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# Dynamics and structure of biopolyelectrolytes in repulsion regime characterized by dielectric spectroscopy

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ABSTRACT

We overview the study of biopolyelectrolytes by dielectric spectroscopy technique by primarily focusing on the case of repulsive regime of intersegment interactions mediated by univalent counterions. Two observed dielectric relaxations in 100 Hz–100 MHz frequency range due to diffusive motion of counterions are related to polyelectrolyte structural properties: the high frequency mode probes the structural organization of the polyion network in solution, while the low frequency mode is correlated with single polyion conformational properties. Open issues are highlighted and prospects for further research with polyvalent counterions are designated in order to study the crossover from repulsive to attractive regime of intersegment interactions.



**Contents**



## 1. Introduction

Polyelectrolytes are macromolecules with ionizable groups that dissociate in an aqueous solution and thus give rise to charged polyions surrounded by an atmosphere of neutralizing counterions [1]. The highly charged nature of polyions is in the origin of the fact that electrostatic interactions are fundamental to their complex structure and dynamics. Biologically relevant polymers such as nucleic acids, polysaccharides, proteins and microtubules are all biopolyelectrolytes whose charge-derived properties are in direct relation to their physical properties and biological functions. In a living cell these biopolymers function in an aqueous environment at room temperature [2]. From the point of view of a solid state physicist, this very limited temperature range contrasts unfavorably with the richness of low temperature physics with vastly different energy scales and quantum effects. On the other hand, a more recent understanding of highly charged molecular systems shows that Coulomb interactions can be so strong as to effectively place us in the realm of low temperature physics [3]. Non-specific theoretical approaches applied to biopolyelectrolytes as well as experiments in a solution have shown that interactions between charged polyions are modulated by other polyions and counterions to the extent depending on factors like the polyion length, concentration, added salt concentration and the counterion valence. In particular, the critical role of counterion valence in modulating inter-polyion forces was comprehended only recently [4]. In the presence of univalent counterions the dominant force between polyions is like-charge repulsion and this case can be well-described by the mean-field theory based on the Poisson–Boltzman approximation [5–7]. These theories show that the primary role of univalent counterions is an electrostatic screening of the polyion's large negative charge. In the limit of low screening polyions keep a stretched conformation, whereas a high screening results in a conformation which is

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best described as a random walk of correlation blobs. One specific hallmark of the latter behavior is a decreased polyion's rigidity [8]. On the other hand, more realistic theoretical models show that the conformations and rigidity strongly depend on increasing the valence of counterions which induce like-charge attraction [4,7]. Strong attractive forces are indeed observed between DNA strands yielding the condensation effects [9].

Coming back to the repulsion regime of electrostatic interactions, we would like to stress that it is important to distinguish two distinct solution regimes: semidilute and dilute one. The semidilute case implies that the chains are long enough to interpenetrate in contrast to the dilute regime in which each chain is well-separated from the others. Following the seminal work by de Gennes [10] polyelectrolyte dynamics in both regimes has been studied extensively yielding different concentration-dependent power law behaviors. These behaviors differ due to a change in mutual balance between electrostatic and elastic energy of the polyelectrolyte chains [5,11].

Over the last several years we have conducted an extensive investigation of dynamics of deoxyribonucleic acid (DNA) and hyaluronic acid (HA) chains in aqueous solutions with univalent sodium (Na) counterions. Samples included polydisperse DNA and HA chains with an average length of 4 μm (long chains) [12–14], and monodisperse 146 bp DNA fragments of 50 nm (short chains) [15,16]. From the standpoint of physics, the long- and short-chain samples survey the electrostatic repulsion regime in semidilute and dilute limits, respectively. In all studied samples we have detected two dielectric relaxation modes due to diffusive motion of polyion counterions in the frequency range 100 Hz–100 MHz. We have found that the parameters which characterize these modes, the dielectric strength and the mean relaxation time, critically depend on the polyion length, charge density, flexibility and concentration, as well as added salt concentration. More importantly, based on the dynamics in kHz and MHz range we have demonstrated how to quantify structural properties of biopolymer solutions and single polyion chains, respectively. In our recent paper [17] we give a comparative overview of all obtained results and suggested interpretations, with a special emphasis on complementary aspects of the dielectric spectroscopy (DS) technique, which covers polyelectrolyte concentrations smaller than 10 mg/mL, and the widely known scattering techniques such as small-angle X-ray (SAXS) and neutron scattering (SANS) which are limited to highly concentrated polyelectrolyte solutions. We showed that characteristic length scales detected in the DS measurements compare well with those extracted from the SAXS measurements in the overlapping concentration range probed by both techniques.

