat different. It is therefore useful to see whether the theoretical expressions for the dielectric increments of polyelectrolyte solutions, derived by van der Touw and Mandel [27] and which have been applied succesfully to several polyelectrolyte systems [1—4] can be used for DNA too. According to this theory a somewhat flexible polyion can represented by a sequency of identical rigid rodlike subunits of uniform charge density. Such a polyion is surrounded by a fraction of its total number of counterions which are closely associated or "domain-bound" to the polyion, i.e. which are assumed to be still mobile in the direction of the chain but bound in the radial direction. In the absence of an electric field by hypothesis these counterions are characterized by an uniform distribution along the polyion contour thus giving rise to no dipole moment. In the presence of an electric field this distribution is slightly perturbed and determines an induced dipole moment. The relaxation of this dipole moment is assumed to occur on two different time-scales; on the shorter one the distribution along the different subunits becomes uniform but the number of counterions associated which each subunit may still differ because in the model these subunits are supposed to be separated by potential barriers opposing the diffusion of the counterions. On the longer time-scale the distribution of the counterions along the complete polyelectrolyte chain then tends to become uniform, the counterions being able to overcome these barriers. The former mechanism determines the high frequency dispersion, the latter the low frequency one in combination with a contribution of the rotational relaxation. Van der Touw and Mandel derived for the two increments of a sufficiently diluted solution of a monodisperse sample of polyions, neglecting end effects of the chains and interactions between the counterions as well as contributions of permanent and conventionally defined induced dipole moments of the macromolecule, the following expressions

$$\Delta\epsilon_s = \sigma\bar{f}N(ze)^2 C_M (12R_g^2 + b^2)/36\epsilon_0 kT \qquad (11)$$

$$\Delta\epsilon_2 = \sigma\bar{f}N(ze)^2 C_M b^2/36\epsilon_0 kT \qquad (12)$$

Here σ represents the effective field correction factor (expressing the ratio of the magnitude of the effective field acting on the associated counterions to the Maxwell field), $\bar{f}$ the average fraction of associated counterions, N the total number of counterions per polyion, ze the charge of the counterions, C_M the number of polyions per m^3, ϵ_0 the absolute electric permittivity of vacuum, k Boltzmann's constant, T absolute temperature and b the length of a rigid rodlike subunit (in m). R_g is defined in terms of the distances q_i of each of the n subunits of the center of mass of the polyion by the equation

$$R_g = \left[\frac{1}{n}\sum_{(k)} q_k^2\right]^{1/2}. \qquad (13)$$

It is related [28] to the conventionally defined radius of gyration $\langle S^2\rangle^{1/2}$ by $12\langle S^2\rangle = 12R_g^2 + b^2$ if the centers of mass of the monomeric units are uniformly distributed along the subunits and the distance between subsequent monomeric units is much smaller than the length of a subunit. Therefore eq. (11) can be rewritten in the case of monovalent counterions

$$\Delta\epsilon_s = \bar{f}N\sigma e^2 C_M \langle S^2\rangle / 3\epsilon_0 kT. \quad (14)$$

For a distribution of molar masses this equation becomes

$$\Delta\epsilon_s = \frac{10^3\sigma\bar{f}e^2N_A C}{3\epsilon_0 kT}\,\frac{\Sigma_{(i)}C_{M,i}N_i\langle S^2\rangle_i}{\Sigma_{(i)}C_{M,i}N_i} = \frac{10^3\sigma\bar{f}e^2N_A C}{3\epsilon_0 kT}\,\overline{\langle S^2\rangle}^w, \quad (15)$$

where $10^3 N_A C = \Sigma_{(i)} C_{M,i} N_i$ is the total number of counterions per m^3, $C_{M,i}$ the number of polyions of molar mass M_i per m^3, N_i the number of counterions per polyions of molar mass M_i and N_A Avogadro's constant. The expression for $\Delta\epsilon_2$ remains unchanged. It is assumed here that b, σ and $\bar{f}$ are independent of molar mass and that the only charged groups in a DNA molecule at neutral pH are the phosphate groups (degree of dissociation unity). The specific increments according to this theory are thus given by

$$\frac{\Delta\epsilon_s}{C} = \frac{\sigma\bar{f}e^2N_A}{3\epsilon_0 kT}\,\overline{\langle S^2\rangle}^w, \qquad \frac{\Delta\epsilon_2}{C} = \frac{\sigma\bar{f}e^2N_A}{36\epsilon_0 kT}\,b^2, \quad (16,17)$$

