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Dielectric behavior of DNA in water–organic co-solvent mixtures

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Abstract

The radiowave dielectric dispersions of DNA in different water–organic co-solvent mixtures have been measured in the frequency range from 100 kHz to 100 MHz, where the polarization mechanism is generally attributed to the confinement of counterions within some specific lengths, either along tangential or perpendicular to the polyion chain. The dielectric dispersions have been analyzed on the basis of two partially different dielectric models, a continuum counterion fluctuation model proposed by Mandel and a discrete charged site model, proposed by Minakata. The influence of the quality of the solvent on the dielectric parameters has been investigated in water–methanol and water–glycerol mixtures at different composition, by varying the permittivity ϵ_m and the viscosity η of the solvent phase. The analysis of the dielectric spectra in solvents where electrostatic and hydrodynamic interactions vary with the solvent composition suggests that both the two models are able, in principle, to account for the observed high-frequency dielectric behavior. However, while some certain assumptions are necessary about the polyion structure within the Mandel model, no structural prerequisite is needed within the Minakata model, where the polarization mechanism invoked considers a radial counterion exchange with the outer medium, which is largely independent of the local polyion conformation.

Keywords: Radiowave dielectric relaxation; Ion fluctuation; DNA in mixed water–organic solvents

1. Introduction

The dielectric properties of DNA aqueous solutions have been widely investigated in the last two decades [1–5] and a well-defined picture, common to other synthetic or natural polyelectrolytes, has been evidenced. Recently, Sun et al. [6] have extended dielectric investigations of DNA aqueous solutions over a wide range of temperatures (from 173 to 263 K), making possible measurements at very low frequencies (of the order of 10^{-2} Hz). These investigations open a new and interesting scenario for the low-temperature behavior and, on the other hand, confirm the previous characterization in the higher temperature range.

In a sufficiently wide frequency interval, the dielectric spectra of DNA aqueous solutions reveal two contiguous, partially overlapping, relaxation processes, attributed to different mechanisms of counterion fluctuation along the polymer chain [7,8].

The partial ionization of the polyion charged groups gives rise to a number of counterions that, owing to the strong electrostatic interactions, can reside, for a sufficiently long time, in the vicinity of the polyion domain and can fluctuate along two typical scale lengths of the polyion chain. Fluctuation within the whole polyion chain causes a dielectric dispersion falling in the low-frequency range (for DNA of 10^2–10^3 kD molecular weight, the typical relaxation frequency is of the order of 1–10 kHz), whereas, counterions that are confined within a smaller length associated to particular segments of the polyion chain [7,8], or can exchange with the bulk solution by a radial diffusion process [9], produce a relaxation in the high-frequency range (typically in the MHz region).

Although the deconvolution of a dielectric spectrum into two or more contributions is a difficult task, particularly if some artifacts are present, as for example, the contribution of the electrode polarization, the dielectric characteristics of the two above stated dispersions have been well-established [10]. For both the two relaxations, the polyion concentration effect on the specific increment (the dielectric strength normalized to the

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polyion content) can be described by empirical equations of the form [10]

$$\frac{\Delta e_j}{C_p} = \frac{A_j}{1 + B_j C_p} \quad (j = 1, 2) \tag{1}$$

where A_j stand for the value of the specific increment extrapolated to infinite dilution, C_p is the polyion concentration expressed in number of monomers per unit volume and B_j take into account the polyion–polyion interaction. Moreover, the low-frequency relaxation is characterized by an average relaxation time, which is molecular weight dependent, whereas the high-frequency relaxation is characterized by a relaxation time independent of the size of the polyion. These findings provide experimental support to the ansazt of different molecular mechanisms responsible of the two contiguous observed dispersions.

The above scenario is, however, less defined for DNA in non-aqueous solutions or in water–organic solvent mixtures, where the role of counterions on the dielectric behavior must be further clarified. Since the electrostatic interactions are mainly governed by the permittivity ϵ_m of the solvent, non-aqueous solvents or water–organic solvent mixtures, that generally possess a lower permittivity than the one of aqueous solvents, are expected to deeply influence the counterion distribution close to the polyion chain and consequently, reflecting in a different dielectric behavior. Moreover, water–organic solvent mixtures experience different viscosimetric properties that, in turn, may alter the ion mobility. Since both the electrostatic (the permittivity ϵ_m) and the diffusional (the viscosity η) processes influence the dielectric behavior of the polyion, the concomitant changes of these two parameters may evidence the role of the electric and hydrodynamic effects in the overall behavior of the system.

