DNA IN AQUEOUS SOLUTIONS WITH REPULSIVE INTERACTIONS: STRUCTURE DETERMINED ON THE BASIS OF DIELECTRIC SPECTROSCOPY MEASUREMENTS

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ABSTRACT

We overview the study of dynamics and structure of the most famous biopolymer deoxyribonucleic acid (DNA) aqueous solutions, which has been conducted primarily in the laboratories at Institut za fiziku in Zagreb during the last six years. For completeness we briefly comment on our study performed on another biopolymer hyaluronic acid (HA) which has been gaining popularity quite rapidly within laymen and large public community recently. The aim of this Academy Report is to bring together the motivation for this research, the description of material preparation, experimental methods and data analysis used, as well as the presentation of the main results. As far as the physics interactions are concerned, our studies were focused until now on the case of repulsive regime of intersegment interactions mediated by univalent counterions. At the end 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. In other words, this Report is a kind of compilation of the material from our peer reviewed articles published in the international physics journals. We consider that the primary achievement of our efforts is the technique of dielectric spectroscopy (DS) developed in the laboratories of Institut za fziku in Zagreb, which enables to study small quantity soft matter and biological samples in solution. We demonstrate that this method, which is basically a method to measure the dielectric relaxation under an applied small ac electric field is a powerful technique to determine the structural properties thanks to a tight connection between these two physical parameters. Moreover, the DS method can serve to successfully discern between the structural properties of DNA solutions composed of many chains, and the single chain structural properties. The latter places the DS method at the level of well developed and widely used single-molecule micro/nano-manipulation techniques like force-measuring laser or magnetic tweezers. Our data consistently show two dielectric relaxations in *100 Hz – 100 MHz* frequency range that can be attributed to diffusive motion of polyion counterions. The overall study as a function of polyion length, concentration and added salt concentration demonstrates that the motion of polyion counterions detected at MHz frequencies probes the structural organization of the polyion network in solution, where-

as the motion at kHz range is correlated with single polyion conformational properties. Fundamental length scales found to characterize the polyelectrolyte structure differ for the dilute and semidilute regime and depend on the strength of repulsive electrostatic interactions and the flexibility; they also compare well with the fundamental length scales predicted by theory.

Keywords: biopoly electrolyte, dielectric response, dynamics and conformational univalent countenious

I. INTRODUCTION

We have been strongly motivated to use physics approaches and its powerful tools to help in characterization of living matter. A full description of life processes is of fundamental importance for speeding up the development of bio-nanotechnology and new approaches in medical sciences for diagnosis and healing.

Charged polyelectrolytes present one of the basic concepts in condensed/soft matter physics and a large amount of research has been dedicated to them in the last decades. At the same time they are fundamental components of biological environment and make their mark in its every structural and functional aspect. Most famous bio-polyelectrolytes range from deoxyribonucleic acid (DNA), ribonucleic acid (RNA), polypeptides and polysaccharides such as hyaluronic acid (HA) and proteins, all the way to molecular aggregates such as bacterial fd viruses and the tobacco mosaic virus[1]. In a living cell these biopolymers function in an aqueous environment at room temperature and their charge-derived properties are in direct relation to their physical properties and biological functions[2]. From the point of view of a condensed matter physicist, this very limited temperature range contrasts unfavourably 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 biopolymers 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 it 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 polyelectrolytes are usually stretched due to electrostatic repulsions and therefore statistically assume a rod-like configuration[8], whereas a high screening results in a conformation which is best described as a random walk of correlation blobs. Polyvalent counterions on the other hand can turn electrostatic repulsion into attraction[9]. This *s.c.* correlation effect is one of the most important features of the polyvalent counterions and can strongly reduce the rigidity of charged polymers which then collapse into highly compact states[10]. DNA is in many respects a paradigm of a semiflexible highly charged polymer whose complex behavior was studied in great detail. In aqueous solutions it assumes a conformation of an extended statistical coil, whereas in vivo long genomic DNA is usually folded in dense and compact states to fit within the micron-sized nucleus of eukaryotic cells or even smaller nano-scale viral capsids[11]. This behavior is associated to strong attractive forces between DNA strands yielding the condensation effects[12]. Such wide range of complex behaviors of DNA is due to its connectivity, stiffness and strong electrostatic interactions. As we mentioned above, to a large degree this behavior seen in vivo can be closely reproduced in vitro by tuning the DNA concentration, varying the amount

of added salt, and in particular by increasing valency of the counterions[5,11]. A full description and understanding of single DNA chain structure together with the structural organization of DNA chains in aqueous solutions are of fundamental importance in the study of living systems.

In biology, it is widely accepted that the conformational properties of cellular components play a key role in determination of their functional behavior. Thus, over the years much effort has been invested to develop more advanced tools for structural determination, most of them being single-molecule techniques. Nevertheless, since the extraordinary conformational and dynamical properties of polyelectrolytes are tightly related, another route can be taken which involves measurement of dynamical properties of many polyelectrolyte chains in solution (s.c. tube experiment). The question which immediately arises is: can the spectroscopic tools applied in case of the tube experiment provide information about the single-chain structure? It turns out that the dielectric spectroscopy (DS) technique is a powerful tool, which is concomitantly able to detect and discern structural organization of the solution as an ensemble composed of many chains, as well as structural properties of a single chain. Over the last several years we have conducted an extensive investigation of dynamics of DNA and HA chains of different lengths in aqueous solutions with univalent sodium (Na) counterions. 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*[13-17]. 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. Quantification of the effects of diffusion and electrostatics was also studied and results were obtained showing valuability of current theoretical models[18]. In our recent review papers we have discussed more in detail different aspects of this research not published previously [19,20]. The aim of this Report is to describe the main aspects of materials used, experimental methods applied and the results obtained. The Report is organized as follows. In Section I we give a brief state-of-the-art in the research of dynamical and structural aspects of bio-polyelectrolytes. In Section II we describe materials and methods used for their characterization and preparation for DS experiments. Section III is dedicated to the Dielectric spectroscopy (DS) technique. Results are presented and discussed in Section IV. In the last section we summarize main aspects of our achievements and lay out the study we have started recently with the intention to delineate the future of DS research of the polyelectrolytes in aqueous solutions.

II. MATERIALS AND CHARACTERIZATION METHODS

Salmon testes and calf thymus lyophilized Na-DNA threads were obtained from Sigma and Rock-land, respectively. The nucleosomal Na-DNA fragments were provided by F.Livolant from Laboratoire de Physique des Soldes, University Paris-South. In the remainder of this Report former samples are referred as Na-DNA samples (or simply DNA samples), while the latter are referred as 146bp Na-DNA fragments (or simply 146bp DNA). We have also studied the hyaluronic acid sodium salt from streptococcus equi sp. Fluka 53747 obtained from Sigma-Aldrich. Here we give the basic information, while the remaining specifics can be found in our published papers.

The following materials have been studied: polydisperse Na-DNA whose most of the fragments were 2-20 kbp implying the contour length between 0.7-7 µm; nucleosomal Na-DNA i.e. 146bp fragments with the contour length of 50 nm and polydisperse Na-HA with 4000 monomers in average
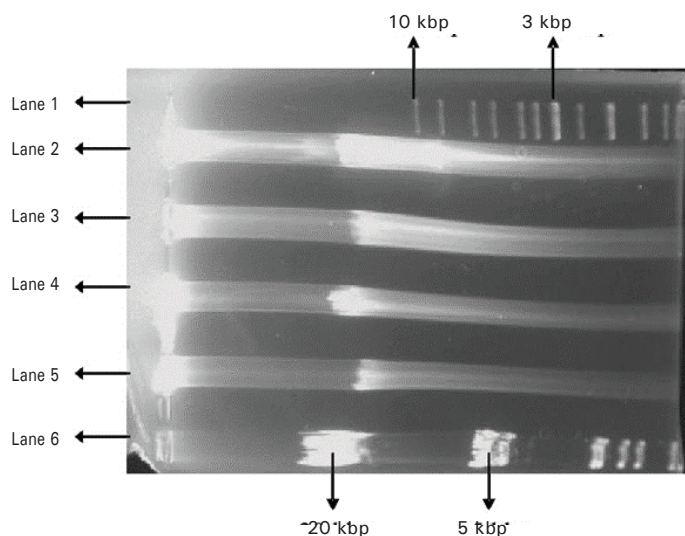
Figure 1. The representative image of the agarose gel with DNA samples. In lanes 2, 3 and lanes 4, 5 are DNA pure water solution samples at concentrations of 0.26 g/L and 0.16 g/L, respectively. For comparison in lanes 1 and 6 are shown comercioal DNA size markers for the 100 to 10000 bp range and 125 to 21226 bp range, respectively.

and 4 μm in length. We note that while the contour length of studied HA samples was provided by the producer, gel electrophoresis was performed in laboratories in Zagreb in order to estimate the average length of polydisperse DNA fragments. This method is commonly used for separation of biological macromolecules of different sizes[21]. 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 to 10 000 base pairs (bp), and another for the 125 to 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 2-20 kbp. Since the scale of 0.34 nm corresponds to one base pair, we estimated that the average DNA fragments are 4 μm long.

