Dielectric spectroscopy of DNA in vitro

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1 Spectroscopy in General

Spectroscopy is the science that describes the interactions of various types of radiation with matter [1]. The data that are obtained through spectroscopical measurements are spectra which describe radiation intensity as the function of wavelength or frequency.

The early beginnings of spectroscopy date back to the 1666 when Isaac Newton showed that the white light that passes through the prism can be dispersed into a continuous series of different colors (Figure 1). To describe the rainbow of colors that he obtained, he used the word "spectrum". For this experiment, he used a small aperture which defined a beam of light, a lens which collimated it, a glass prism which dispersed the white light and a screen where he displayed the resulting spectrum [3].

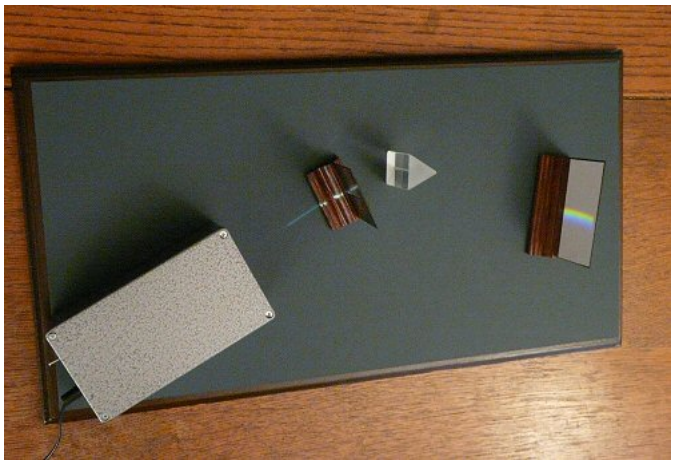


Figure 1: White light dispersion on the prism[2]

In the beginning, spectroscopy was used to examine only the interactions of light, i.e. electromagnetic radiation, and matter. Up to these days, various spectroscopical techniques were developed that include interactions of matter with other forms of energy, for example acoustic waves and beams of particles, such as ions and electrons [1].

Common classification of spectroscopy is based on type of radiation, nature of the interaction, type of material that is used and specific implementations and applications. Some types of interactions between the radiation and the sample include absorption, emission and slowing the transmittance of energy [1, 4].

Absorption spectroscopy measures the absorption of radiation as a function of frequency. The sample absorbs the photons of the incident radiation, comes to the excited state and transmits the rest. The results of these measurements are absorption spectra which are fractions of incident radiation absorbed by the sample. The fragments of the incident radiation that are more likely to be absorbed correspond to the frequencies that represent energy differences between two quantum states in atoms or molecules.

Emission spectroscopy measures the intensity of radiation which is emitted by the sample. This intensity is again usually represented as the funtcion of frequency. Emission occurs when the sample transitions from the excited state to the one with the lower energy. This results with the emission of photons with the frequency which corresponds to the energy difference between the two states. The emission can be spontaneous or stimulated.

Slowing the transmitance of energy is examined by dielectric spectroscopy.

2 Dielectric spectroscopy - Brief Overview [5, 6, 7]

Dielectric spectroscopy, often refered to as electrochemical impedance spectroscopy, examines dielectrical properties of the sample as the function of frequency. It measures the impedance of the sample over a range of frequencies, and thus it determines the frequency response of the sample.

2.1 Electrical Circuit Elements

The idea behind dielectrical spectroscopy is to model the behaviour of electrochemical systems by the known electrical circuits. Thus, the obtained data are analyzed by fitting to an equivalent circuit model. The common elements in the models are resistors, capacitors and inductors.

The resistor is often used to model the cell's solution resistance. Its impedance equals the resistence:

$$Z = R \tag{1}$$

This means that the impedance of the resistor has only a real component from which is possible to conclude that the current through the resistor is in phase with the potential.

The relationship between the current and the potential of the inductor is given by equation (2):

$$U = L\frac{dI}{dt}, \tag{2}$$

where L is the inductance of the inductor. The impedance of these elements has only an imaginary component

$$Z = i\omega L, \tag{3}$$

thus the current through the inductor is phase-shifted for $-\frac{\pi}{2}$ with respect to the potential. Since the impedance is proportional to the frequency, for larger frequencies, larger the impedances of the inductors are measured.

Current through the capacitor and potential depend on each other by

$$I = C\frac{dU}{dt}, \tag{4}$$

where C stands for capacitance of the capacitor. Capacitors also have only an imaginary component of the impedance:

$$Z = \frac{1}{i\omega C}. \tag{5}$$

The difference between these elements and inductors is that the current passing through the capacitor iz phase-shifted for $\frac{\pi}{2}$ with respect to potential. Also, for higher frequencies, lower values for impedance are measured, because of their reversly proportional relationship.

If two or more of these elements are combined in series, as shown in Figure (2), then the net impedance is given by the equation (6):

$$Z = Z_1 + Z_2 + Z_3 + \cdots = \sum_i Z_i \tag{6}$$

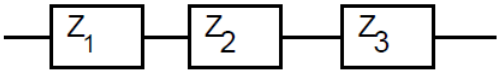


Figure 2: The serial combination of impedances [6]

If elements are parallely combined, as shown in Figure 3, the net impedance is given by

$$Z = \frac{1}{Z_1} + \frac{1}{Z_2} + \frac{1}{Z_3} + \cdots = \sum_i \frac{1}{Z_i} \tag{7}$$

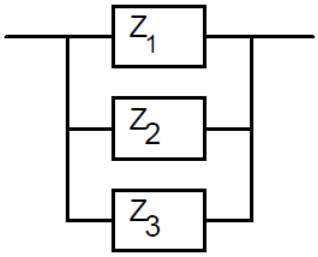


Figure 3: The parallel combination of impedances [6]

It follows from the equations (1)-(7) that impedance and resistance increase in the serial combination, while the capacitanse decreases. The opposite behaviour is observed in paralell combination.