To this end, we have investigated the dielectric properties of DNA in water–methanol and in water–glycerol mixtures, varying the solvent composition from pure water to about 0.8 water molar fraction mixture. In this way, the permittivity ϵ_m of the solvent varies from 80 (pure water) to about 65 (water–methanol mixture 41.7 % wt/wt or water–glycerol mixture 56.5% wt/wt), influencing the overall counterion concentration and their electrostatic interactions with the bulk solution and/or with the ionized polyion chain. For the same mixed solvents, the viscosity varies from about 1 cP for pure water to about 1.8 cP for water–methanol 41.7% wt/wt and to about 8.7 cP for water–glycerol mixture 56.5% wt/wt. On the other hand, both glycerol and methanol are known to affect DNA dynamics. Glycerol is generally regarded as one of the best preservant at cryogenic temperature and forms a very rigid structure that increases energy barriers for conformational transitions. Methanol alters the melting temperature leading to a higher thermal temperature in the presence of some kind of monovalent ions, such as for example Cs^+ ion, whereas divalent cations at methanol concentration higher than 50% cause DNA condensation and denaturation, even at room temperature.

The influence of the permittivity ϵ_m and the viscosity η of the solvent on the dielectric properties of DNA in water–organic solvent mixtures are analyzed in the light of two different models [11–14] (herein, referred to as Mandel and Minakata models, respectively) that have been previously proposed to take into account the dielectric properties of polyelectrolyte aqueous solutions. Both models attribute to the counterions the effect of inducing a non-uniform electrical polarization along the chain, causing a relaxation process under the influence of an external electric field. The role of the counterions spatially restricted in the vicinity of the polyion chain is discussed in the light of two different mechanisms, at molecular level, considering a concentration fluctuation along some restricted lengths of the polyion chain or a radial diffusional polarization towards the bulk medium. Although in the two stated models the origin of the dielectric relaxation is similar, invoking the role of counterions in the vicinity of the polyion chain, the extent of the effects depends differently on the counterion concentration. The choice of varying the quality of the solvent, influencing the amount of counterions present in the system, could offer the opportunity to prove which of them furnishes a better picture of the experimental behavior. Both models are, in principle, able to account for the observed dielectric dispersions, with a set of appropriate values of the characteristic parameters. However, our findings, on the basis of a good degree of self-consistency, give support to the use of the Minakata model to describe the dielectric behavior of DNA in solvents of varying quality.

2. Theoretical background

The dielectric properties of DNA aqueous solutions will be analyzed on the basis of counterion-fluctuation mechanism models formerly proposed by Oosawa [15,16], which has been successfully employed in the analysis of the dielectric behavior of natural and synthetic polyelectrolyte solutions. In particular, we will briefly review here a continuum dielectric model proposed by Mandel [11,12] and a discrete charged site model proposed by Minakata [13,14].

In the former model [11,12], the polyion is represented by a sequence of rigid subunits of identical length b that, in solution, owing to the partial flexible structure, assumes a conformation characterized by a radius of gyration R_g. In the case of a linear polyion, such as a DNA chain, counterion fluctuation occurs on two typical scale lengths, the overall length $L=(12R^2{}_g+b^2)^{1/2}$, causing the low-frequency relaxation process, and the subunit length b, causing the high-frequency relaxation process. The basic idea of this model is that counterions are constrained in a cylindrical potential wall around each subunit due to the electrostatic field arising from the net charge on the chain. While ion motion along the subunit is unrestricted, electrostatic forces prevent ions from leaving the neighborhood of the polyion. Between two consecutive subunits, there are potential barriers due to the angle they form with each other and which cause a certain hindrance to the motion of ions along the whole chain. On the basis of this model, the dielectric increments of the low-frequency and high-frequency dielectric dispersions $\Delta\epsilon_1$ and $\Delta\epsilon_2$, respectively, are expected to be proportional to the average square end-to-end distance $<L^2>$ of the whole polyion and to the square of the length b of the subunit, respectively.

Our experimental investigation is confined within the frequency range between 100 kHz to 100 MHz, where the high-frequency relaxation falls. Consequently, we will consider only the high-frequency process whose characteristic dielectric parameters (the dielectric increment $\Delta\epsilon_2$ and the relaxation frequency f_{ion}) are given by

$$\Delta e_2 = \frac{(\mathrm{ze})^2 C F_{\mathrm{M}} N \gamma b^2}{36 e_0 K_{\mathrm{B}} T} \tag{2}$$

$$f_{\mathrm{ion}} = \frac{\pi u K_{\mathrm{B}} T}{2b^2} \tag{3}$$

where C is the concentration of the polyion per unit volume, N the total number of charged sites per polyion chain, F_{M} the average fraction of associated counterions, ze their charge, ϵ_0 the dielectric constant of free space, u the mobility of counterions along the subunit b and $K_{\mathrm{B}}T$ the thermal energy. Finally, γ is the internal field correction factor, assumed in dilute solutions to be, as usually, unity.