In order to apply these expressions to the experimental results it should be kept in mind that the former were derived for a system in which also polyion-polyion interactions are negligible, i.e. at infinite dilution. In view of the strong concentration dependence of the experimental specific increments such conditions are not yet reached even at the lowest concentrations investigated. Therefore the extrapolated values $(\Delta\epsilon_s/C)_0$ and $(\Delta\epsilon_2/C)_0$ obtained from (7) should rather be used.

Assuming as is usually done that $\sigma \sim 1$, the specific increments are determined by the three parameters $\bar{f}$, $\overline{\langle S^2\rangle}^w$ and b. The fraction of associated Na^+ can be found from "activity coefficient" measurements in the polyelectrolyte system. Using the Katchalsky approach [29], $\bar{f} = 1 - \gamma^P_{Na}$, where γ^P_{Na} represents the operationally defined activity coefficient of the Na ion due to its interaction with the polyelectrolyte, which can directly be measured with the help of a Na-sensitive electrode in a salt-free polyelectrolyte solution and which can be calculated for polyelectrolyte/salt solutions from the total measured Na activity by application of the additivity rule. This approach yielded [30] for both Na-DNA systems investigated the same value $\bar{f} = 0.68 \pm 0.06$. Using this value of $\bar{f}$, and applying (16) and (17) to $(\Delta\epsilon_s/C)_0$ and $(\Delta\epsilon_2/C)_0$ respectively, values of $\overline{\langle S^2\rangle}^w$ and b may be calculated. These values for DNA I/H_2O and DNA II/3×10^{-4} M NaCl have been collected in table 4.

Table 4
Molecular parameters of DNA determined from dielectric data

		DNA I/H_2O	DNA II/3×10^{-4} M NaCl
$\overline{\langle S^2\rangle}^w$ a)	(nm)	38 ± 4	23 ± 2
b a)	(nm)	86 ± 8	55 ± 6
$\tau_{2,w}\times10^6$ b)	(s)	0.6 ± 0.1	0.25 ± 0.06

a) Calculated from (16) and (17) respectively, with $\bar{f} = 0.68 \pm 0.06$.
b) Calculated from (18) with $u = u_0 = 3\times10^{11}$ m s^{-1} N^{-1}.

In accordance with expectations, the values of the mean radius of gyration in H_2O is larger than the value in the salt solution where the repulsive electrostatic forces along the chain may already be screened off to some extend thus reducing the average dimensions. Also the value of b, roughly a measure of the stiffness of the chain, is found to be larger in the former as compared to the latter. However, if we compare the mean radii of gyration thus determined from dielectric experiments with the values of $\overline{\langle S^2\rangle}^w$ for a solution at 0.2 M NaCl, estimated for a wormlike chain of persistence length $a = (55 \pm 10)$ nm and the distribution of molar masses as revealed by the electron microscopy (see table 1), the former are definitely smaller, while larger values are to be expected because of the extension of the polyions with decreasing salt concentration. It should however be kept in mind that the values derived from the dielectric parameters are only rough estimates in view of all the approximations involved in the theoretical van der Touw-Mandel treatment (such as $\sigma = 1$, neglect of counterion-counterion interactions, etc.). Also the extrapolation procedure to $C \to 0$ according to (7) does not necessarily yield dielectric parameters for a truely infinitely diluted DNA solution. It cannot be excluded that at very low C, where measurements cannot be performed due to lack of sensitivity, deviations with respect to (7) may occur for these systems at vanishing small ionic strength, such that the extrapolated specific increments rather refer to a hypothetical state where some polyion-

polyion interaction effects have disappeared but the average conformational state of the polyions is not that prevailing at infinite dilution. Therefore the average dimensions determined from these extrapolated dielectric increments may be smaller than expected at a truely infinite dilution and vanishingly small ionic strength. The fact that the right order of magnitude of the radius of gyration with respect to the maximum value (at maximum extension) is found, is gratifying.