2.2 Data representation

Since the impedance is a complex number, its real part can be plotted on the x-axis and the imaginary part on the y-axis. This is called the Nyquist plot (Figure 4).

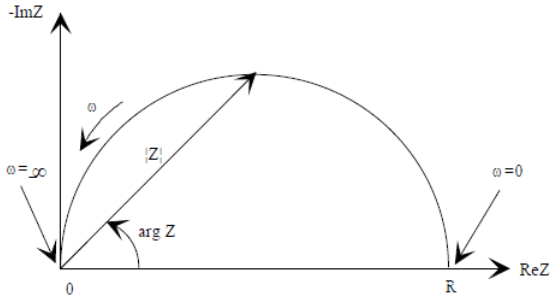


Figure 4: Example of Nyquist plot [6]

In this plot the y-axis is negative and each point on the plane represents an impedance at the certain frequency. Also, from Figure 4 it is clear that the data for the lower frequencies are found on the right and for the higher frequencies on the left side. In Nyquist plot, impedance is represented as a vector defined by it's length $|Z|$ and an angle ϕ between the positive x-axis. The angle ϕ is the phase-shift between two sine waves. Despite it's elegance, Nyquist plot doesn't display information about the frequency at which the impedance is measured. For this purpose, Bode plot (Figure 5) is used.

It is usually a combination of two plots: a Bode magnitude plot and a Bode phase plot. As it can be seen from the Figure 5, Bode magnitude plot has logarithmic values of impedance $\log Z$ on y- and frequencies ω on x-axis, while Bode phase plot shows the phase angle ϕ on the y- and frequency ω on the x-axis.

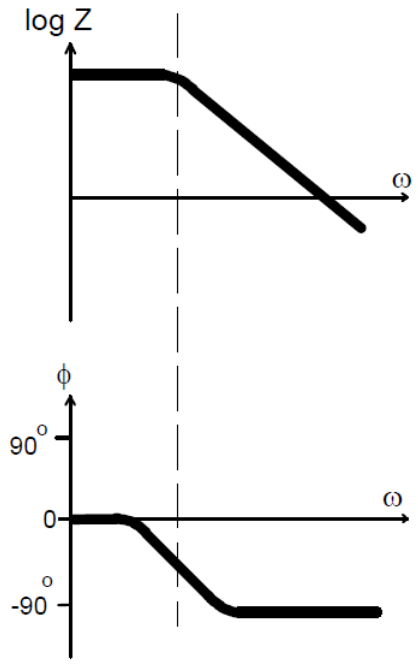


Figure 5: Example of Bode plot [6]

2.3 Impedance

The idea is to model the behaviour of electrochemical systems by known electrical circuits. An electrical circuit is a closed path in which electrons flow from the source of voltage or current. It consists from the passive and active elements. The passive components of the electrical circuit do not generate the current and include resistor, inductor and capacitor (Figure 6).

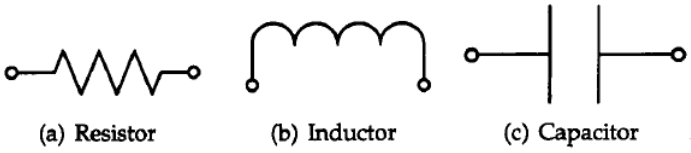


Figure 6: Passive components of electrical circuit [7]

All these elements oppose to the flow of the electrical current. The relationship between the resistance (R) of the ideal resistor, voltage (V) and electrical current (I) is given by the Ohm's law:

$$R = \frac{V}{I} \tag{8}$$

The ideal resistor follows the Ohm's law at all current and voltage levels, it's resistance doesn't depend on frequency and alternating current and voltage are in the phase. In reality, there is no such thing as the ideal resistor. Thus, the concpet of the resistance described by the Ohm's law is improved by introducing the impedance, which is a measure of the ability of a circuit element to resist the flow of current. It isn't strictly bound to the resistor and it isn't limited by the properties of the ideal resistor. Electrochemical impedance is measured by applying an alternating current voltage to an electrochemical cell and then measuring the current through the cell. Electrochemical cell is the system that either generates electrical energy from chemical reactions or catalyzes chemical reactions by through introduction of electrical energy.

We can assume that the sinusoidal voltage is applied. The response of the system to this excitation is an alternating current and it can be analyzed as a sum of sinusoidal functions, employing the Fourier analysis. Voltage that is used for measuring the impedance is usually small. This ensures that the system's response is pseudo-linear, meaning that the current response to the sinusoidal potential is a sine wave with the same frequency but shifted in phase.