DNA solutions with different concentrations and different added salt ionic strengths were prepared according to two protocols as described below. Similar protocols were applied to prepare HA solutions.

I *Pure water DNA solutions*: Dry DNA threads were dissolved in pure water for 48 hours at 4°C so that the solutions within concentration range $0.01 \leq c \leq 15$ g/L were obtained.

II *DNA solutions with added salt:*

II1 NaCl was added to DNA water solution with a chosen c (prepared according to I), so that the added salt ionic strength was achieved in the range 0.01 mM$\leq I_s \leq$ 4 mM.

II2 DNA solutions with concentrations in the range $0.1 \leq c \leq 1.25$ g/L and with the added salt ionic strength $I_s = 1$ mM were prepared starting from a stock DNA solution in which DNA was dissolved in 10 mM NaCl for 48 hours at 4°C. Stock solution was dialyzed against 1 mM NaCl during 24 hours at 4°C.

Next, UV spectrophotometry measurements of the absorbance intensity of the DNA were used to measure the DNA purity. DNA absorbs UV light at 260 nm and aromatic proteins absorb UV light at 280 nm. The DNA sample is considered pure that is free from protein contamination if it

has the A_{260}/A_{280} ratio at 1.8 or smaller. We have verified that the protein content in our samples was low as declared by producers: A_{260}/A_{280}= 1.65 – 1.70 (Sigma) and A_{260}/A_{280}= 1.87 (Rockland).

Further, the measurements at 260 nm were done in order to verify nominal DNA concentrations. The method is based on the following considerations. The intensity of light passing through the solution decreases exponentially as described by the Beer-Lambert $I_t= I_0 e^{-\varepsilon cl}$ where I_0 is the incident light intensity, I_t is the transmitted light intensity, ε [L/gcm] is the extinction coefficient, l [cm] is the path length, and *c* [g/L] is the solution concentration. The difference between the incident and transmitted intensity is the intensity of absorbed light. The Beer-Lambert law is therefore often expressed in the form in which the absorbance is determined as: A = *log(I_0/I_t)* = ε *c l*. It should also be noted that the Beer-Lambert law is valid under the assumption that absorbance is proportional to the concentration. This is why we have been using three different cuvettes of path lengths 10 mm, 1 mm and 0.2 mm in order to measure absorbance in three DNA concentration ranges: 0.01 g/L < *c* < 0.08 g/L, 0.08 g/L < *c* < 0.5 g/L and 0.5 g/L < *c* < 2.5 g/L, respectively. We have verified by performing routine measurements of A_{260} as a function of the concentration of DNA that the absorbance was a linear function of the concentration of DNA in all three concentration regions. Thus, if the path length is known and the absorbance is measured, the DNA concentration can be deduced.

The concentration was determined assuming double-stranded conformation that is the extinction coefficient at 260 nm equal to 20 L/gcm. The measured concentrations for DNA solutions II2, performed on 10 aliquots, were consistently smaller by about 20% than the nominal ones. On the other hand, 146bp DNA showed measured concentrations which were smaller by about 40% than the nominal ones. We interpret this difference to be due to water content not taken into account by the spectrophotometry approach. Indeed, we have verified that lyophilized DNA if kept at 110°C for 30 min lost about 20% in weight, while we have found that 146bp DNA lost between 30 – 40% in weight. Throughout this Report we will refer to these measured (real) concentrations as the DNA concentrations. Note that this differs if compared with our publications on DNA samples where the results were given in terms of nominal concentrations (while the results on 146bp DNA were already given in terms of real concentrations).

We have also used UV spectrophotometry in order to study the stability of the double-stranded conformation of DNA in solutions with different concentrations and added salt ionic strengths. It is well known that the extinction coefficient of DNA strongly depends on the conformation. While an extinction coefficient at 260 nm ε = 20L/gcm corresponds to the double-stranded conformation of DNA, its value is 40% larger in the denatured single-stranded state[11]. The issue of DNA conformation in pure water solutions is of paramount importance: basically, the question is whether DNA at very low salt conditions is in double-stranded or single-stranded form. UV spectrophotometry experiments performed previously by Record[22] on T4 and T7 phage DNA have shown that ds-DNA denaturation depends not only on added salt concentration, but also on the concentration of intrinsic DNA counterions. Moreover, ds-DNA was found to be stable at 25°C dissolved in nominally pure water for the concentration of intrinsic counterions larger than 0.2 mM. This question has not been fully resolved yet, partially due to the fact that it appears that the answer depends on the experimental method applied. Namely, while dielectric spectroscopy results strongly indicate that the double stranded form of DNA is stable in all pure water solutions studied[14], UV spectrophotometry indicates that this may not be so (see Fig. 2)[23]. Of course, a precise and definitive information on the polyelectrolyte intrachain conformation of DNA solutions would demand the small-angle neutron scattering and/or X-ray scattering experiments performed at the same conditions.

At the end of this section we find useful to note the relationship which connects the molar concentrations and the concentrations by weight of monomers as well as of intrinsic counterions. For double-stranded DNA, monomers correspond to base pairs of molecular weight 660 g/mol and
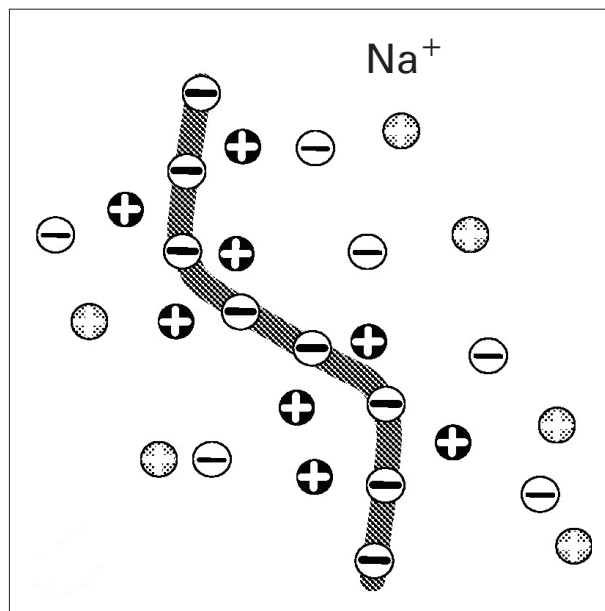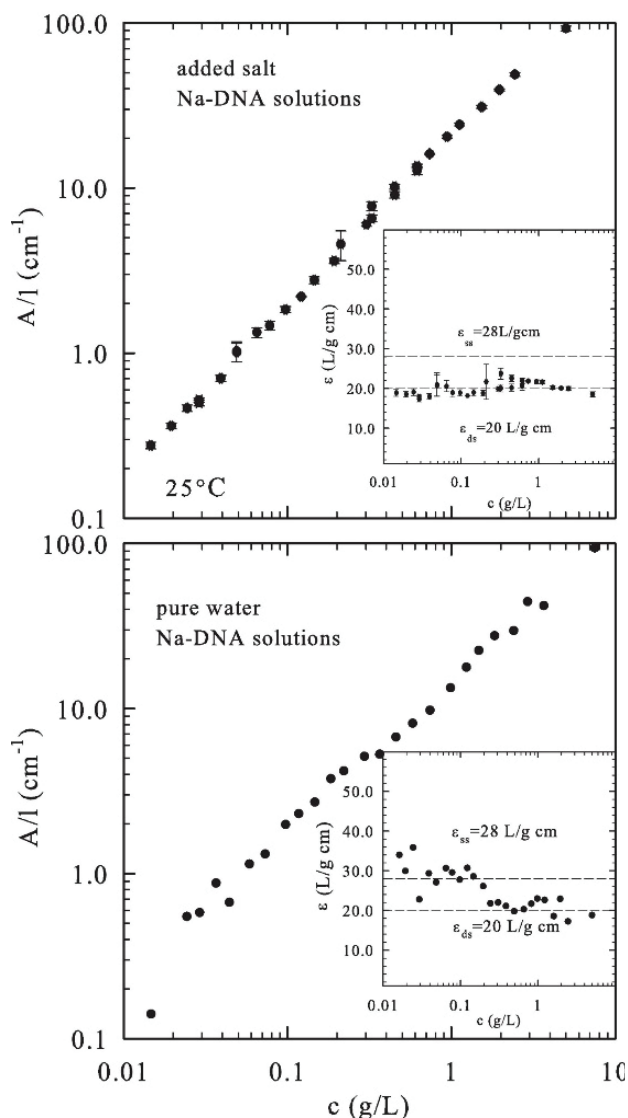
Figure 3. Schematic representation of a bio-polyelectrolyte: a negatively charged chain is surrounded by a cloud of positive sodium counterions (black circles). White and grey circles stand for the electrolyte ions in which the biopolymer is dissolved.