Some internal consistency in the theoretical analysis of the dielectric data of DNA can be found by considering the mean relaxation time τ_2 of the high-frequency dispersion. It has been pointed out by van der Touw and Mandel [1,27] that this relaxation time should be primarily determined by the diffusion of the associated counterions along the *subunits*. The relaxation time of unidimensional counterion diffusion has been obtained by Oosawa [31] and Wyllie [32] by solving the diffusion equation using a Fourier expansion. The first mode of this expansion determines almost completely the relaxation. For the case where counterion-counterion interactions are neglected the Wyllie treatment which has physically better justified boundary conditions yields the following expression of the diffusional relaxation time of a counterion along a subunit of length b

$$\tau_{2,w} = b^2/\pi^2 ukT. \tag{18}$$

Here u is the mobility of a counterion moving along the subunit. With eq. (18), using the value for b calculated from the dielectric increment $(\Delta\epsilon_2/C)_0$ and as an estimate for u the mobility of Na^+ in an infinitely diluted electrolyte solution (i.e. $u = u_0 = 3.1 \times 10^{11}$ m s^{-1} N^{-1} at 22°C), $\tau_{2,w}$ may be calculated. It can be seen in table 4 that these values are in reasonable agreement with the mean relaxation times $(\tau_2)_0$ obtained by extrapolation according to (8), as was also the case for other polyelectrolytes investigated.

In as far as the mean low-frequency relaxation time is concerned, the theoretical treatment applied so far predicts that the corresponding relaxation mechanism should be determined by rotational diffusion (τ_r) of the polyion and/or the diffusion of the associated counterions along the whole contour of the macromolecule (τ_q) for a molecular weight dependent τ_1. In case these mechanisms can be treated independently and the corresponding correlations functions may be approached by an exponential, the relaxation time τ_1 is given by

$$(\tau_1)^{-1} = (\tau_r)^{-1} + (\tau_q)^{-1} \tag{19}$$

If one of these relaxation times τ_r or τ_q is significantly smaller than the other, τ_1 will be determined mainly by this relaxation time. Experimental values of τ_r have been obtained for DNA of different molar masses by Hornick and Weill [33] and Ding et al. [34] from flow birefringence and electric dichroism respectively. By interpolation the value of τ_r was estimated for DNA I and DNA II to be 1.6×10^{-4} s and 1.3×10^{-4} s respectively. Houssier et al. [35] have determined from electric birefringence experiments however values of τ_r for sonicated DNA ($M \sim 5 \times 10^5$ g mol^{-1}) which are about three times smaller. These values obtained at finite dilution seem to be at least one order of magnitude larger than τ_1 found from dielectric experiments (see table 2). Although the latter may somewhat increase at still lower concentrations it is not likely that rotational relaxation is the main contributor to τ_1. In this stage no definite statement about τ_1 can be made particularly as no theoretical expression for τ_q is yet available in the case of a wormlike chain. However there are no indications that the experimental values of τ_1, which are of the same order of magnitude as τ_1 found with other polyelectrolytes of comparable contour length, would be inconsistent with the theoretical analysis applied to the DNA dielectric data.

We therefore reach the conclusion that the dielectric behaviour of our low-molecular weight Na-DNA in solution of vanishingly small ionic strength is qualitatively the same as of other polyelectrolytes and that no specific effects have to be taken into consideration in understanding the frequency or concentration dependence. Here, as well as for other polyelectrolyte solutions, the dielectric properties seem to be determined by induced dipole moments arising from the perturbation of the inner ion atmosphere by the electric field. From a quantitative point of view the theoretical analysis of Van der Touw and Mandel also can account for the dielectric parameters observed at least for their values extrapolated to infinite dilution.

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