In the latter model [13,14], the electric-field induced polarization is due to fluctuation of the counterion concentration in a linear lattice having N charged sites corresponding to the ionic groups of the polyion. According to the Minakata model [13,14], the Fourier expansion of the thermal fluctuation of the counterion concentration produces a dielectric relaxation described by

$$e'(\omega) = e_{\mathrm{m}} + \frac{4\pi\gamma N(\mathrm{ze})^2 l^2 n_0(1-n_0)}{3NK_{\mathrm{B}}T} \times \sum_q \frac{1}{(1+Nn_0(1-n_0))(\Phi(q))(1+\omega^2\tau_q^2)} \tag{4}$$

where

$$\Phi(q) = -(1-\cos q)\frac{(\mathrm{ze})^2 \ln(1-2\cos(q)\exp(-k_D h)+\exp(-2k_{\mathrm{D}}h))}{e_{\mathrm{m}} l K_{\mathrm{B}} T} \tag{5}$$

is the counterion interaction energy in the Debye–Hückel approximation and

$$\tau_q = \frac{1}{(2W(1-\cos q) + C/n_0)(1 + Nn_0(1-n_0)\Phi(q)/(1-\cos q))} \tag{6}$$

is the relaxation time of the q mode. The other symbols have the following meaning. k_{D} is the inverse of the Debye thickness layer, $l=Nh$ is the length of the linear polyion, h the average charge spacing along the axis of the polyion chain, n_0 is the average number of counterions at a site ($0 \le n_0 \le 1$) and q is the wave mode number given by $q=2\pi m/N$ ($m=1, 2 \ldots N/2$).

In this model, two characteristic parameters appear, i.e., the rate constant W of ion transfer between adjacent lattice points and the constant C that takes into account the radial ion diffusion. These parameters give rise to distinct relaxation regions through their influence on the relaxation time τ_q of the q mode. The addition of the term C/n_0 results in the decrease of τ_q and the high-frequency side of the relaxation is shifted towards higher frequencies, leading to the separation of the overall relaxation curve into two separate relaxation processes. For a given polyion shape, the possibility of use a range of solvents of different quality (water–organic co-solvent mixtures) allows us to test separately the influence of the permittivity and the viscosity on the extent of the radial ion distribution which, in turn, should reflect in a change of the high-frequency dielectric dispersion.

3. Experimental

3.1. Materials

Calf thymus DNA (ctDNA) was purchased from Sigma Chem. Co. An appropriate amount of DNA was diluted in deionized water to a concentration of 5 mg/ml and fragmented by sonication with a Vibra Cell sonifier (Sonic and Materials Inc). The molecular weight distribution of the DNA sample, as determined by agarose gel electrophoresis, was widely spread, covering the range from 0.5 to 1 kilobase. The final DNA concentration was 1.5 mM in terms of phosphates. The ionic strength of the samples, as deduced from electrical conductivity measurements, was equivalent to a 0.5 mM NaCl solution, indicating that only a fraction of the Na^+ ions deriving from the DNA dissociation are free enough to contribute to the ionic conductivity. The methanol and glycerol were commercial products. The solutions were prepared by weighing.

3.2. Dielectric measurements

Permittivity $\epsilon'(\omega)$ and dielectric loss $\epsilon_{\mathrm{d}}''(\omega)$ were measured by means of a computer controlled Hewlett-Packard impedance analyzer, model 4194A, in the frequency range 10^5–10^8 Hz. The measuring cell is a section of a cylindrical waveguide partially filled with the sample solution. The system behaves as a wave-guide excited far beyond its cut-off frequency mode and, therefore, only the stray-field of the coaxial line/wave-guide transition is used in the measurement. The input reflection coefficient $\rho^*(\omega)$ (in magnitude and angle) is measured at the input of the dielectric cell for each frequency of interest. Cell constants were determined by measurements with standard electrolyte solutions of known conductivity, following a well-defined procedure reported elesewhere [17]. Because of the invariance of the cross-ratio in the relation between the complex dielectric constant $\epsilon^*(\omega)$ and the refection coefficient $\rho^*(\omega)$, the following relationship holds

$$\frac{[e^*(\omega)-e_1^*(\omega)][e_2^*(\omega)-e_3^*(\omega)]}{[e^*(\omega)-e_2^*(\omega)][e_1^*(\omega)-e_3^*(\omega)]} = \frac{[\rho^*(\omega)-\rho_1^*(\omega)][\rho_2^*(\omega)-\rho_3^*(\omega)]}{[\rho^*(\omega)-\rho_2^*(\omega)][\rho_1^*(\omega)-\rho_3^*(\omega)]} \tag{7}$$

The cell is filled in turns with three different electrolyte solutions of known permittivities, ϵ_j ($j=1, 2, 3$), and conductivities, σ_j ($j=1, 2, 3$), and the reflection coefficient ρ_j^* ($j=1, 2, 3$) measured.