Figure 2. Upper panel: Normalized absorption (A/l, where l is the light path length) of Na-DNA in 1mM added salt as a function of DNA concentration. Inset: extinction coefficient versus DNA concentration indicating double-stranded conformation in the whole concentration range. Lower panel: The absorption of Na-DNA pure water solutions as a function of DNA concentration. Inset: extinction coefficient versus DNA concentration indicating double-stranded conformation may not be preserved at concentrations lower than 0.1 g/L.

there are two Na+ ion per DNA monomer. This means that the molar DNA concentration and the DNA concentration by weight are related by $c_{DNA}[mM] = c_{DNA}[g/L] \times 1.5$ µmol/mg. On the other hand, the concentration of intrinsic DNA counterions and the DNA concentration by weight are related by $c_{int}[mM]=c_{DNA}[g/L]\times 3$ µmol/mg. As for HA, disaccharide monomer is of molecular weight 401 g/mol and there is one Na+ ion per HA monomer. This implies that the molar concentrations of HA monomers, as well as Na+ counterions are related by $c_{HA}[mM]=c_{int}[mM]= c_{HA}[g/L]\times 2.5$ µmol/mg to the HA concentration by weight.

III. DIELECTRIC SPECTROSCOPY TECHNIQUE

Many applications in chemical, biological and medical areas rely on the response of the poly-ion to applied dc electric fields. On the other hand, the information on the structural features of the polyion can be also extracted from the response of the polyelectrolyte intrinsic counterions to applied ac electric fields by means of the DS technique[24]. 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[25,26]. Its main advantage is that 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. Note that 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[27,28].

In the following we describe the basics of DS technique and give detailed procedure of the measurement and data analysis. As we said above, DS probes the dynamics of counterion atmosphere which surrounds each biopolymer in a solution. Biopolymers like DNA and HA usually come as highly asymmetric salts with positive counterions such as sodium (Na) counterions. In aqueous solutions phosphate groups on DNA and carboxyl groups on HA are completely dissociated, making them charged polyelectrolytes. Intrinsic sodium counterions form the counterion atmosphere, and the dynamics of this fluctuating charge cloud, being sensitive to applied electric fields, can be studied by the DS. For the strongly charged polyelectrolytes such as DNA (implying that the relative strength of electrostatic interactions is larger than thermal motion of the counterions, see last part of Section IV) two distinct types of counterions can be distinguished. The first type are condensed counterions which are tightly bound to polyions, and the second type are free counterions distributed in a larger volume around the polyions. These counterion types should be considered transient, meaning that there is a constant dynamic exchange between them. On the other hand, in the case of weakly charged polyions such as HA the thermal energy is much stronger than electrostatic interactions and consequently only free counterions form the counterion atmosphere. Schematically, we can represent the biopolymer as a negatively charged chain in a cloud of positive sodium counterions (Fig. 3).

In DS experiments DNA (HA) solutions samples were studied. Two physically distinctive cases are of note. In the semidilute regime the chains are long enough to be entangled with each other, whereas in the dilute regime each segment is well-separated from the others (Fig. 4 (a), (b)).
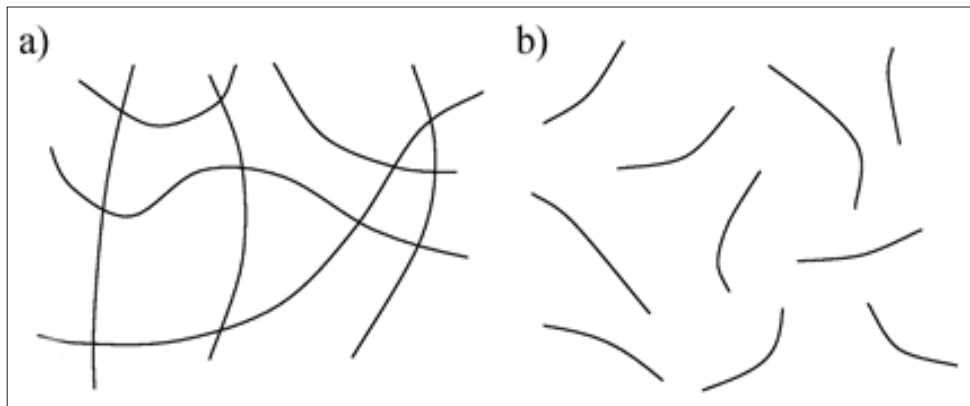

Figure 4. Long overlapping chains form a semidilute solution (a), whereas short chains or chains at much smaller concentrations are well separated from each other forming a dilute solution (b).

It is exactly that these two distinct solution regimes need to be distinguished in the case of dominant repulsive electrostatic interactions. Following the seminal work by de Gennes[29] 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].

**Figure 5**. Dielectric spectroscopy equipment at the Institut za fiziku in Zagreb.

Figure 6. Real part $G_{exp}(\omega)$ (upper panel) and imaginary part $C_{exp}(\omega)$ (lower panel) of the measured admittance versus frequency at T = 25°C of a representative 0.08 g/L pure water DNA solution. Insets: frequency dependence of the conductance (upper panel) and capacitance (lower panel) for 0.08 g/L pure water DNA solution. ($G_{exp}(\omega)$, $C_{exp}(\omega)$) and the matching reference NaCl solution of 0.14mM ($G_{ref}(\omega)$, $C_{ref}(\omega)$).
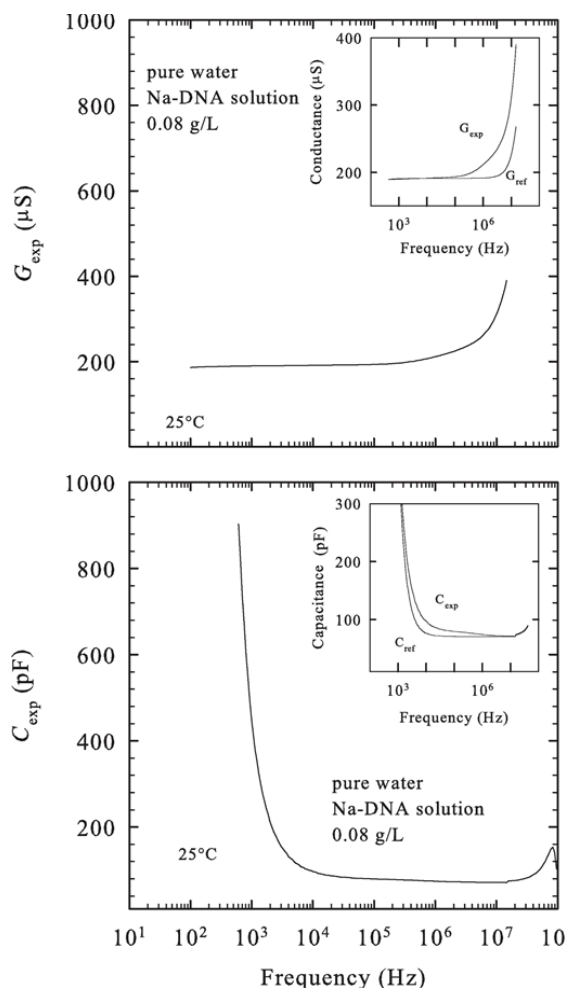
Under the applied small ac electric field the counterions oscillate and, since their displacements take place by diffusive motion, the dielectric response is basically characterized by the mean relaxation time $\tau_0 \propto L^2/D_{in}$, where L is the associated length scale, and D_{in} is the diffusion constant of counterions. Experimental data[31] and theoretical estimates[32] show that the renormalization of the diffusion constant of bulk ions due to the presence of polyions is negligible, leading to a value of $D_{in} = 1.33 \cdot 10^{-9}$ m²/s. In other words, if one can measure the relaxation time τ_0, then one can easily calculate corresponding length scales involved in counterion oscillations. As will become clearer in what follows, these length scales are defined by conformational features of either a single polyion chain or the structure of a many chain ensemble in solution. The former means that DS can be used as an alternative to the single molecule techniques.