The applied potential has the form

$$U(t) = U_0 \cos(\omega t), \tag{9}$$

where $U(t)$ stands for the potential at the time t, U_0 is it's amplitude and ω radial frequency $\omega = 2\pi f$. If the response of the system is linear, then $I(t)$ is shifted in phase for ϕ and has an amplitude I_0:

$$I(t) = I_0 \cos(\omega t + \phi). \tag{10}$$

Using the expression analogous to the Ohm's Law (8), one gets the expression for the impedance Z:

$$Z = \frac{U(t)}{I(t)} = \frac{U_0}{I_0}\frac{\cos(\omega t)}{\cos(\omega t + \phi)} = Z_0\frac{\cos(\omega t)}{\cos(\omega t + \phi)}. \tag{11}$$

From the equation (11), it is evident that the impedance is expressed in terms of it's amplitude Z_0 and the phase shift ϕ. Thus, if one plots the excitation potential on the x-axis and the response of the system on the y-axis, one gets an ellipse (Figure 7), which is a specific Lissajous curve when both frequencies are equal, only the waves are phase-shifted.

Using the Euler's relation for complex numbers

$$e^{i\phi} = \cos\phi + i\sin\phi, \tag{12}$$

the potential and the current can be written as:

$$U(t) = U_0 Re\left[e^{i\omega t}\right] \tag{13}$$
$$I(t) = I_0 Re\left[e^{i\omega t}e^{i\phi}\right] \tag{14}$$

The impedance thus can be represented as a complex number

$$Z = \frac{U}{I} = Z_0 e^{i\phi} = Z_0\left(\cos\phi + i\sin\phi\right) \tag{15}$$

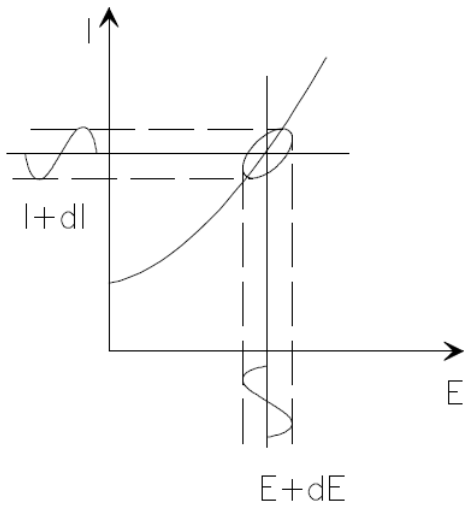


Figure 7: Ellipse - Lissajous curve for two phase-shifted sine waves of equal frequency. E is the applied voltage and I the current [7]

2.4 Representation Domains

Data can be represented in different domains. It is common to use two of them: time and frequency.

In the time domain, amplitudes of signals are represented with respect to time, while in the frequency domain, the same amplitudes are plotted as the function of frequency.

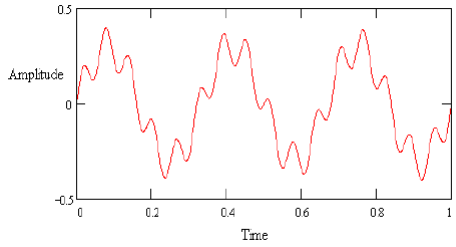
It is possible to shift between these two domains using the Fourier analysis. If the signal is given as the function of time $f(t)$, its equivalent representation in the frequency domain is represented with

$$f(\omega) = \int_{-\infty}^{\infty} f(t)e^{i\omega t}dt. \tag{16}$$

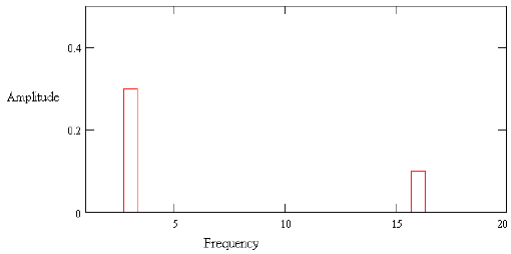
The similar equation gives the transformation from the frequency-domain representation of $f(\omega)$ to its time-domain representation:

$$f(t) = \int_{-\infty}^{\infty} f(\omega) e^{-i\omega t} d\omega. \tag{17}$$

The signal that consists of two sine waves is shown in both time and frequency domain on the Figures 8a and 8b.



(a) Time domain representation



(b) Frequency domain representation

Figure 8: Signal that consists of two sine waves in different domain representations [6]

2.5 Electrochemical Systems

Electrochemical cells aren't linear systems. Since the linear systems are much easier to analyse, it is possible to assume only a small part of the voltage versus current curve. In this small interval, the curve, thus the system, appears to be linear (Figure 9).

Thus, the potential that is usually applied to the system is a small alternating current signal.

Measuring the electrochemical impedance spectrum often requires many hours. In order to obtain relevant data, the system has to remain in the steady state. This is the main source of the problems when examining the behaviour of the system using dielectric spectroscopy. The system can adsorb solution impurities, grow an oxide layer, reaction products can build up in the solution, temperature can change ...

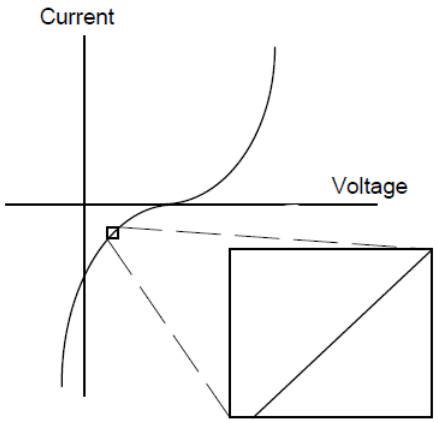


Figure 9: Technique of approximating non-linear system by the linear [6]