The technique allows the determination of the real part $\epsilon'(\omega)$ and imaginary part $\epsilon''(\omega)$ of the complex dielectric constant and of the low-frequency limit σ_0 of the electrical conductivity $\sigma(\omega)$. The errors are within 1–2%. The relaxation contribution $\epsilon_d''(\omega)$ to the total dielectric loss $\epsilon''(\omega)$ was calculated by subtracting the conductivity term $\sigma_0/\epsilon_0\omega$, where $\omega=2\pi f$ is the angular frequency of the applied electric field and ϵ_0 the dielectric constant of free space. In the low-frequency tail of the frequency window investigated, the electrode polarization effect, causing a frequency-dependent increase of the permittivity $\epsilon'(\omega)$ due to an ion accumulation close to the electrode interface, was corrected by means of the "subtraction" method, measuring the dielectric response of a single electrolyte solution whose electrical conductivity was matched to the one of the sample to be investigated.

All the measurements were carried out at the temperature of (20.0 ± 0.1) °C. In the frequency window employed, the mixed solvents do not show any dielectric dependence on the frequency, the orientational dielectric relaxation falling at frequencies well above 100 MHz.

3.3. Analysis of the dielectric spectra

The dielectric measurements were carried on aqueous solutions of DNA in the presence of an organic compound (methanol or glycerol) as a co-solvent, in the frequency range 0.1–100 MHz. In this frequency interval, the bio-polymer shows a typical relaxation, due to local oscillations of counterions at the interface solute/solvent. The composition of the solvent was varied by increasing the weigh percentage of the co-solvent up to about 40% in methanol and 60% in glycerol. As a matter of fact, circular dichroism [CD] spectra in the wavelength range 220–320 nm ensure the maintenance of the native structure of DNA. Typical CD spectra for DNA in water–glycerol solutions at different composition from 0% to 56.5% wt/wt are shown in Fig. 1. DNA in water–methanol solutions behaves similarly. Moreover, the co-solvent concentrations employed realize the same change in the permittivity ϵ_m of the solvent, whereas the viscosity of water–glycerol mixture exceeds by about a factor 4 the one of water–methanol mixture.

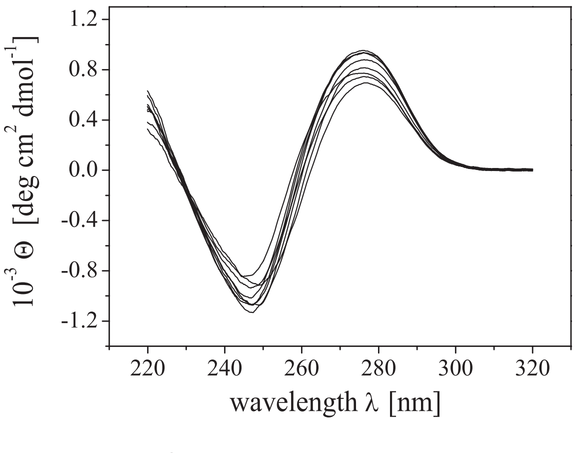

Fig. 1. The Ellipticity Θ of DNA in water–glycerol mixtures of different composition as a function of the wavelength λ, at the temperature of 20.0 °C. The glycerol concentration is varied from 0% to 56.5% wt/wt.

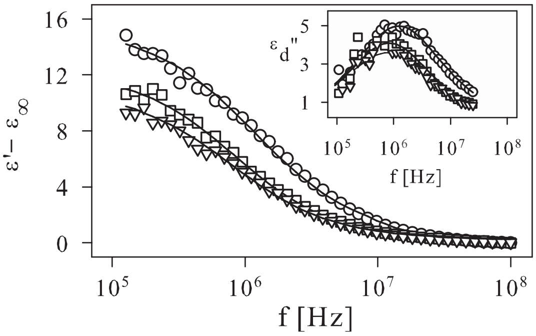

Fig. 2. The dielectric increment $\epsilon'-\epsilon_\infty$ of DNA in water–methanol solutions as a function of frequency at some selected values of the co-solvent concentration: (○): water, (□): 31.8% wt/wt; (▽): 41.7% wt/wt. The DNA concentration is 1.5 mM in nucleotide. The continuous lines are the calculated values on the basis of the Cole–Cole relaxation function. The inset shows the dielectric loss $\epsilon_d''(\omega)$ obtained subtracting the conductivity loss contribution $\sigma_0/\epsilon_0\omega$ from the total loss.

Figs. 2 and 3 show some typical dispersion curves of DNA in water–methanol and water–glycerol mixtures at different compositions. The experimental data were analyzed on the basis of the Cole–Cole relaxation function

$$e^*(\omega) = e_\infty + \frac{\Delta e}{1 + (i\omega\tau_{\text{ion}})^{(1-\alpha)}} \tag{8}$$

where $\epsilon^*(\omega)$ is the complex dielectric constant, ω is the angular frequency of the applied electric field, τ_{ion} the relaxation time, $\Delta\epsilon$ the dielectric increment, ϵ_∞ the permittivity extrapolated at high frequency and α is an empirical parameter taking into account the spread of relaxation times around the mean value [18]. The results of the fitting procedure, reported in Tables 1 and 2, show a progressive reduction of the dielectric increment $\Delta\epsilon$ with a consequent shift of the relaxation frequency $f_{\text{ion}}=1/(2\pi\tau_{\text{ion}})$ toward lower values as the amount of the co-solvent (methanol and glycerol) is progressively increased. The values