The aim of the present work is to summarize the state-of-the-art in the DS research of dynamical and structural aspects of biopolyelectrolytes; we describe the previously unpublished details of experimental procedure in the study of polydisperse 4 μm long DNA chains with univalent counterions dissolved in aqueous solutions and discuss unsolved issues from a current perspective. At the end, we lay out the study we have started recently with the intention to delineate the future of DS research of the polyelectrolytes in aqueous solutions.

## 2. Materials and experimental methods

Many applications in chemical, biological and medical areas rely on the response of the polyion to applied dc electric fields. This response can be studied by electrophoresis [18] and by the electric dichroism or birefrigence experiments [19,20]. On the other hand, the counterion response can be detected by the dielectric spectroscopy technique in the kHz–MHz frequency range [21]. DS has been somehow neglected as a modern tool to study the dynamics and structure of polyelectrolyte solutions despite its proven numerous abilities to quantify many aspects of the ion-solvent or macromolecule-solvent interactions [22,23]. Its advantage is twofold. Firstly, it is noninvasive thanks to the small ac electric fields (0.5 V/cm) applied to the specimen which enables detection of the sample response in linear regime. This does not hold for the electric dichroism or birefrigence techniques which use more than three orders of magnitude larger dc electric fields to induce a torque on DNA molecules or to align them. The second powerful advantage of DS is brought by the two relaxation modes of counterion response, one defined by oscillations throughout the polyion network, and the other along single polyion chains. Hence, DS is at the same time able to detect two fundamental length scales, one characterizing the structural organization of the solution composed of many chains, and the other pertinent to structural properties of a single chain. The latter means that DS can be used as an alternative to the single molecule techniques.

In the study of dynamics of long DNA chains, we have used lyophilized genomic Na-DNA. Specifics concerning studied materials have been given previously [13]. In this paper we concentrate on the details of the gel electrophoresis characterization and the procedure applied in order to extract the dielectric response of DNA from the bare data obtained in the DS measurements. Gel electrophoresis was performed to estimate the average length of polydisperse DNA fragments. This method is commonly used for separation of biological macromolecules of different sizes [18]. Since the electrophoretic mobility of a long polyelectrolyte in solution is independent of its total size, the electrophoresis needs to be used on gel which acts as a sieving medium, retarding the passage of molecules as a function of their size. We have carried out electrophoresis experiments on 0.5% agarose gel at room temperature. Measurements were performed on DNA dissolved in pure water and in NaCl electrolyte with different ionic strengths. Linearity of the DNA migration was ensured by a low amplitude (6 V/cm) electric field. Because of an expectedly wide distribution of DNA molecules two distinctive commercial size markers have been used: one for the size range between 100 and 10 000 base pairs (bp), and another for the 125 and 21226 bp range. We used bromophenol blue in order to track the migration of DNA fragments during the course of electrophoresis. In addition, the DNA fragments in the gel were stained with ethidium bromide whose molecules fluoresce under ultraviolet light. Once the electrophoresis was completed an image was taken under ultraviolet light of 302 nm (Fig. 1). Molecular sizes of migrated DNA are estimated from position and intensity of light stripes. The majority of migrated DNA fragments was in the range between 2 and 20 kbp. Since the scale of 0.34 nm corresponds to one base pair, we estimated that the average DNA fragments are 4 μm long.

Dry DNA samples were dissolved in either pure water or NaCl aqueous solution. A DNA solution droplet of 100 μL was applied between platinum electrodes of a home-made capacitive chamber. The chamber is closed and connected to the temperature control unit and the Agilent 4294A precision impedance analyzer which operates in 40 Hz–110 MHz frequency range. The measured quantities were conductance $G_{\exp}(\omega)$ and capacitance $C_{\exp}(\omega)$, both as functions of frequency $\omega = 2\pi\nu$.