2.6 Modelling the System

When modelling the system, solution resistance between the reference and the working electrode has to be considered. The resistence of electrolyte depends on the concentration of ions, type of ions, temperature and the geometry of the area in which current is carried. If we suppose that the uniform current passes through an area A with length l, resistance is given by equation (18)

$$R = \rho \frac{l}{A}, \tag{18}$$

where ρ is the solution resistivity. It is common to use conductivity of the solution κ, which is defined as $\kappa = \frac{1}{\rho}$. Thus:

$$\rho = R\frac{A}{l} \tag{19}$$

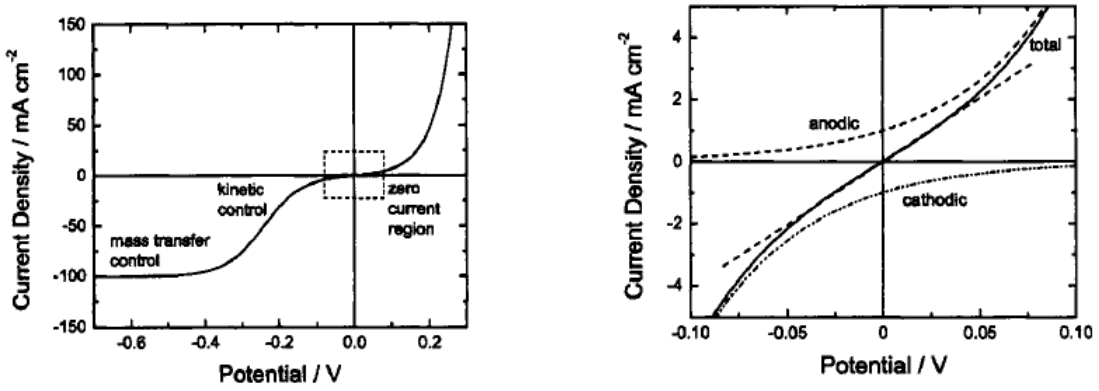
$$\kappa = \frac{l}{RA} \tag{20}$$

Expressions (19) and (20) are valid only if the current through the observed area is uniform. In reality, this isn't the case. Thus, the major problem in determining the conductivity and resistance of the solution is to discover the current flow path and the geometry of the electrolyte that carries the current.

On the interface between the electrode and the surrounding electrolyte, an electrical double layer is formed. The double layer forms when ions from the solution stick on the electrode surface. The electrode becomes charged and separated from the rest of the ions. This is how the capacitor is formed. The value of its capacitance depends on many variables,

such as electrode potential, temperature, concentrations of ions and their types, formation of oxide layers, impurity adsorption ...

If the potential of electrode is forced away from it's value in an open-circuit ($I = 0$), it is said that the electrode is being polarized. The polarization of electrode causes the current to flow through electrochemical reactions that occur at the electrode surface. This current can be controlled by the kinetics of the reactions and the diffusion of reactants. The non-linear current-voltage curves, i. e. polarization curves, are depicted on the Figures 10a and 10b bellow:



(a) broad range of potential

(b) expanded representation of zero-current region

Figure 10: Example of polarization curves [6]

The polarization curve is devided in three regions: region where the current equals zero, region where current is controlled by reaction kinetics and the region where the current is controlled by mass transfer. From the expanded view on the Figure 10b, it can be seen that the positive current which is contributed by the anodic reaction is balanced by the negative current contributed by the cathodic reaction. If these reactions represent forward and backward rates of the same reaction, the zero current is obtained under the condition of reaction equillibrium. Thus, $I_a = -I_c$, where I_a stands for the anodic and I_c for cathodic current.

If the zero current is achieved through balancing of different reactions, net rate for each reaction isn't zero and equillibrium isn't attained. This is often the case when electrodes undergo corrosion. The potential at which zero current occurs for different electrochemical reactions is called the mixed potential, or in the case of metal dissolution, the corrosion potential. When observing the corrosion, the potential is related to the current by the

Butler-Volmer equation 21:

$$I = I_{corr}\left(e^{\frac{2.303(U-U_{oc})}{\beta_a}} - e^{-\frac{2.303(U-U_{oc})}{\beta_c}}\right), \tag{21}$$

where I is the measured current, I_{corr} the corrosion current at which the corrosion occurs, U_{oc} an open-circuit voltage, and β_a and β_c are anodic and cathodic beta coefficients respectively. If the small signal aproximation is applied to the above equation, one gets:

$$\begin{aligned} I &= I_{corr}\left(1 + \frac{2.303\,(U-U_{oc})}{\beta_a} - 1 + \frac{2.303\,(U-U_{oc})}{\beta_c}\right) \\ &= I_{corr}\cdot 2.303\left(\frac{1}{\beta_a} + \frac{1}{\beta_c}\right)(U-U_{oc}) \qquad (22) \\ I_{corr} &= \frac{I}{2.303\,(U-U_{oc})\left(\frac{1}{\beta_a}+\frac{1}{\beta_c}\right)} \\ &= \frac{\beta_a\beta_c}{2.303(\beta_a+\beta_c)}\frac{1}{R_p} \qquad (23) \end{aligned}$$

where $R_p = \frac{U-U_{oc}}{I}$ is the polarization resistance.