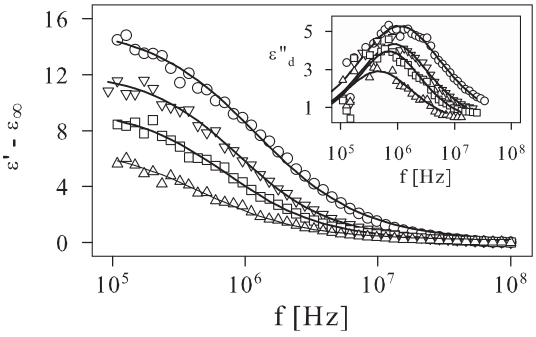

Fig. 3. The dielectric increment $\epsilon'-\epsilon_\infty$ of DNA in water–glycerol solutions as a function of frequency at some selected values of the co-solvent concentration: (○): water, (▽)): 18.0% wt/wt; (□): 34.6% wt/wt; (△): 56.6% wt/wt. The DNA concentration is 1.5 mM in nucleotide. The continuous lines are the calculated values on the basis of the Cole–Cole relaxation function. The inset shows the dielectric loss $\epsilon_d''(\omega)$ obtained subtracting the conductivity loss contribution $\sigma_0/\epsilon_0\omega$ from the total loss.

Table 1
Dielectric parameters of DNA in water–methanol solutions

Methanol % (wt/wt)	f_{ion} [MHz]	$\Delta\epsilon$	α
0.0	1.20±0.06	15.6±0.4	0.26±0.02
6.40	1.07±0.07	14.5±0.5	0.29±0.02
12.6	0.99±0.08	14.2±0.5	0.24±0.04
14.2	0.92±0.04	13.7±0.4	0.21±0.02
22.5	0.83±0.06	12.4±0.4	0.23±0.03
31.8	0.81±0.08	11.1±0.6	0.27±0.02
37.7	0.79±0.07	10.8±0.5	0.24±0.04
41.7	0.86±0.06	10.4±0.4	0.26±0.02

of the parameter α are, to a first approximation, unaffected by the increasing amount of co-solvents and are probably related to the heterogeneity of the system due to the relatively wide distribution of DNA fragments investigated.

4. Results

The measured spectra cover the high-frequency region of the dielectric relaxation and consequently only the dielectric dispersion associated with the local fluctuation of counterions has been observed. We analyze now separately the data of the two set of experiments, carried out employing methanol and glycerol as co-solvents, respectively.

4.1. DNA in water/methanol solution

According to Mandel model [11,12], from Eqs. (2) and (3), we have evaluated the two characteristic parameters, the length *b* of the subunit and the fraction F_M of bound counterions. We have assumed for the parameter *u* the value of the mobility of Na^+ ions at infinite dilution in water ($u=3.10^{11}$ m s^{-1} N^{-1} at 20 °C). Moreover, we assume that the mobility *u* is inversely proportional to the viscosity *η* of the solvent. This assumption yields that the ratio between the length of subunit in water/methanol mixture and the one in pure water is given by $(b/b_0)^2=\eta_0 f_{\text{ion}0}/\eta f_{\text{ion}}$. The values of *b* are shown in Fig. 4 as a function of the permittivity ϵ_m of the solvent.

The fraction of counterions, fluctuating at the polymer interface can be deduced from Eq. (2), by using the data of the observed dielectric increments $\Delta\epsilon_2$ and the values of subunit length b derived from Eq. (3). The results are shown in Fig. 5, where the fraction F_M is shown as a function of the permittivity ϵ_m of the solvent.

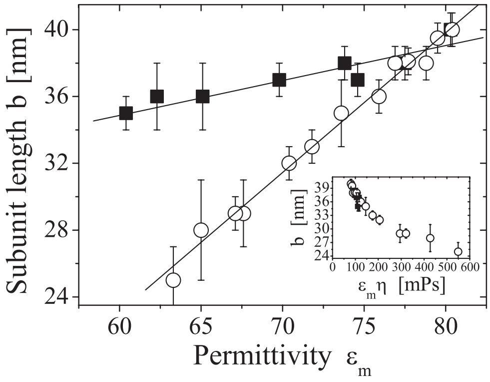

Fig. 4. Dependence of the subunit length *b* according to the Mandel model on the permittivity of the solvent: (■): water–methanol mixture; (○): water–glycerol mixture. The inset shows the same data plotted as a function of the product $\epsilon_m\eta$.