DNA dielectric properties have been studied by a number of authors since the early 1960s (see[14] for references); however, no previous experimental work has been done in such a broad range of DNA concentrations and added salt and no work has been able to detect concomitantly two dispersions and associate them with the solution and single-chain structural properties. In addition, specially designed DS measurements were used to study DNA behavior before and after thermally induced denaturation in order to check the stability and integrity of the double helix in pure water DNA solutions. These studies indicated that ds-DNA was never denatured into two spatially distinguishable and well-separated single strands (see Section II for more information). An additional study of polydisperse, average fragments 4 μm long HA chains with much weaker electrostatic in-
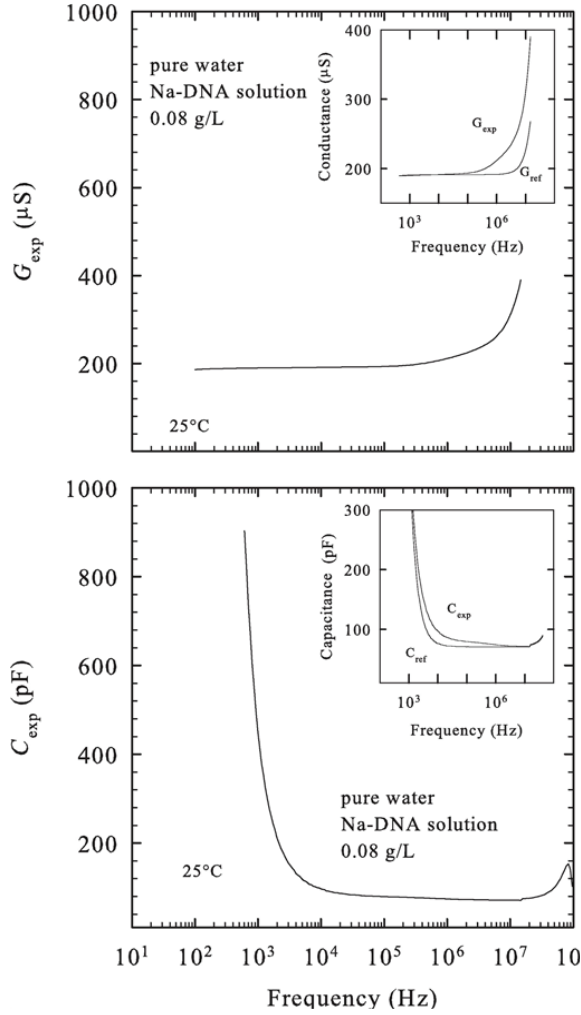
Figure 7. Real part $G(\omega)$ (upper panel) and imaginary part $C(\omega)$ (lower panel) of the differential admittance versus frequency at T = 25°C of a representative 0.08 g/L DNA pure water solution. The correction constants are denoted G^{corr} and C^{corr}.

teractions and much higher chain flexibility as compared to DNA clearly showed the relevance of these two parameters [16]. What follows is a description of how the parameters characterizing the DNA counterion dynamics from DS measurements are extracted and how they are connected with polyelectrolyte structural properties predicted by theoretical models.

Dielectric spectroscopy measurements were performed at room temperature (25°C) using a setup which consists of a home-made parallel platinum plate capacitive chamber and temperature control unit, in conjunction with the Agilent 4294A precision impedance analyzer operating in ν =40 Hz - 110 MHz frequency range. The capacitive chamber enables reliable complex admittance measurements with reproducibility of 1.5% of samples in solution with small volume of 100 µL and with conductivities in the range of 1.5 - 2000 µS/cm (Fig.5). Low ac amplitudes of 50 mV were employed in order to probe the DNA response in the linear regime, once we verified that for ac signal levels in the range between 20 mV and 500 mV the result was essentially the same. Admittance is standardly sampled at 201 frequencies at 27 points per frequency decade. At each frequency, admittance is sampled 10 times and averaged. In addition, three consecutive frequency sweeps are taken in order to average out the temperature variations. Total time for described measurement amounts to 60 sec[33].

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 a representative DNA concentration in pure water solution are shown in Fig. 6. 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 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. Insets in Fig. 6 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. The DNA response is displayed in Fig. 7 for 0.08 g/L DNA pure water solution.

Finally, the real and imaginary parts of dielectric function are extracted using relations

$$\varepsilon''(\omega) = (l / S) \, (G_{exp}(\omega) - G_{ref}(\omega) - G_{corr}) / \omega\varepsilon_0 \tag{1}$$
$$\varepsilon'(\omega) = (l / S) \, (C_{exp}(\omega) - C_{ref}(\omega) - C_{corr}) / \varepsilon_0 \tag{2}$$

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Figure 8. Double logarithmic plot of the frequency dependence of the imaginary (ε'') and real (ε') part of the dielectric function at T = 25°C of (a, b) pure water DNA solutions and (c, d) DNA water solutions with added salt Is = 1 mM for representative a1-a3 (2, 0.08, 0.01 g/L) and b1-b3 (0.66, 0.25, 0.1 g/L) DNA concentrations. The full lines are fits to the sum of the two Cole-Cole forms; the dashed lines represent a single CC form.

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 ε_0 is the permittivity of vacuum. Note that 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. 7 and taken into account in the subsequent fitting procedure. 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 ε_∞ is the high-frequency dielectric constant, $\Delta\varepsilon$ is the dielectric strength, τ_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 usually demonstrated with Cole-Cole plots (not shown here). Fig. 8 and 9 show the frequency dependent real and imaginary part of the dielectric function for selected DNA concentrations and added salts ionic strengths of Na-DNA and 146bp Na-DNA solutions, respectively. The main features of this response are two broad modes, whose amplitude and position in frequency depend on the DNA concentration and added salt. The param-