In the region of kinetic control, current densities are exponential functions of potential. We consider the metal which is in contact with an electrolyte. The metal electrolitically dissolves into the electrolyte according to

$$R \Longleftrightarrow O + ne^{-}, \tag{24}$$

where R is a reducant, O oxidant and n the number of electrons transferred. In the forward reaction, electrons enter the metal and metal ions diffuse into the electrolyte. This means that charge is being transferred. This process has certain speed, which depends on the kind of reaction, the temperature, the concentration of the reaction products and the potential. To describe the influence of the potential on the current, the Butler-Volmer equation (25) is used:

$$i = i_0\left(\frac{C_o}{C_o^*}e^{\frac{\alpha nF\eta}{RT}} - \frac{C_R}{C_R^*}e^{\frac{-(1-\alpha)nF\eta}{RT}}\right), \tag{25}$$

where i is the current density, i_0 exchange current density, C_o and C_o^* concentrations of oxidant at the electrode surface and in bulk respectively, C_R and C_R^* concentrations of reductant at the electrode surface and in bulk respectively, η overpotential (which is the difference between the applied and the open-circuit potential $U_{app} - U_{oc}$), F Faraday's constant, T temperature, R gass constant, α reaction order and n number of electrons transferred. If the concentrations in the bulk and on the electrode surface are the same, (25) reduces to

$$i = i_0\left(e^{\frac{\alpha nF\eta}{RT}} - e^{\frac{-(1-\alpha)nF\eta}{RT}}\right). \tag{26}$$

If the overpotential is very small and the system is at equilibrium, (26) becomes:

$$\begin{aligned} i &= i_0 \left(1 + \frac{\alpha n F}{RT}\eta - 1 + (1-\alpha)\frac{nF}{RT}\eta\right) \\ &= i_0 \frac{nF\eta}{RT} \qquad (27) \\ \frac{i}{\eta} &= i_0 \frac{nF}{RT} \qquad (28) \\ R_{ct} &= \frac{\eta}{i} = \frac{RT}{i_0 nF} \qquad (29) \end{aligned}$$

In (29) R_{ct} is the charge transfer resistance.

In the region of mass-transfer control, the rate of reactions is limited by the finite rate at which the reacting species are carried to the electrode surface. The exchange current density is now proportional to the concentration at the interface of the reacting species with the power of stochiometric coefficient that matches the reacting species. The reciprocal value of this current can be expressed as the sum of the reciprocal values of limiting current and the kinetic current. The kinetic current is based on bulk concetration, and the limited is restricted by the mass transfer. It is influenced by the bulk concentration and the diffusivity of the limiting reactant, by the extent of convenction and by the cell geometry.

Diffusion of reactants creates the Warburg impedance. It depends on the frequency and potential perturbation. At high frequencies the reactants don't have to move far and the Warburg impedance is small. The opposite occurs at low frequencies. The equation for the infinite Warburg impedance is:

$$Z_W = \sigma\omega^{-\frac{1}{2}}(1-i), \qquad (30)$$

where σ is Warburg coefficient and i is the imaginary unit. The Warburg coefficient has the form of

$$\sigma = \frac{RT}{n^2F^2A\sqrt{2}}\left(\frac{1}{C_0^*\sqrt{D_0}} + \frac{1}{C_R^*\sqrt{D_R}}\right) \qquad (31)$$

in which ω is radial frequency, D_o and D_R diffusion coefficients of oxidant and reductant, A surface area of the electrode and n number of electrons involved. The expression for infinite Warburg impedance (30) holds only if the diffusion layer has an infinite thicknes. If the diffusion layer is bounded, the impedance at lower frequencies is described in terms of finite Warburg impedance:

$$Z_0 = \sigma\omega^{-\frac{1}{2}}(1-i)\tanh\left(\delta\left(\frac{i\omega}{D}\right)^{\frac{1}{2}}\right), \qquad (32)$$

where δ is Nernst diffusion layer thickness and D the average value of diffusion coefficients of the diffusion species.

The coating layer on electrodes can cause the formation of a capacitor. Generally, a capacitor is formed when two conducting plates are separated by a dielectric. The capacitance is a function of the size of the plates, their distance and the properties of the dielectric. Thus, it is given by

$$C = \frac{\epsilon_0 \epsilon_r A}{d}, \tag{33}$$

where A is the area of each plate, d their distance and ϵ_0 and ϵ_r the permittivity of vacuum and the dielectric respectively.
Capacitors in dielectric spectroscopy measurements aren't ideal, but behave like a constant phase element. This means that their impedance is defined as

$$Z_{CPE} = \frac{1}{(i\omega)^{\alpha} C}, \tag{34}$$

where α is the emphirical constant that generally has the value between 0.9 and 1. For the ideal capacitor, $\alpha = 1$.

3 Application to Polyelectrolytes

When the electric field is applied to the system, it responds by polarization, which is the net dipole density of the system. This can be characterized by the system's permittivity $\epsilon(\omega)$ which is possible to determine from measured impedances at various frequencies. Different procedures exist depending on the frequency range [8].

The spectrum is often devided in two regions: the one with the low frequancy and the other with high.

In the low frequency range the system can be modelled by the equivallent circuit on the Figure 11.

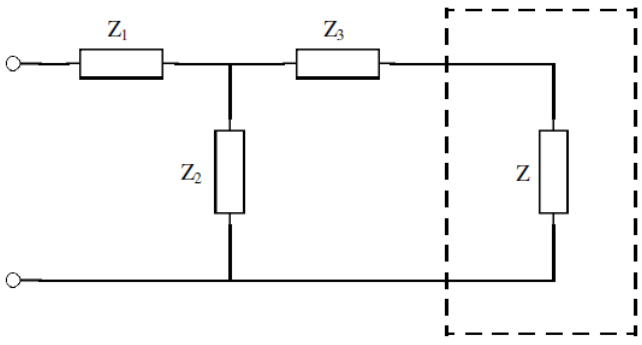


Figure 11: The equivalent circuit of the measuring cell for low-frequency measurements [8]

The capacitance C and resistance R of the sample can be expressed in terms of the sample permitivity ϵ' and conductivity σ as:

$$C = C_0\epsilon' \tag{35}$$

$$\frac{1}{R} = C_0\frac{\sigma}{\epsilon_0} \tag{36}$$

where C_0 is the capacitance of the free cell [8]. On the Figure 11 R_s and L_s represent series resistance and inductance respectively which can be neglected at low frequencies.