The complex dielectric function is extracted from the measured $G_{\exp}(\omega)$ and $C_{\exp}(\omega)$ as follows. The measured conductance and the capacitance as a function of frequency for three representative DNA concentrations in pure water solutions are shown in Fig. 2. Reference samples were also measured in order to minimize stray impedances, including the free ion contribution and electrode polarization effects. Since the admittance contribution of each component in the solution is in good approximation

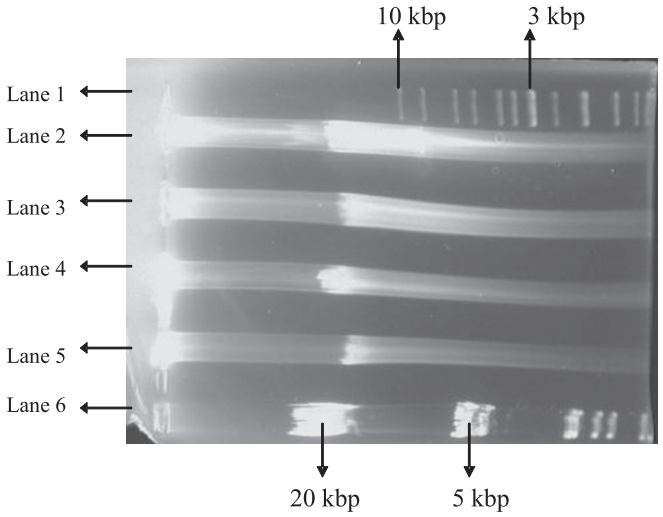


**Fig. 1.** The representative image of the agarose gel with DNA samples. In lanes 2, 3 and lanes 4, 5 are DNA pure water solution samples at concentrations of 0.33 mg/mL and 0.2 mg/mL, respectively. For comparison in lanes 1 and 6 are shown commercial DNA size markers for the 100–10 000 bp range and 125–21 226 bp range, respectively.

additive, the DNA response is given by $G(\omega)=G_{exp}(\omega)-G_{ref}(\omega)$ and $C(\omega)=C_{exp}(\omega)-C_{ref}(\omega)$, where $G_{ref}(\omega)$, $C_{ref}(\omega)$ is the response of a reference sample.

The DNA response is displayed in Fig. 3 for 0.1 mg/mL DNA pure water solution. Insets show the measured conductance $G_{exp}(\omega)$ and capacitance $C_{exp}(\omega)$ together with $G_{ref}(\omega)$, $C_{ref}(\omega)$ data obtained for a 0.14 mM NaCl reference solution of matching conductance and capacitance at 100 kHz and 10 MHz, respectively. Finally, the real and imaginary parts of dielectric function are extracted using relations

$$\varepsilon''(\omega)=(l/S)(G_{exp}(\omega)-G_{ref}(\omega)-G_{corr})/\omega\varepsilon_0 \quad (1)$$

$$\varepsilon'(\omega)=(l/S)(C_{exp}(\omega)-C_{ref}(\omega)-C_{corr})/\varepsilon_0 \quad (2)$$

where $l$ is the electrode separation ($l$=0.1021 cm) and $S$ the surface area (0.98 cm$^2$) for a 100 μL droplet, and $\varepsilon_0$ is the permittivity of vacuum.

Due to the imperfect matching of the reference solution small corrections $G_{corr}$ and $C_{corr}$ remain. They are read from the data in Fig. 3 and taken into account in the subsequent fitting procedure. The calculated Cole–Cole plots are presented in Fig. 4. The observed dielectric response can be well fitted by a sum of two Cole–Cole forms

$$\varepsilon(\omega)-\varepsilon_\infty=\frac{\Delta\varepsilon_{LF}}{1+(i\omega\tau_{0,LF})^{1-\alpha_{LF}}}+\frac{\Delta\varepsilon_{HF}}{1+(i\omega\tau_{0,HF})^{1-\alpha_{HF}}} \quad (3)$$

where $\varepsilon_\infty$ is the high-frequency dielectric constant, $\Delta\varepsilon$ is the dielectric strength, $\tau_0$ the mean relaxation time and $1-\alpha$ the symmetric broadening of the relaxation time distribution function of the low frequency (LF) and high frequency (HF) dielectric mode. Measured data were analyzed by using the least-squares method in the complex plane meaning that the same set of parameters fits both the real and imaginary spectra. The Kramers–Kronig consistency is demonstrated with Cole–Cole plots (Fig. 4). The LF mode contributes as the dominant arch, while the smaller HF mode is found near the origin of the axes. The DNA concentration dependences of dielectric strengths, broadening parameters and mean relaxation times as a function of DNA concentrations are shown in Fig. 5.