Figure 9. Double logarithmic plots of the frequency dependence of the imaginary (ε'') and the real part (ε') of the dielectric function at T = 25°C of pure water 146bp DNA solutions for DNA concentrations a1-a3 (5, 0.5, 0.05 g/L) and for 146bp DNA solutions with added salt Is = 1 mM for DNA concentrations b1-b3 (1.5, 0.8, 0.3 g/L). The full lines are fits to the sum of two Cole-Cole forms; the dashed lines represent the single form.
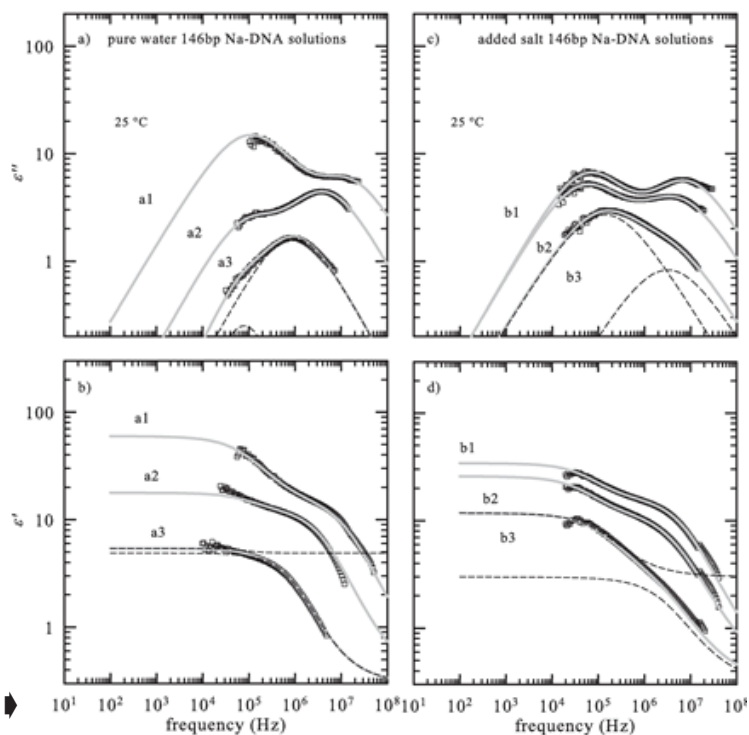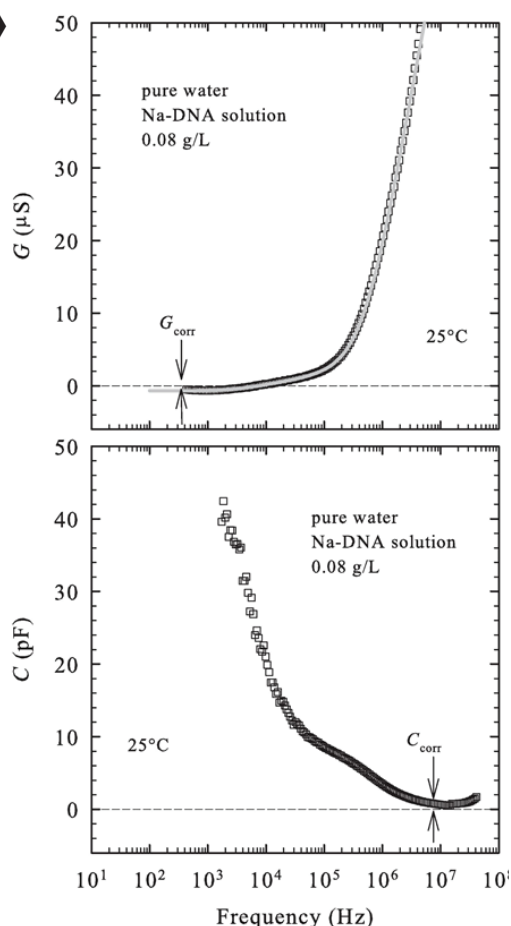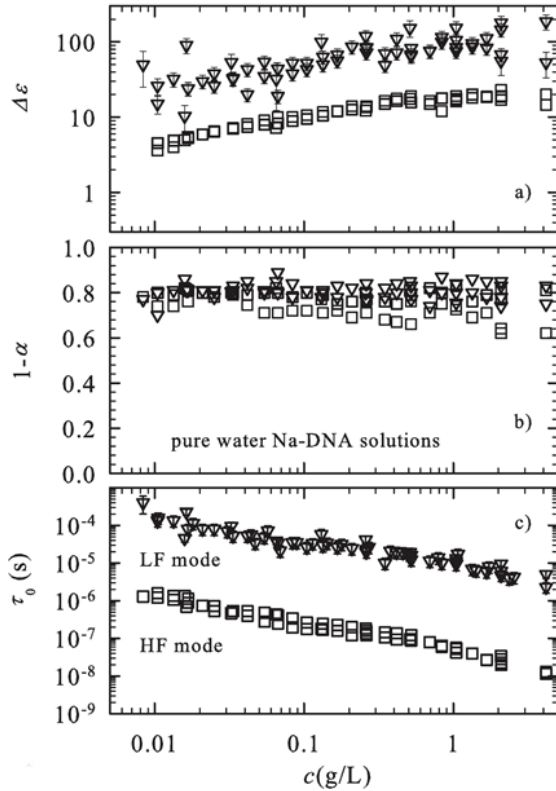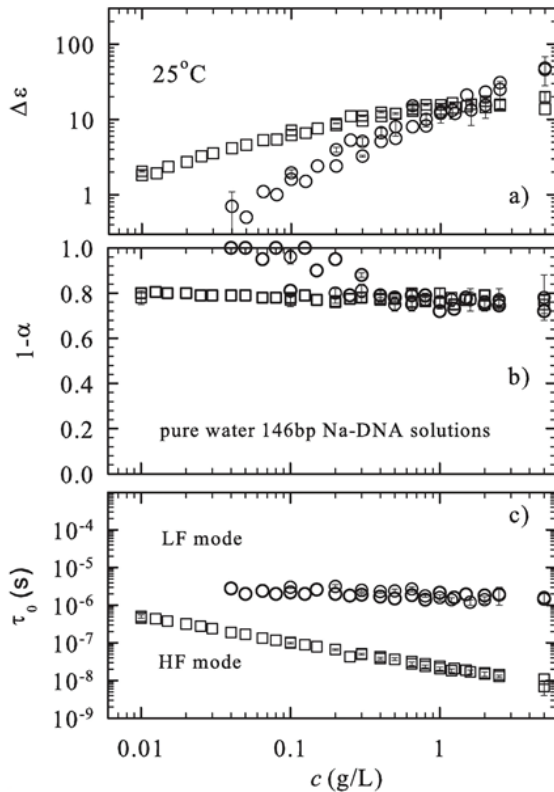
Figure 10. (a) Dielectric strength, (b) broadening parameter and (c) mean relaxation time of DNA pure water solutions as a function of DNA concentration. Open triangles and squares stand for the *LF* and *HF* mode, respectively.

eter $1 - \alpha$, which describes the symmetrical broadening of the relaxation time distribution function, is concentration independent and similar for both modes $1 - \alpha \approx 0.8$. The mode centered at higher frequencies is characterized by smaller dielectric strength than the mode centered at lower frequencies. In the remainder of this Report, we will refer to these modes as the high-frequency (*HF*) and low-frequency (*LF*) mode, respectively. Finally, the DNA concentration dependences of dielectric strengths, broadening parameters and mean relaxation times for the case of pure water solutions (for DNA and 146bp DNA), as a function of DNA concentrations are shown in Fig. 10 and 11. In the following section, we will describe how the parameters characterizing the counterion dynamics from DS measurements are extracted and how they are connected with polyelectrolyte structural properties predicted by theoretical models.

IV. RESULTS AND DISCUSSION

First, we extract the characteristic length scales from the measured relaxation times using the expression $\tau_0 \propto L^2/D_{in}$ (see section 3). We point out that a numerical coefficient in the scaling relationship between τ_0 and L is not known. In our work we have used this formula without any prefactor which turned out to be justified. 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. These three fundamental length scales are the Debye screening length in 1 mM added salt (10 nm), the structural persistence length of DNA


Figure 11. (a) Dielectric strength, (b) broadening parameter and (c) mean relaxation time of 146bp Na-DNA pure water solutions as a function of DNA concentration. Open circles and squares stand for the *LF* and *HF* mode, respectively.

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(50 nm) and the contour length of 146bp DNA fragments (50 nm). 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 (see the last part of this section for more information).

In what follows we show that the characteristic length scales observed in DS experiments compare nicely with the fundamental length scales predicted by existing theoretical models which are valid in the regime of repulsive electrostatic interactions. Our first important result is that the HF mode in MHz range probes the collective properties of the polyelectrolyte solution. The characteristic length scales for the case of Na-DNA in semidilute regime and 146bp Na-DNA in dilute regime are shown in Fig. 12.

In pure water polyelectrolyte solutions, the characteristic length L_{HF} decreases with increasing DNA concentration. In the case of Na-DNA in semidilute regime we find that at high DNA concentrations L_{HF} follows the power law $L_{HF} \propto c^{-0.5}$. This dependence corresponds to the solution mesh size or the de Gennes-Pfeuty-Dobrynin (dGPD) correlation length ξ, as theoretically expected for salt-free semidilute polyelectrolyte solutions[5,29]. The correlation length was first introduced by de Gennes as the length scale delimiting the space around each polyion in the solution from length on the polyelectrolyte density is obtained by Dobrynin et al. by minimization of the total interaction energy which consists of the electrostatic part and the elastic contribution due to the stretching of the chain. The interpretation of this scaling result is that for volumes smaller than ξ^3 the polyelectrolyte chain is stiffened by electrostatic interactions, whereas for scales larger than ξ, it behaves as a free-flight chain (random walk). In other words, the chain is a random walk of correlation blobs. Earlier, de Gennes approach was based on the argument that for long chains local properties should be independent of the degree of polymerization (N) implying that the correlation length is independent of N. Taking into account that at the crossover concentration, where dilute and semidilute regimes meet $c* \propto 1/L_c^2$, the correlation length is comparable to the contour length L_c, and assuming the scaling form $\xi \propto L_c (c*/c)^m$ one gets $\xi \propto c^{-0.5}$.