The different parameters that characterize the equivalent circuit can be obtained by means of proper calibration procedures, using different standard liquids of known permittivity and conductivity [8]. This can be achieved by measuring the cell impedance in three different configurations: shorted cell electrodes, the empty cell and the cell filled with a standard sample of known dielectric permittivity and conductivity. Combining the results of the measurements and equations (35) and (36), it is possible to obtain the condactivity σ and dielectric permittivityϵ' of the sample [8].

In the high frequency range the cell can be modelled by means of equivalent T-network. This approach uses the result that impedance of the sample can be represented as the bilinear function of measured impedance. Using the fact that the composition of two bilinear transformations in the complex plane is still a bilinear transformation, the complex permittivity ϵ of the unknown sample can be determined straightforwardly from the measured complex impedance [8].

3.1 Dielectric response

Dielectric response $\epsilon(\omega)$ of a given system to an external oscillatory electric field of angular frequency ω is given by equation 37:

$$\left\langle \vec{J} \right\rangle = i\omega\epsilon_0\epsilon(\omega)\vec{E}, \tag{37}$$

where $\left\langle \vec{J} \right\rangle$ the averaged current density over the sufficiently large volume of the system and $\vec{E}$ the applied electric field [8].

Dielectric response constant $\epsilon(\omega)$ is complex and it is related to the time correlation function $\phi(t)$ of the macroscopic dipole moment of the volume V in time t in the absence of an applied electric field, by the one-side Fourier transform given by equation 38:

$$\frac{\epsilon(\omega) - \epsilon_\infty}{\epsilon_s - \epsilon_\infty} = 1 - i\omega\int_0^\infty \phi(t)e^{-i\omega t}dt, \tag{38}$$

where ϵ_s and ϵ_∞ are the limiting low- and high-frequency permittivities, respectively [8]. If $\phi(t)$ is characterized by a single exponential decay with a time constant τ, equation gives the well-known Debye relaxation function

$$\frac{\epsilon(\omega) - \epsilon_\infty}{\epsilon_s - \epsilon_\infty} = \frac{1}{1 + i\omega\tau} \tag{39}$$

An extensively used empirical modification of the equation is

$$\frac{\epsilon(\omega) - \epsilon_\infty}{\epsilon_s - \epsilon_\infty} = \frac{1}{\left[1 + (i\omega\tau)^\beta\right]^\alpha}, \tag{40}$$

which for parameters $\alpha = 1$ and $\beta \in \langle 0, 1\rangle$ leads to the Cole–Cole relaxation function [8].

If correlation function isn't written in terms of single-exponential decay, equation (39) may be written by introducing a distribution of relaxation times $g(\tau)$ as

$$\frac{\epsilon(\omega) - \epsilon_\infty}{\epsilon_s - \epsilon_\infty} = \int \frac{g(\tau)d\tau}{1 + i\omega\tau} \tag{41}$$

If two or more relaxation processes overlap, two or more distinct relaxation functions may be employed.

If a polymer is given with degree of polymerization N, the response function $\phi(t)$ is written as a normalized correlation function

$$\phi(t) = \frac{\sum_{i=1}^{N} \langle \mu_i(t)\mu_i(0)\rangle}{\sum_{i=1}^{N} \langle \mu_i(0)\mu_i(0)\rangle} \tag{42}$$

In (42) $\mu_i(t)$ is the dipole moment of the ith repeat unit at time t [8].

3.2 Dielectric Response as Complex Function

The complex dielectric constant $\epsilon(\omega)$ is usually written as

$$\epsilon(\omega) = \epsilon'(\omega) - i\frac{\sigma(\omega)}{\epsilon_0\omega}, \tag{43}$$

where ω is the angular frequency, $\epsilon'(\omega)$ is the real part of the complex dielectric constant, $\sigma(\omega)$ is the total conductivity and ϵ_0 is the permittivity of vacuum. The measured dielectric loss $\frac{\sigma(\omega)}{\epsilon_0\omega}$ is made up of two components: one is due to the dielectric process $\epsilon''(\omega)$ and the

other due to the DC electrical conductivity σ_0, which is the low-frequency limit of $\sigma(\omega)$. The general expression of equation (43) is given by

$$\epsilon(\omega) = \epsilon'(\omega) - i\left[\epsilon''(\omega) + \frac{\sigma_0}{\epsilon_0\omega}\right]. \tag{44}$$

The DC conductivity loss $\frac{\sigma(\omega)}{\epsilon_0\omega}$ increases with decreasing frequency and often obscures $\epsilon''(\omega)$ due to the dielectric processes of interest at the lowest frequencies [8].

The fundamental effects in polyion solutions that influenc dielectric response in different frequency ranges are classical dipole orientation, polarization of the counterion atmosphere (counterions are an atmosphere of ions of the opposite charge that surround polyelectrolyte), polarization associated with the internal degrees of freedom of the polymer chain and dielectric relaxation caused by fast chemical relaxation processes. They provide information about structural characteristics (dipole moments, rotational diffusion coefficients) but also on properties of the counterion atmosphere (ion mobility and density, 'effective' dielectric constant) as well as on kinetic parameters (chemical relaxation time, rate constants) and on the influence of an electric field on the chemical equilibria in the solution [8].