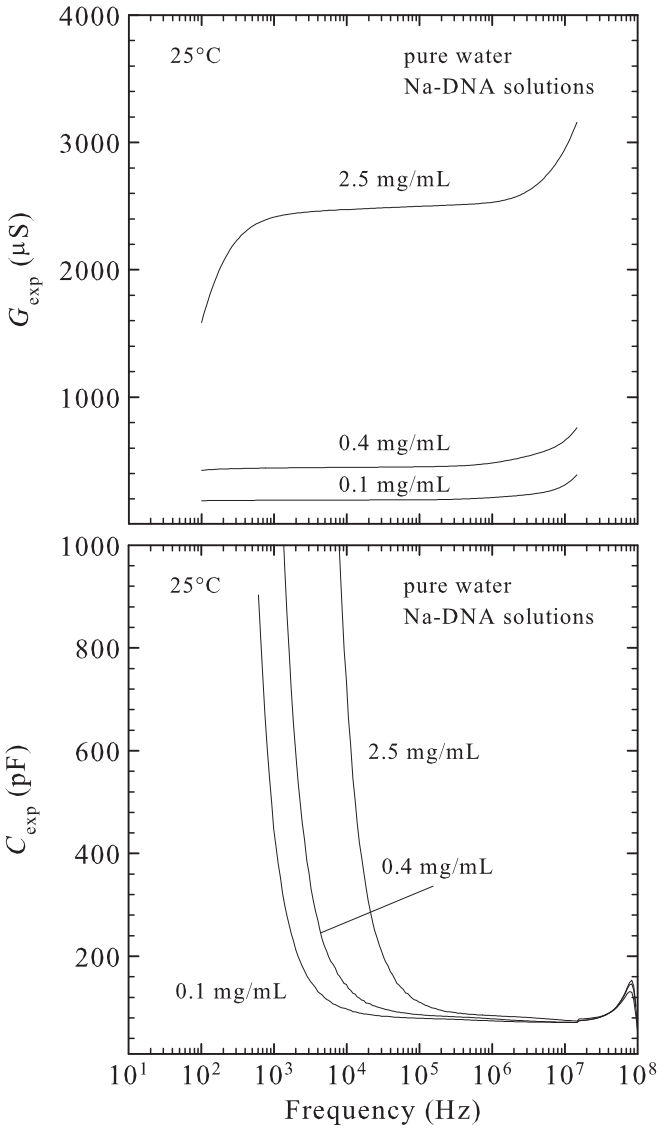


**Fig. 2.** Real part $G_{exp}(\omega)$ (upper panel) and imaginary part $C_{exp}(\omega)$ (lower panel) of the measured complex admittance versus frequency at $T$=25 °C of pure water DNA solutions for representative 0.1, 0.4, 2.5 mg/mL DNA concentrations.

## 3. Discussion and open issues

The characteristic length scale $L$ along which counterions oscillate was calculated from the measured mean relaxation time $\tau_0$, using the relationship $\tau_0 \propto L^2/D$, where $D$ was the diffusion constant of counterions. Two remarks are in order concerning the use of this formula. The first one is that a numerical coefficient in the scaling relationship between $\tau_0$ and $L$ is not known. In our work we have used this formula without any prefactor which turned out to be justified. The second remark concerns the most appropriate choice of the diffusion constant of oscillating counterions. We have analyzed the dielectric relaxation data by approximating the diffusion constant of counterions with the one of bulk ions. In the case of sodium counterions this means $D=1.33\times10^{-9}$ m$^2$/s. This approach was warranted by theoretical estimates of Bordi et al. [24]. In addition, a recent experimental work by Angelini et al. [25] gave evidence that this approach should be valid not only for the free counterions but for the condensed counterions as well [26]. However, the latest theoretical consideration by Manning seems to suggest that the renormalization of diffusion constant of condensed counterions cannot be neglected