The analysis of the data within the discrete charge site model of Minakata [13,14] requires caution since Eqs. (4)–(6) furnish the overall dielectric profile of the dielectric relaxation, without resolving the spectra into the two distinct processes predicted by the model. Since we have investigated only the high-frequency behavior, the comparison should be carried out considering only the contribution associated to the higher-frequency process. This can be made, to a first approximation, by putting the rate constant *W* equal to zero and by selecting an appropriate value of the constant *C* in order to make overlapped, within the experimental uncertainties, the predicted average relaxation time to the one observed experimentally. Once the high-frequency characteristic parameters have been evaluated, it is possible to reconstruct the full spectra by adding the low-frequency contribution, mainly governed by the rate constant *W*. An example of the results from this procedure is shown in Fig. 6, where we report the measured increment of the permittivity of DNA in water–methanol 31.8 wt/wt solution compared to the expected whole dielectric spectrum, according to the Minakata model.

Table 2
Dielectric parameters of DNA in water–glycerol solutions

Glycerol % (wt/wt)	f_{ion} [MHz]	$\Delta\epsilon$	α
0.0	1.19±0.06	15.7±0.4	0.26±0.02
3.00	1.14±0.05	15.3±0.4	0.21±0.02
5.40	1.12±0.06	14.7±0.5	0.27±0.02
9.30	1.03±0.04	13.9±0.3	0.22±0.02
12.2	0.96±0.06	12.8±0.4	0.23±0.02
18.0	0.89±0.07	12.2±0.5	0.22±0.04
23.8	0.80±0.08	10.6±0.7	0.22±0.04
29.8	0.72±0.05	9.7±0.4	0.28±0.04
34.6	0.63±0.06	8.9±0.4	0.22±0.02
43.6	0.51±0.06	7.5±0.4	0.25±0.04
45.4	0.48±0.05	6.8±0.4	0.24±0.03
51.7	0.37±0.07	6.2±0.7	0.21±0.05
56.6	0.35±0.07	5.9±0.5	0.23±0.04

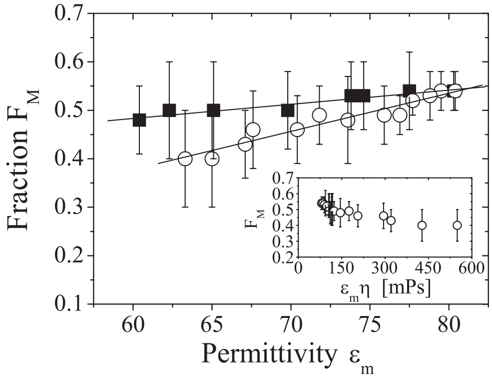

Fig. 5. Dependence of the fraction F_M of counterions according to the Mandel model on the permittivity of the solvent: (■): water–methanol mixture; (○): water–glycerol mixture. The inset shows the same data plotted as a function of the product $\epsilon_m\eta$.

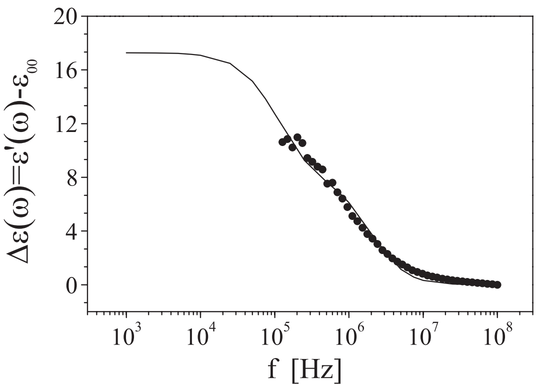

Fig. 6. The permittivity spectra (the dielectric increment $\Delta\epsilon(\omega)$ as a function of the frequency of the applied electric field) of DNA in water–methanol 31.8 wt/wt mixture. The full curve is calculated according to the Minakata model with the following parameters: $W=10^6\ \mathrm{s}^{-1}$,$C=1.5\ 10^4\ \mathrm{s}^{-1}$.

A value of the parameter C, of the order of $C=1.5\ 10^4\ \mathrm{s}^{-1}$, produces a high-frequency contribution centered around 1 MHz, which is approximately the value where the high-frequency dispersions of the samples investigated fall. It must be noted that the constant C should depend on the quality of the solvent through its permittivity ϵ_m and its viscosity η. However, the comparison with the experimental spectra by the relaxation time is not accurate enough to allow to evidence a dependence of C on the solvent parameters. Consequently, we have assigned to C a constant value for all the solvents investigated. The average number n_0 of counterions associated to a charged site can be evaluated by fitting Eq. (4) in the limit $\omega<<1/<\tau_q>$ to the dielectric increment $\Delta\epsilon_2$ experimentally observed. The results are reported in Fig. 7.