4 Deoxyribonucleic Acid (DNA)

DNA (deoxyribonucleic acid) is a molecule which is a carrier of genetic information [9].

Its 3D structure was discovered in 1953 by James Watson and Francis Creek based on the x-ray crystallographic measurements performed by Maurice Wilkins and Rosalind Franklin. The analysis led to the conclusion that the DNA is a two-stranded biopolymer. The strands are coiled around each other and form a double helix. They consist of series of nucleotides which are composed of nitrogen-containing nucleobases, that can be either adenine, guanine, cytosine or tymine, monosaccharide sugar deoxyribose and a phosphate group. Nucleotides in the DNA are oriented in such a way that the bases are found inside the helix and the sugar-phosphate backbone is positioned towards the outside. The two strands are connected via hydrogen bonds which form between the two opposite nucleobases. The basepairing is highly specific and occurs only in the way that adenine (A) pairs with guanine (G) and cytosine (C) with tymine (T).

DNA is a negatively charged molecule since its components are phosphate groups. Each phosphate group contributes with $q = 1e^-$ to the net charge. To the each negatively charged phosphate group, a positively charged counterion is attached. These counterions are often Na^+, K^+ or Mg^+.

In aqueous solution, phosphate groups are completely dissociated and DNA is a charged polymer. It is surrounded by the cloud of positive counterions (Figure 12) Two types of counterions can be distinguished: condensed and free. Condensed counterions are tightly

bound to polyions, while the free are distributed in a larger volume around them. These counterion types are transient. This means that there exists the constant dynamic exchange between them [14].

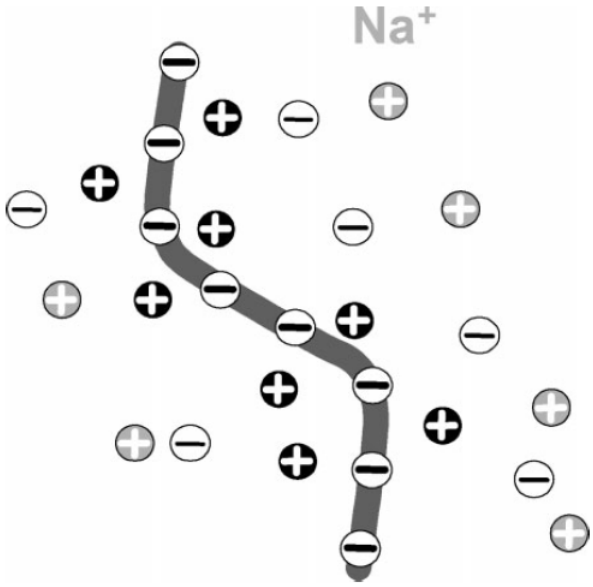


Figure 12: Counterion atmosphere which surrounds the DNA [14]

5 Description of DNA Behaviour in Aqueous Solutions

Using dielectric spectroscopy, it is a goal to understand and model the behaviour of DNA in the cell. This is done by trying to mimic the environment in which the DNA is found and probing the dynamics of counterion atmosphere which surrounds DNA in a solution [14].

5.1 Theory Overview

Behaviour of DNA in aqueous solutions with different concentrations of salt is observed. In semidilute regime the chains are long and entangled with each other, while in the dilute regime each segment is well-separated from others (Figure 13) [14].

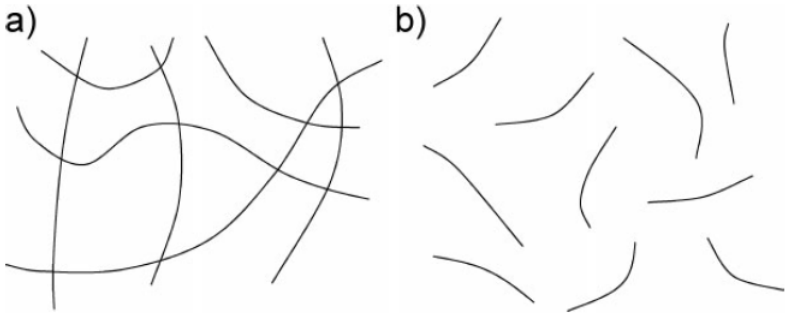


Figure 13: a) long overlapping chains form a semidilute solution, b) short chains or chains at much smaller concentrations in dilute solution [14]

Theoretical descriptions that examine the single charged polymer chain or semidilute solutions of many charged polymer chains include the Manning-Oosawa (MO) counterion condensation, the Odijk-Skolnick-Fixman (OSF) electrostatic persistence length, and the de Gennes–Pfeuty–Dobrynin (dGPD) correlation length of a polyelectrolyte solution [11].

In the MO counterion condensation theory the counterions accumulate in the condensed layer in such a way that the effective charge density parameter $\eta = \frac{zl_B}{b} > 1$ is reduced to 1. In this equation z is the valency of the counterion, b is the linear charge spacing and l_B is the Bjerrum length, which is the separation at which the electrostatic interaction between two elementary charges is comparable in magnitude to the termal energy scale k_BT . The condition that $\eta = 1$ means that the effective separation between charges is increased from b to l_B. If salt is added to the system, the mobile salt ions screen electrostatic interactions between fixed charges along DNA. If the fixed charge density is small, the screening is quantified by a screening or Debye length κ^{-1}, which is defined by $\kappa^2 = 8\pi l_B n$, where n is the density of added salt. If the salts are monovalent, both effects, ionic screening and counterion condensation, coexist [11, 12].