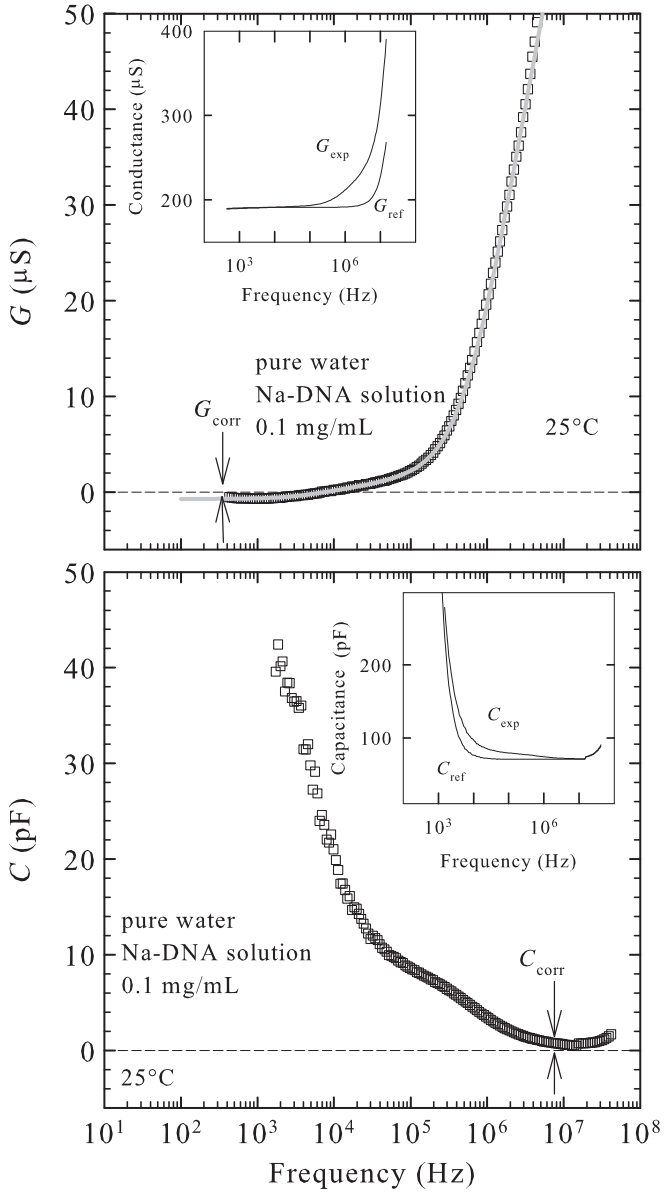


**Fig. 3.** Real part $G(\omega)$ (upper panel) and imaginary part $C(\omega)$ (lower panel) of the complex admittance $((G_{exp}(\omega)-G_{ref}(\omega),\ C_{exp}(\omega)-C_{ref}(\omega))$ versus frequency at $T=25$ °C of pure water DNA solutions for a representative 0.1 mg/mL DNA concentration. The correction constants are denoted $G_{corr}$ and $C_{corr}$. Insets: frequency dependence of the conductance (upper panel) and capacitance (lower panel) for 0.1 mg/mL DNA solution ($G_{exp}(\omega)$, $C_{exp}(\omega)$) and the matching reference NaCl solution of 0.14 mM ($G_{ref}(\omega)$, $C_{ref}(\omega)$).