Coming back to the DS results in semidilute regime of long DNA, it is noteworthy that at low DNA concentrations the correlation length scales as $c^{-0.33}$ (see Fig. 12, left panel) which is typical
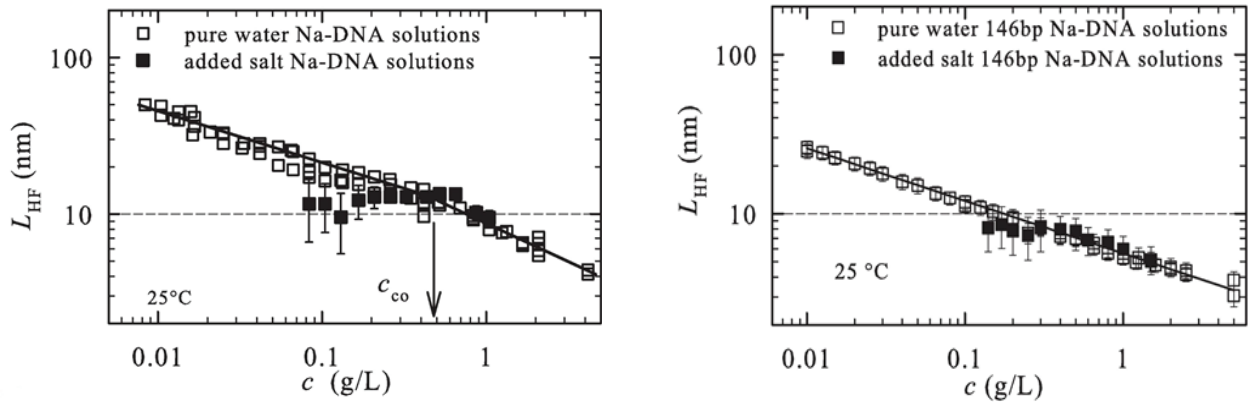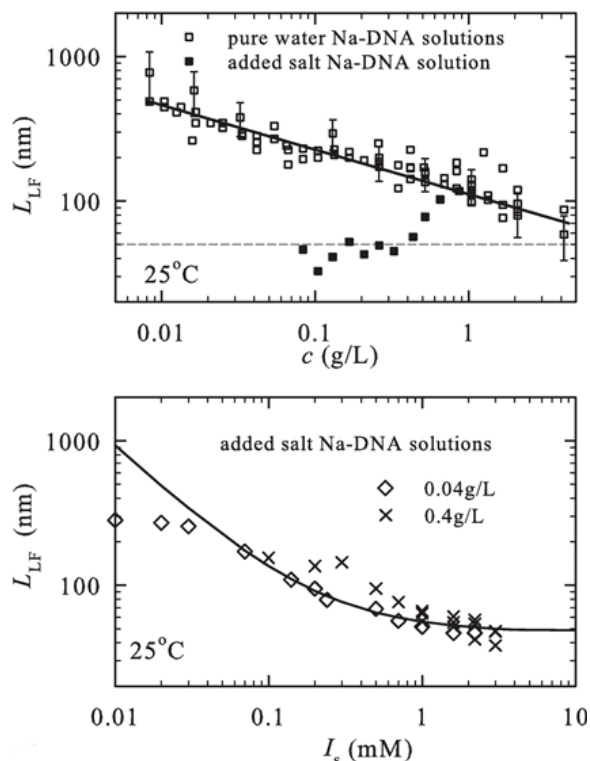

FIG. 12. Left panel: Characteristic length of the *HF* mode (L_{HF}) for pure water Na-DNA solutions (open squares) and for Na-DNA solutions with added salt *Is* = 1 mM (full squares) as a function of DNA concentration (c). The full line is a fit to the power law $L_{HF} \propto c^{-0.33}$ and $\propto c^{-0.5}$ for c smaller and larger than $c_{co} \sim 0.5$ g/L, respectively. Right panel: Characteristic length of the *HF* mode (L_{HF}) for pure water 146bp Na-DNA solutions (open squares) and for solutions with added salt *Is* = 1mM (full squares) as a function of DNA concentration (c). The full line is a fit to the power law $L_{HF} \propto c^{-0.33}$. Dashed line stands for the theoretically expected Debye lengths.

for semidilute solutions of charged chains with hydrophobic cores. The interpretation of this result is that at these low DNA concentrations and at the time scales of DS experiment local conformational fluctuations (DNA denaturation bubbles[34,35]) partially expose the hydrophobic core of DNA. We speculate that DNA denaturation bubbles with their exposed nitrogen bases could play the roles of hydrophobic cores as in the model of weakly charged polyelectrolytes[5]. In other words, we think that this DNA state is basically different from the denatured state as the ground state of DNA (see section II for more information). Finally, upon adding salt the dGPD correlation length gives way to the Debye screening length as a new relevant length scale for HF mode (full squares in Fig. 12, left panel). This occurs exactly when sufficient salt is added so that the corresponding Debye screening length becomes comparable to and eventually smaller than the dGPD correlation length.

In the case of short 146bp DNA fragments in dilute regime the characteristic length scale decreases with increasing concentration following power law $c^{-0.33}$ as expected for the average distance between chains in this regime (Fig. 12, right panel). On the other hand, the size of the observed characteristic length scale is smaller than the chain contour length of 50 nm (not expected in dilute regime) and can thus be ascribed to the reduced average distance between chains. It is noteworthy that these results are in nice agreement with the predictions of the theoretical model by Deshkovski et al.[36] and Dobrynin et al.[5] developed for polyelectrolytes in dilute regime where chains are as far away from one another as possible, due to the long-range repulsive interactions between them. In this model, the polymer is placed at the center of a cell that is subdivided into two zones: a smaller cylindrical one, inside which the electrostatic interaction energy is large, and a larger spherical zone where the electrostatic interactions are described by the low-coupling Debye-Hückel approximation. In the latter zone the counterions are distributed almost uniformly due to rather small variations of the electrostatic potential there. The response of DNA counterions to an applied ac field is mostly conned to the smaller cylindrical volume, thus their oscillation naturally reflects the cylinder size (as found in DS experiments) instead of the size of the sphere which corresponds to the average distance between chains. At the end, note that upon adding salt the average distance between chains, similarly as the correlation length, apparently shows a levelling-off, with a limiting value close to the Debye length (full squares in Fig. 12, left panel).

In what follows, results are shown which demonstrate that the LF mode in kHz range probes single-chain properties of the polyelectrolyte solution.

First, we address the *LF* mode in the semidilute regime. In the case of Na-DNA chains which are strongly charged and semiflexible, the characteristic length L_{LF} decreases with increasing DNA concentration following the power law $L_{LF} \propto c_{DNA}^{-0.25}$ (Fig. 13, upper panel). Note that this is true within data error bar, while the exact fitting exponent is -0.29 ± 0.04. This dependence corresponds to the average size of the chain R, as theoretically expected for the salt-free semidilute polyelectrolytes solutions[5,29]. In this low added salt limit, DNA acts as its own salt. We note that in this regime the polyelectrolyte is theoretically expected to behave as a random walk of correlation blobs. In our case, at $c = 1$ g/L the correlation length L_{HF} is about 10 nm (Fig. 12, left panel), while the chain size L_{LF} is about 100-150 nm (Fig. 13, upper panel). One easily calculates that a chain behaving as a random walk of correlation blobs of the size $\xi = 10$ nm and of about n = 250 steps would have size $R = \sqrt{n\xi} = 150$ nm, very close to L_{LF} that we found. Further, this chain would have a contour length somewhat larger than $n \cdot \xi = 2500$ nm, and we remind that the contour length of Na-DNA that we studied was 4 micrometres in average. Thus, the relationship between the correlation length and the chain size holds quantitatively within our data-set. For DNA solutions with added salt, $I_s = 1$ mM (full squares in Fig. 13, upper panel), the measured L_{LF} coincides with the characteristic length found in the salt free case only in the regime of DNA concentrations where the ionic strengths of DNA is larger than $2I_s$ of bulk added salt ions. In the opposite case the