4.2. DNA in water/glycerol solution

We have varied the composition of the water–glycerol mixture in order to obtain the same changes in the permittivity ϵ_m as those occurring in the water–methanol mixture. We report in Figs. 4 and 5) the values of subunit length b and fraction F_M of oscillating counterions as function of the permittivity of the solvent. The trend of b and F_M is qualitatively the same as the one observed in methanol. Analogously, the results obtained within the Minakata model, summarized in the dependence of the fraction n_0 on the solvent composition, are shown in Fig. 7. Interestingly, Sun et al. [6] have recently measured DNA in water–glycerol solution in a very low temperature range (from 203 to 263 K). They found a deep analogy of the observed dielectric behavior with the one of pure glycerol, suggesting that the dynamics of DNA solutions are dominated by the solvents. Our findings, even though in a different temperature range, support some further evidences in this direction.

5. Discussion

The overall results we present clearly evidence the effects of the solvent on the dielectric behavior of DNA solutions. As the electrostatic interactions are progressively enhanced as the permittivity of the solvent is reduced, there is a decrease in the length of the subunits, indicating an increased flexibility of the whole chain, accompanied by a concomitant reduction of the fraction of associated fluctuating counterions. It has to be noted, however, that the viscosity of water–glycerol mixtures is higher than the one of water–methanol mixtures. This fact may be related to the observed differences in the parameters b and F_M estimated by the model of Mandel for the two co-solvents. When these quantities are plotted as a function of the product $\epsilon_m\eta$, the values scale on the same curve (see the insets of Figs. 4 to 5), indicating that both the hydrodynamic (through the viscosity η) and electrostatic (through the permittivity ϵ_m) parameters contribute to the dielectric properties and that the polyion conformation described by a sequence of subunits b and the overall counterion distribution depend on the product $\epsilon_m\eta$ similarly.

The value of the subunit b compares favorably well with the one we have previously evaluated in the case of Ag–DNA in $AgNO_3$ aqueous solutions [19]. In that case, we observed a decrease of the length b with the increase of the salt concentration or, equivalently, with the increase of the screening effect induced by the added salt. In the present case, the decrease of the permittivity ϵ_m, in the limit of very low polyion concentration, induces stronger interactions between phosphate groups and their associated counterions, increasing the neutralization of the charged groups of the DNA chain and consequently reducing the internal electrostatic repulsion. This should favor an enhanced polyion flexibility and a following decrease in the subunit length. However, it must be pointed out that this result strongly depends on the assumption that the counterion mobility varies as the inverse of the viscosity of the solvent. Whereas this assumption is certainly valid in bulk solutions, it may be questionable close to the DNA–solvent interface, where the counterion diffusion might be influenced to a lesser extent by the bulk properties.

The fraction of fluctuating counterions derived from the high-frequency dispersion within the Minakata model increases with the decrease of the permittivity of the solvent phase, i.e., when the electrostatic interactions increase. The perpendicular diffusion process, which provides the additional dispersion in the high-frequency region, results in dielectric relaxations with

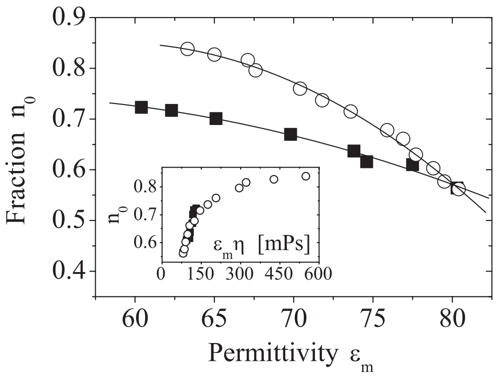

Fig. 7. Dependence of the fraction n_0 of counterion according to the Minakata model on the permittivity of the solvent: (■): water–methanol mixture; (○): water–glycerol mixture. The inset shows the same data plotted as a function of the product $\epsilon_m\eta$.

reduced strength owing to the stronger electrostatic interactions between counterions and lattice charged groups. This qualitative picture is accounted for by the behavior of n_0 as a function of ϵ_m. Also in this case, the values of n_0 for the two different solvents collapse into an unique dependence when the data are plotted as a function of the product $\epsilon_m\eta$ (see the inset of Fig. 7).

A final comment is in order. In a highly charged polyion chain, independently of the model adopted for the polyion chain, according to the counterion condensation theory of Manning [20–22], a fraction of counterions are confined close to the polyion charged groups, forming a well-defined condensed counterion phase. The parameter that governs the counterion condensation is the ratio ξ between the Bjerrum length $l_b = e^2/(\epsilon_m K_B T)$ and the average distance between the charged groups along the polyion chain. If $\xi > 1/z$, where z is the valence of the counterion, condensation occurs and a fraction f_M of counterions will condense

$$f_M = 1 - \frac{1}{z\xi} \tag{9}$$

Assuming that the DNA is in the B-form, with an average distance between phosphate pairs of 1.7 Å, we have $\xi = 4.17$ and, consequently, in an aqueous phase, a fraction $f_M = 0.76$ of counterions will condense. If the permittivity ϵ_m is reduced as in the case of mixed water–organic cosolvent mixtures, this fraction increases, reaching a value of about $f_M = 0.82$, at the highest concentration of the co-solvent investigated.