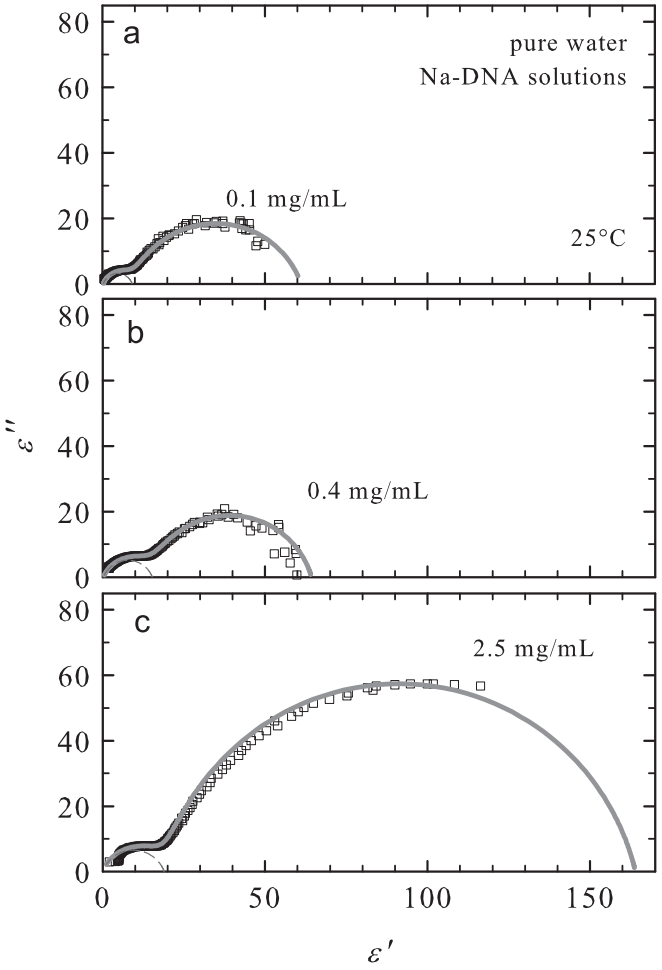


**Fig. 4.** (a)–(c) Cole–Cole plots of the dielectric response for representative 0.1, 0.4, 2.5 mg/mL DNA concentrations. The full line is a fit to the sum of the two Cole–Cole forms; the dashed line represents a single Cole–Cole form of the HF mode.

due to a prominent electrostatic term [27]. Nevertheless, our DS results indicate that the use of a bulk diffusion constant is justified for both the free and condensed DNA counterions. This conclusion is upheld by the fact that the three relevant fundamental length scales extracted from our DS measurements correspond surprisingly well to the theoretically expected values at the quantitative level, which justifies our choice of the diffusion constant and the use of formula $\tau_0 \propto L^2/D$ without any prefactor. These three fundamental length scales are the Debye screening length in 1 mM added salt (10 nm), the structural persistence length [28] of DNA (50 nm) observed in the study of long DNA chains [13] and the contour length [29] of 146 bp DNA fragments (50 nm) [15]. The contour length was detected due to the diffusive motion of condensed counterions along single polyion chains, while Debye screening length and the structural persistence length were observed in added salt conditions in which both condensed and free counterions oscillated along single polyion chains. In short, our work clearly showed that both kinds of counterions participate in the dielectric response of DNA. An opposing view was raised by Penafiel et al. on the basis of DS measurements on polyacrylic acid with different degrees of ionization [30], claiming that only condensed counterions contributed to the relaxation since they are associated with the polyion, while the free counterions were indistinguishable from bulk ions of the same species. This view was somehow adopted by Manning who suggested that free counterions might not contribute to the dielectric relaxation in polyelectrolytes in general [27]. In any event, these confronting views on the role of free counterions and the relevant value of the diffusion constant deserve further attention.

Next, we comment on some aspects of relevant fundamental length scales which follow power laws as a function of polyion concentration and added salt concentration. The observed exponents can be nicely understood in the framework of existing theoretical models which are valid in the regime of repulsive electrostatic interactions. It is noteworthy that the behavior observed by DS showed some features that are specific for DNA solutions. The first one is the appearance of locally fluctuating regions (DNA denaturation bubbles) with exposed hydrophobic cores at low DNA concentrations [13]. We think that this DNA state is basically different from the denatured state as a "ground state" of DNA. The second one is an extremely high flexibility for