Figure 13. Upper panel: Characteristic length of the *LF* mode (L_{LF}) for pure water DNA solutions (open squares) and for DNA solutions with added salt Is = 1 mM (full squares) as a function of DNA concentration (c). The full line is a fit to the power law $L_{LF} \propto c^{-0.29\pm0.04}$.; Lower panel: Characteristic length of the *LF* mode (L_{LF}) for DNA solutions with varying added salt (*Is*) for two representative DNA concentrations: *c* = 0.04 g/L (diamonds) and *c* = 0.4 g/L (crosses). The full line is a fit to the expression $L_p = L_0 + a \cdot I_s^{-1}$ s with $L_0 = 47$ nm and *a* = 9 nm mM.

measured L_{LF} starts to deviate from the line $L_{LF} \propto c_{DNA}^{-0.25}$ and decreases to attain a value of about 50 nm. Note that this value corresponds to the DNA structural persistence length.
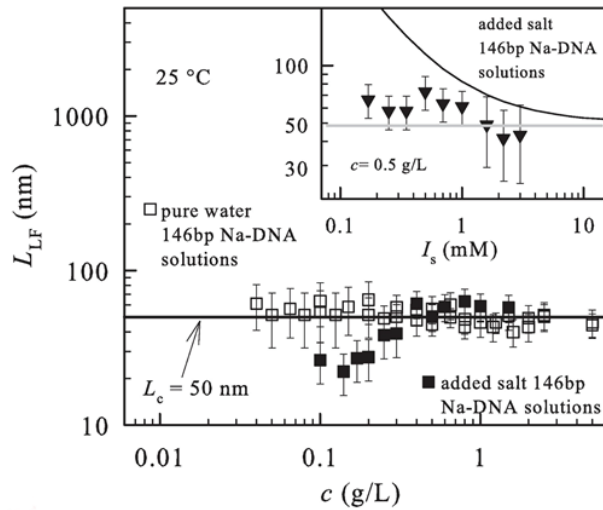
On the other hand, in the limit of high added salt the characteristic length L_{LF} behaves as the persistence length $L_p = L_0 + aI_s^{-1}$ (Fig. 13, lower panel) where $L_0 = 50$ nm is the structural persistence length and a I_s^{-1} is Odijk-Skolnick-Fixman electrostatic persistence length contribution, proportional to the inverse of the added salt ionic strength I_s, a quadratic function of the Debye length[37,38]. The persistence length is the correlation length of the polymer's molecular axis; in other words, this is the length over which the original directional correlation is lost. It is a measure of the chain flexibility and can be understood as the boundary between rigid behavior over short distances and flexible over large distances. The results in Fig. 13 demonstrate our next important result that concerns the issue of the respective roles of intrinsic DNA counterions and ions from the added salt in ionic screening. It is noteworthy that the OSF model applies as long as the ionic strength of added salt is larger than the ionic strengths of DNA. The data for *c* = 0.04 and 0.4 g/L deviate from the OSF behavior for $I_s < 0.05$ mM and for $I_s < 0.5$ mM, respectively. At these low added salt values ($I_s < 0.4 I_{DNA}$), where $I_{DNA} = 3c$, we expect that the intrinsic counterions become dominant in determining the behavior of L_{LF} and indeed it attains the same value as in pure water DNA solutions (see Fig. 13, upper panel). Our data thus seem to indicate that for the *LF* mode the characteristic length in our system in the low added salt limit is given by the average size of the chain, i.e. the DNA polyelectrolyte chain behaves as a Gaussian chain with a size $\propto c^{-0.25}$, and with added salt goes smoothly into the OSF form of the persistence length. This means that when salt is added to the system the role of screening is transferred from other chains and counterions, thus from DNA as its own salt, to the added salt ions and thus emerges as the OSF value of the persistence length. Very similar conclusions have been reached also by Record[22] while analyzing T4 and T7 phage DNA denaturation. He observed that in this system too the screening depends not only on added salt concentration, but also and more importantly on the concentration of intrinsic DNA counterions - on the DNA as its own salt.

At the end, we address the *LF* mode in the dilute regime which was studied for the case of short 146bp DNA fragments. Theoretical models describe them as nonuniformly stretched chains due to the fact that interchain interactions are negligible compared to intrachain interactions (in contrast to the semidilute regime). Indeed, our observations confirm this expectation and reveal the concentration-independent contour length of 50 nm as the fundamental length scale (see Fig. 14). This result holds as long as DNA ionic strength ($I_{DNA} = 3c$) is larger than the added salt ionic

strength (I_s). Once the added salt prevails, an unexpected behavior emerges: the single-chain length scale shrinks in size, becoming two times smaller than the nominal contour length of the chain. This effect cannot be due to a decrease of rigidity as quantified by the persistence length, since structural persistance length is 50 nm for ds-DNA. The interpretation of this result is that it is due to incipient dynamic dissociation which induces short bubbles of separated strands. Model calculations on short DNA fragments indeed showed[34,35] that these local denaturations can occur at physiological temperatures and despite being rare and transient lead to a lower value for the persistence length of short DNA segments. Independent Fluorescence Energy Transfer (FRET) and SAXS experiments performed on a set of short DNA fragments between 10bp to 89bp in size (3 - 30 nm) confirm this result[39]. Moreover, these experiments also reveal that DNA appears softer as its length decreases and that this base-pair breathing happens at millisecond time scales.
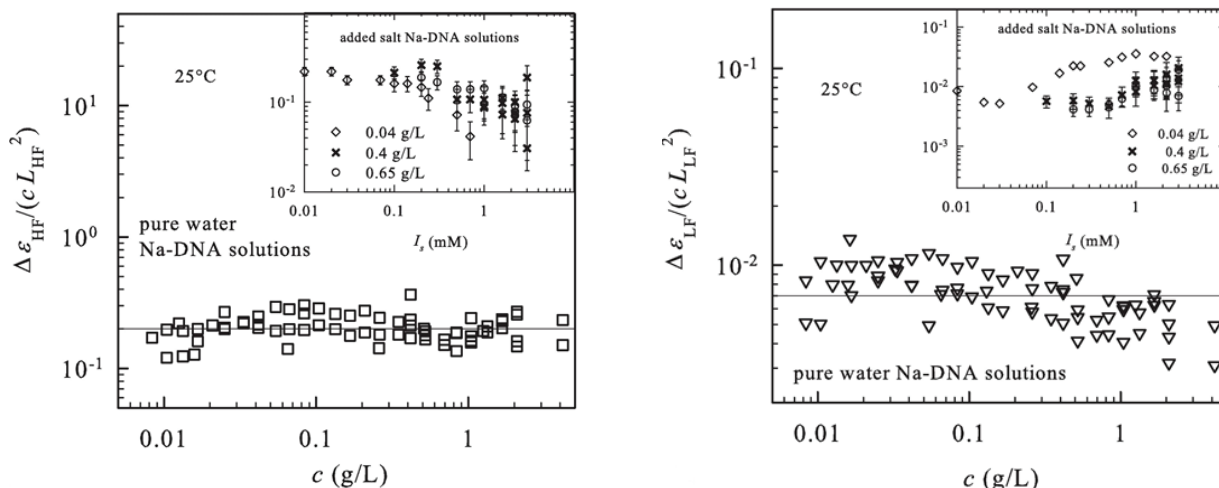
Additional data (inset of Fig. 14) for $c = 0.5$ g/L with varying added salt show that L_{LF} does not vary with I_s in most of the measured range of added salt. When added salt concentration is larger than DNA concentration, the behavior of L_{LF} indicates only a minor decrease, in contrast to a more substantial one shown by 1 mM data (main panel of Fig. 14). This discrepancy might be ascribed, similarly as for the *HF* process, to a poor accuracy of former data, which are severely influenced by high salt environment. The added-salt-independent behavior in the limit of low added salt is not surprising since the contour length of the short-fragment DNA chains is on the order of the DNA intrinsic persistence length. This fact immediately excludes the effects of electrostatic interactions on the persistence length as predicted by OSF theory and shown for comparison in inset of Fig. 14.

In the last part of this section we present the behavior of dielectric strength defined as $\Delta\varepsilon \approx f c_{in} \cdot \alpha$, where f is the fraction of counterions participating in the *HF* or in *LF* process, c_{in} [mM] $= c_{DNA}$ [mg/mL] $\times 3$ μ mol/mg (as explained in Section II) and α is the corresponding polarizability. The polarizability α is given by the scaling form $\alpha \propto e^2 \cdot L^2 /(\varepsilon_0 kT)$. Therefore, the fraction of counterions f participating in the *HF* and *LF* process is proportional to $\Delta\varepsilon/(c \cdot L^2)$. In Fig. 15 and 16 we show the dependence of f on DNA concentration for the Na-DNA and for the 146bp Na-DNA, respectively.