This fraction differs from the one derived from the Mandel model in a different context. The counterion condensation in the Manning view derives in the attempt to avoid the physically unrealistic divergence of the Helmholtz free energy of the polyion–ion system at short distance when the density charge exceeds a critical value. In this light, condensed counterions include all the charges localized by a logarithmic potential appropriate for an infinitely long charge line and consequently their amount can exceed the number of counterions that are to be considered as confined close to the polyion chain, but able to fluctuate in concentration under an external electric field, according to the Mandel model. For example, in the water–glycerol mixture, we find $f_M \simeq 0.82$, but about one half ($F_M \simeq 0.4$) contribute to the high-frequency dielectric relaxation.

As far as the values of the parameter n_0 are concerned (Fig. 7), in the presence of two organic solvents, we observe an increase, even if with different slopes, with the decrease of the solvent permittivity. This finding can be qualitatively justified considering that to an increase of the electrostatic interactions (following the decrease of the permittivity), it corresponds a larger amount of the counterions residing in the neighborhood of the charged groups. This effect is further enhanced when the viscosity effect dominates (water–glycerol solvent at the highest concentrations). However, this effect may be partially caused by the fact that we have assumed, as already stated, a value of the parameter C independent of the viscosity of the solvent. It is worth noting that, in these conditions, in the Minakata model, the term $n_0(1-n_0)$ accounts for the decrease of the dielectric increment, as observed experimentally. Also in this case, the dependence of n_0 on the parameter $(\epsilon_m\eta)$ is approximately the same for methanol– or glycerol–water mixtures.

Both the Mandel and Minakata models appear able, in principle, to justify the observed dielectric dispersions and to explain several qualitative characteristics of these effects even though the values of the parameters involved and their dependencies on both the permittivity ϵ_m and the viscosity η of the solution are a somewhat different. However, whereas the Mandel model requires an ad hoc parameter, introduced by a subunit structure that has never observed by other experiments, whose length apparently decreases with the decrease of the solvent permittivity, the Minakata model attributes the high-frequency dielectric dispersion to interactions of all the bound counterions with the outer medium, introducing a radial polarization, which may be largely independent of the local polyion conformation. Moreover, the dependence of n_0 on the permittivity ϵ_m of the solvent is in qualitative agreement with what expected with the increase of the electrostatic interactions. Finally, this picture is consistent with several qualitative features of interfacial polarization mechanisms such as those described by Maxwell–Wagner effects.

References

[1] S. Takashima, C. Gabriel, R.J. Sheppard, E.H. Grant, Biophys. J. 46 (1984) 29–34.

[2] K.R. Foster, B.R. Epstein, M.A. Gealt, Biophys. J. 52 (1989) 421–425.

[3] T. Umehara, S. Kuwabara, S. Mashimo, S. Yagihara, Biopolymers 30 (1990) 649–656.

[4] F. Pedone, A. Bonincontro, Biochim. Biophys. Acta 1073 (1991) 580–584.

[5] D.J. Bakrwell, I. Ermolina, H. Morgan, J. Miller, Y. Feldman, Biochim. Biophys. Acta 1493 (2000) 151–158.

[6] M. Sun, S. Pejanovic, J. Mijovic, Macromolecules 38 (2005) 9854–9864.

[7] F. Van der Touw, M. Mandel, Biophys. Chem. 2 (1974) 218–230.

[8] F. Van der Touw, M. Mandel, Biophys. Chem. 2 (1974) 231–241.

[9] K. Ito, A. Yagi, N. Ookubo, R. Hayakawa, Macromolecules 23 (1990) 857–863.

[10] Th. Vreugdenhil, F. Van der Touw, M. Mandel, Biophys. Chem. 10 (1979) 67–80.

[11] M. Mandel, N.Y. Acad. Sci. 303 (1977) 74–87.

[12] M. Mandel, Biophys. Chemist. 85 (2000) 125–139.

[13] A. Minakata, Ann. N.Y. Acad. Sci. 303 (1977) 107–120.

[14] A. Minakata, Biopolymers 11 (1972) 1567–1582.

[15] F. Oosawa, Polyelectrolytes, Mercel Dekker, Inc., New York, 1971.

[16] F. Oosawa, Biopolymers 9 (1971) 677–688.

[17] T.W. Athey, M.A. Stuckly, S.S. Stuckly, IEEE Trans., vol. 30, MTT, 1982, pp. 82–86.

[18] J.B. Hasted, Aqueous Dielectrics, Chapman and Hall, 1973.

[19] A. Bonincontro, C. Cametti, A. Di Biasio, F. Pedone, Biophys. J. 45 (1984) 495–501.

[20] G.S. Manning, Q. Rev. Biophys. II, 2 (1973) 179–246.

[21] G.S. Manning, Biophys. Chem. 9 (1978) 65–70.

[22] G.S. Manning, Biophys. Chem. 7 (1977) 95–102.