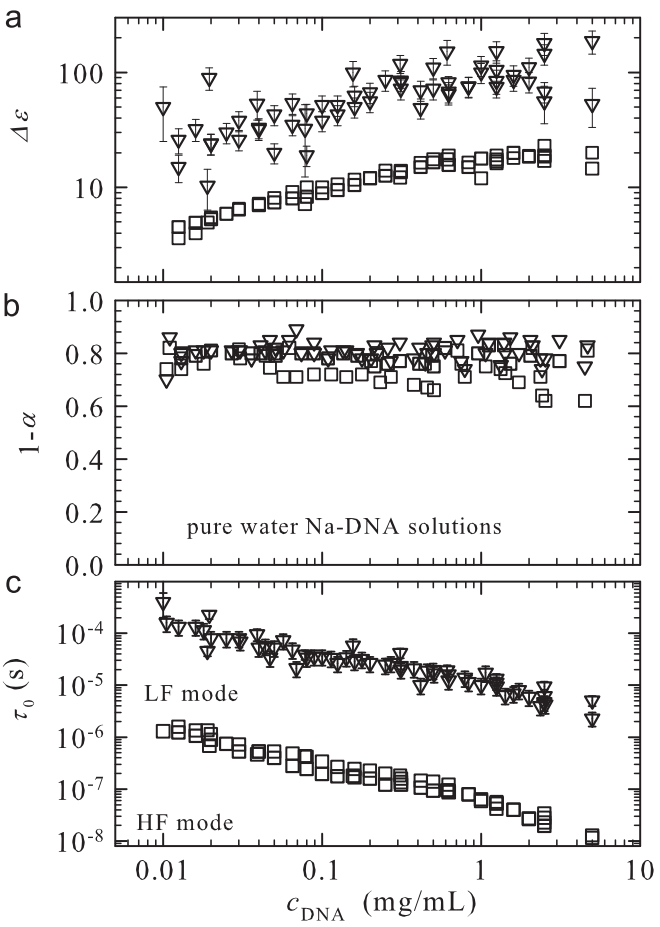


**Fig. 5.** (a) Dielectric strength, (b) broadening parameter and (c) mean relaxation time of DNA pure water solutions as a function of DNA concentration. Open triangles and squares stand for the LF and HF modes, respectively.

short ds-DNA fragments [15] which might be quantified by the persistence length as small as 25 nm for 146 bp DNA. This behavior has also been observed by Fluoroscence resonance energy transfer (FRET) and SANS techniques [31]. In contrast, several groups in the past [19,20] have determined persistence length for short DNA fragments using electric dichroism and birefrigence experiments and reported the persistence length values of about 50 nm. Moreover, they found that 146 bp DNA segments remain fully extended in 0.2–2 mM added salt range. This result does not comply with DS measurements which indicated extremely high 146 bp DNA flexibility. The apparent contradiction can be reconciled by noting that these different techniques, DS on one side and birefringence and electric dichroism on the other, place the DNA in drastically different environments. If only a small ac electric field is applied, an addition of salt induces a shorter length scale associated with incipient dynamic dissociation of the two strands of DNA, while a much larger dc field aligns DNA fragments which remain fully extended with added salt.

A few words are in order concerning the comparability of single polyion chain conformation properties obtained by the DS technique and single molecule techniques. A non-technical aspect of this issue reduces to the question whether the conformational properties of a single (isolated) molecule remain the same when it is placed in the midst of other molecules and counterions. A comparison of our DS measurements of the DNA persistence length as a function of added salt concentration and force-measuring laser tweezers experiments by Baumann et al. [32] unfortunately does not answer this question. The results obtained by these two techniques were qualitatively similar and agree with the behavior predicted by the Odijk–Skolnick–Fixman (OSF) theory [33,34]. However, the effective charge densities obtained through the OSF expression differ for the two techniques, and at the same time neither corresponds to the theoretically expected value. To our best knowledge, this issue is still open for discussion.

The final remark concerns the relevance of our experiments for the biological conditions. Namely, our measurements are done in the range between 0.01 and 5 mM added salt concentrations, while the salinity in the living cell is of the order of 100 mM. We consider that in order to develop physical concepts for a unified conceptual picture which would explain the relationship between structure and functions of biological matter, it is certainly helpful to also understand the structure of cell components like DNA in the several decades wide "low salt limit", which is also studied by other groups with different methods [35]. As pointed out by these authors, investigations in the absence of the added salt but in concentrated polyelectrolyte regimes might be relevant for biological systems.

## 4. Prospects

The ability of rather stiff and highly charged DNA polyions to condense into compact structures in the presence of multivalent counterions has been recognized to be of major biological interest for a long time [36]. Only fairly recently did this problem captured the interest of physicists who proposed that DNA condensation might be a purely electrostatic phenomenon. Interestingly, Shklovskii invoked the idea of electronic Wigner crystal from solid-state physics and suggested that a strongly correlated liquid of counterions in the vicinity of DNA surface is at the origin of DNA condensation [37]. Similarly, others proposed theoretical explanations of like-charge attraction [4,7] through counterion correlations since mean-field treatment of the electrostatic interactions on the Poisson–Boltzmann level always lead to repulsive interactions between like-charged molecules.