Our results reveal that the standard theoretical approaches based on the Manning-Oosawa (MO) counterion condensation theory describe well the dielectric response of the counterion atmosphere around polyions. The charge density parameter η thus measures relative strength of electrostatic interactions vs. thermal motion and is strongly dependent on the valency of the counterions. $\eta = z l_B/b$, where z is the valency of the counterion, b is the linear charge spacing and l_B is the Bjerrum length, defined as $l_B = e_0^2 /(4\pi\varepsilon\varepsilon_0 kT)$. In the MO theory the counterions accumulate in the condensed layer exactly to such an extent that the effective charge density parameter η is reduced to 1[11], i.e. the effective separation between charges is increased from b to l_B. The condensed counterions in the MO theory are still assumed to be perfectly mobile. Because

Figure 14. Main panel: Characteristic length of the *LF* mode (L_{LF}) for pure water 146bp Na-DNA solutions (open squares) and for solutions with added salt I_s = 1mM (full squares) as a function of DNA concentration (c). The full line denotes the contour length $L_c \approx 50nm$. Inset: L_{LF} for 146bp Na-DNA solutions with varying ionic strength of added salt for $c = 0.5$ g=L (full triangles). The grey and full lines denote the contour length of studied 146bp Na-DNA and the persistence length as predicted by the OSF theory, respectively.

Figure 15. Normalized dielectric strength of the *HF* (left panel) and *LF* (right panel) mode as a function of DNA concentration for pure water Na-DNA solutions. The full line is a guide for the eye. Insets show normalized dielectric strength of the modes vs. ionic strength of the added salt for three representative DNA concentrations: $c = 0.04$ g/L (diamonds), $c = 0.4$ g/L (crosses) and $c = 0.65$ g/L (circles).

of counterion condensation the effective charge of DNA is reduced by a factor $r = 1 - 1/(z\eta)$. In the case $z = 1$ this implies that the fraction of condensed counterions is $1-f = 1-1/\eta$, while the fraction of free counterions is given by $f = 1/\eta$. Note that the Manning parameter is concentration-independent and this implies the concentration - independent $f = 1/\eta$ exactly as found in our experiments on DNA pure water solutions. Since the *HF* relaxation happens at the length scale ξ which describes the density correlations between DNA chains, this result also indicates that it is the free counterions as opposed to condensed counterions, that can hop from chain to chain in the volume ξ^3, that are the relaxation entities participating that the *LF* relaxation happens at the length scale of the average size of the polyelectrolyte chain suggests that the *LF* relaxation engages mostly condensed counterions along and in close vicinity of the chain.
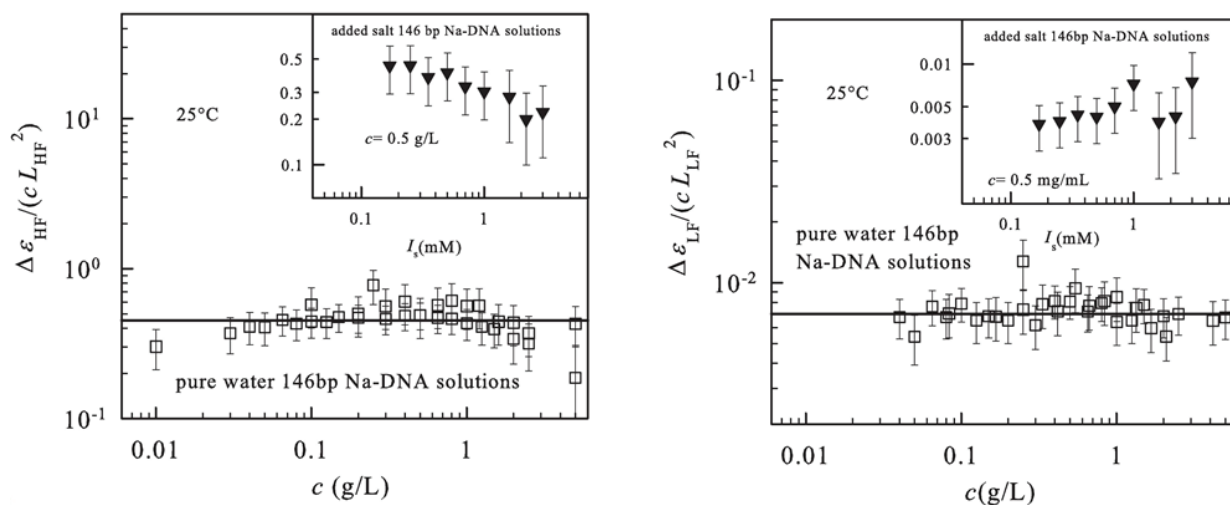
Figure 16. Normalized dielectric strength of the *HF* (left panel) and *LF* (right panel) mode as a function of DNA concentration for pure water 146bp Na-DNA solutions. The full line is a guide for the eye. Insets show normalized dielectric strength of the modes vs. ionic strength of the added salt for representative DNA

On the other hand, changes in behavior are observed upon addition of salt (see insets of Fig. 15 and 16). First let us consider the HF mode. The data suggest that the fraction of intrinsic counterions active in the HF mode remains constant when salt is added to the DNA solution as long as c is substantially larger than I_s. However, as soon as the ionic strength of added salt ions prevails over the ionic strength of DNA ($I_{DNA} = 3c$), f starts to decrease. A plausible suggestion would be that the salt renormalization of f is a consequence of screening due to added salt ions that seem to diminish the effective number of counterions that can participate in the chain - chain hopping process. On the other hand, an opposite behavior is found for the *LF* mode. Namely, the data suggest that the fraction of counterions participating in the *LF* process becomes larger in the case of added salt solutions, compared to the pure water case, if the I_s becomes larger than I_{DNA}. This means that at least some of the free counterions join the relaxation of the condensed counterions along the segments of the same chain. It is thus impossible to completely separate condensed counterions from free counterions in their contribution to the *LF* relaxation mode. This conclusion bears crucially on the assumption that the scale of polarizability is given by $L_{LF}(I_s)$. It is noteworthy that for the *HF* and *LF* modes the addition of salt changes the effective number of participating counterions in the opposite way for both DNA and 146bp DNA samples. This might be attributed to the increased screening for the interchain *HF* relaxation, and to the intrinsic counterion atmospheres squeezed closer to the chains due to reduced Debye length for the *LF* relaxation along the chain.

V. SUMMARY AND PROSPECTS

In summary, our results obtaines in the tube experiments demonstrate that dielectric spectroscopy is a powerful tool which reliably reveals the structural features of a single polyelectrolyte chain as well as structural organization of the polyelectrolyte solution composed of many chains. Since the DS technique can be applied at low polyelectrolyte concentrations below 10 g/L, it stands out as an important experimental method which complements scattering techniques like SAXS and SANS limited to highly concentrated polyelectrolyte solutions. The experiments are done in the repulsive regime (univalent counterions) and in both the dilute and the semidilute limit. The question however remains, how specific is the observed behavior of DNA and whether some of these results can be taken as generic properties of biopolyelectrolytes? Obviously, de Gennes-Pfeuty-Dobrynin solution correlation length, which describes collective properties of semidilute solutions, stands out as a generic property of diverse polyelectrolytes. On the other hand, some features like extremely high flexibility of 146bp DNA and locally fluctuating regions of exposed hydrophobic cores of DNA are certainly very specific. Furthermore, the chain flexibility is revealed to be the key parameter which determines scaling of the electrostatic persistence length: the scaling is a quadratic function of the Debye length as rationalized by the OSF formula for the rigid and semi-flexible chains. Conversely, for flexible chains such as *HA* the scaling becomes linear.

An intriguing route to be taken in further research will focus on polyelectrolytes, specifically DNA, with polyvalent counterions in order to study the behavior in the vicinity of the attractive (correlation) regime of electrostatic interactions. 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. 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. Finally, a standing challenge remains to compare the behavior of polyelectrolytes in repulsive and attractive regimes with results of single-molecule experiments in solutions.

Acknowledgments

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