Usually the appearance of strong attractive forces and the consequent collapse of DNA is connected with the presence of tri- and higher valence cations interacting with the phosphate groups of DNA strands [6]. Much less is known about the ability of divalent counterions that usually do not induce attractive interaction and consequently do not cause a collapse of ds-DNA, but do condense ss-DNA. We have thus decided to pursue these and similar topics in our research of dynamics and structure of DNA in aqueous solutions in the future. Preliminary results are gratifying and show an increased stability of double-stranded conformation of DNA with divalent counterions as opposed to univalent counterions in pure water solutions.

## Acknowledgments

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## References

[1] F. Oosawa, Polyelectrolytes, Marcel Dekker, New York, 1971.
[2] J.R.C. Van der Maarel, Introduction to Biopolymer Physics, World Scientific, Singapore, 2007.
[3] A.Yu. Grosberg, et al., Rev. Mod. Phys. 74 (2002) 329.

[4] A. Naji, et al., in: W.-B. Hu, A.-C. Shi (Eds.), Understanding Soft Condensed Matter via Modeling and Computations, World Scientific, Singapore, 2010, p. 265.
[5] A.V. Dobrynin, et al., Prog. Polym. Sci. 30 (2005) 1049.
[6] A.G. Cherstvy, Phys. Chem. Chem. Phys. 13 (2011) 9942.
[7] M. Kanduč, et al., J. Chem. Phys. 132 (2010) 224703.
[8] R.R. Netz, et al., Phys. Rep. 380 (2003) 1.
[9] V.A. Bloomfield, Biopolymers 44 (1997) 269.
[10] P.G. de Gennes, et al., J. Phys. (Paris) 37 (1976) 1461.
[11] A.V. Dobrynin, et al., Macromolecules 28 (1995) 1859.
[12] S. Tomić, et al., Phys. Rev. Lett. 97 (2006) 098303.
[13] S. Tomić, et al., Phys. Rev. E 97 (2006) 098303.
[14] T. Vuletić, et al., Phys. Rev. E 82 (2010) 011922.
[15] S. Tomić, et al., EPL 81 (2008) 68003.
[16] T. Vuletić, et al., Phys. Rev. E 83 (2011) 041803.
[17] S. Tomić, et al., Macromolecular Symposia, vol. 305, 2011, p. 43.
[18] J.L. Viovy, Rev. Mod. Phys. 72 (2000) 813.
[19] P.J. Hagerman, Biopolymers 20 (1981) 1503.
[20] H.H. Chen, et al., Nucleic Acids Res. 10 (1982) 3561.
[21] F. Bordi, et al., J. Phys.: Condens. Matter 16 (2004) R1423.
[22] N. Nandi, et al., Chem. Rev. 100 (2000) 2013.
[23] R. Buchner, et al., Phys. Chem. Chem. Phys. 11 (2009) 8984.
[24] F. Bordi, et al., Phys. Rev. E 66 (2002) 021803.
[25] T.E. Angelini, et al., Proc. Natl. Acad. Sci. USA 103 (2006) 7962.
[26] For the strongly charged polyelectrolytes such as DNA two types of counterions can be distinguished: condensed counterions which are tightly bound to polyions and free counterions distributed in a larger volume around the polyions. Under the action of applied small ac field, the former are assumed to move along the polyion chain, whereas the latter oscillate throughout a polyion network.
[27] G.S. Manning, Eur. Phys. J. E 34 (2011) 39.
[28] The persistence length is a measure of the polymer's flexibility and can be described as the correlation length for orientational correlations along the molecular axis.
[29] The contour length of polymer is defined as $L_c = N \cdot a$, where $N$ is the number of monomers and $a$ is the size.
[30] L.M. Penafiel, et al., J. Chem. Phys. 97 (1992) 559.
[31] C. Yuan, et al., Phys. Rev. Lett. 100 (2008) 018102.
[32] C.G. Baumann, et al., Proc. Natl. Acad. Sci. USA 94 (1997) 6185.
[33] T. Odijk, J. Polym. Sci.: Polym. Phys. 15 (1977) 477.
[34] J. Skolnick, et al., Macromolecules 10 (1977) 944.
[35] E. Raspaud, et al., Phys. Rev. Lett. 84 (2000) 2533.
[36] V.A. Bloomfield, et al., Nucleic Acids: Structures, Properties, and Functions, University Science Books, Sausalito, 2000.
[37] B.I. Shklovskii, Phys. Rev. Lett. 82 (1999) 3268.