DNA is also a stiff molecule. Its stiffness can be described in terms of OSF theory. It is characterized by the persistence length L_p which can be decomposed into a structural L_0 and electrostatic L_e contribution as

$$L_p = L_0 + L_e = L_0 + \frac{l_B}{(2b\kappa)^2} \tag{45}$$

The usually accepted value of L_0 for DNA is about 500 Å. The persistence length describes the length along which DNA retains the direction. It measures the chain flexibility and can be understood as the boundary between rigid behavior over short distances and flexible over large distances. This OSF result applies for monovalent counterions. If no salt is

added and electrostatic screening is weak, DNA behaves as semiflexible charged chain in a semidilute polyelectrolyte solution and acts like a random walk of correlation blobs. These correlation blobs are conformational fluctuations that expose the hydrophobic interior of dsDNA [10, 11, 12, 14].

According to the dGPD theory, in the semidilute polyelectrolyte solution the dGPD correlation length ξ is important. Above the semidilute overlap concentration, DNA chains maintain their highly extended conformation up to the scale of ξ. On the larger scales the chains behave like random walks. In semidilute polyelectrolyte solutions, ξ scales as $c^{-0.5}$, where c is the concentration of DNA. The dGPD correlation length is expected to be proportional to the screening length due to both free DNA counterions and added salt ions. Also, the correlation length divides the semidilute polyelectrolyte solution into decorrelated volumes of size ξ^3 [11, 12].

5.2 What Is Measured and How

The small AC signal is applied to the sample (thus it is possible consider the linearity of the system) and the counterions start to oscillate).

Their displacments are results of the diffusive motion. Thus, the dielectric response can be characterized by the mean relaxation time which is described by the following:

$$\tau_0 \sim \frac{L^2}{D}, \tag{46}$$

where D is the diffusion constant of counterions and L is associated length scale at which counterions oscillate. The diffusion constant of bulk ions due to the presence of polyions can be considered negligible, thus, measuring the relaxation time τ_0 directly gives the corresponding length scales which are involved in counterion oscillations. These length scales are defined by conformational features of either the single polyion chain or the structure of many chain ensemble in solution. Parmeters which are expected to be relevant are valency, chain length, concetrations of polyions and added salt ions [10, 11, 13, 14].

5.3 The Experimental Procedure

First it is ensured that the DNA samples are low protein and that that ds-DNA isn't denaturated in two spacially distinguishible and well-separated single strands [11, 12, 13, 14].

Dielectric spectroscopy measurements are performed at room temperature. Dry DNA samples are dissolved in eather pure water and NaCl aqueous solutions. The DNA solution droplet is applied between platinum electrodes of a home-made capacitive chamber. The chamber is closed and connected to the temperature control unit and the impedance analyzer which operates in the 40 Hz-110 MHz frequency range. The properties that are measured are

the conductance $G_{sample}(\omega)$ and capacitance $C_{sample}(\omega)$. Reference samples are measured in order to minimize stray impedances such as free ion contribution and electrode polarization. It is assumed that the conductivity of each entity in the solution is additive. Thus, the polyelectrolyte reposnse is given by (47) and (48):

$$G(\omega) = G_{sample}(\omega) - G_{ref}(\omega) \quad (47)$$

$$C(\omega) = C_{sample}(\omega) - C_{ref}(\omega) \quad (48)$$

The real and imaginary parts of dielectric function are extracted by equations (49) and (50):

$$\epsilon'(\omega) = \frac{l}{S}\frac{C(\omega)}{\epsilon_0} \quad (49)$$

$$\epsilon''(\omega) = \frac{l}{S}\frac{G(\omega)}{\epsilon_0} \quad (50)$$

where $\frac{l}{S}$ is a chamber constant and ϵ_0 permittivity of the vacuum [12, 14, 15].

5.4 What Is Observed

The relaxation time τ_0 is measured and the characteristic length scale L is extracted using the equation $\tau_0 \sim \frac{L^2}{D}$, where D is the diffusion constant of counterions. This equation is used with prefactor 1 and the bulk diffusion constant is used for D. The use of these quantities is justified by dielectric spectroscopy experiments for both free and condensed counterions [14, 15].

The three fundamental length scales are extracted from the measurements and these are the Debye screening length in 1 mM added salt (10 nm), the structural persistence length of DNA (50 nm) observed in the study of long DNA chains and the contour length of short DNA fragments that are 146 bp long (50 nm). The contour length is the result of diffusive motion of condensed counterions along single polyion chains. Debye screening length and the structural persistence length come from the oscillations of both condensed and free counterions along single polyion chains. This means that both kinds of counterions take part in the dielectric response of DNA [10, 11, 12].

The dielectric function spectra of long and short DNA chains exhibit two prominent broad modes in MHz (high-frequency - HF) and kHz (low-frequency - LF) frequency range. The data is fitted to the sum of two Cole-Cole forms:

$$\epsilon(\omega) - \epsilon_\infty = \frac{\Delta\epsilon_{LF}}{[1 + (i\omega\tau_{0,LF})]^{1-\alpha_{LF}}} + \frac{\Delta\epsilon_{HF}}{[1 + (i\omega\tau_{0,HF})]^{1-\alpha_{HF}}}, \quad (51)$$

where ϵ_∞ is the high-frequency dielectric constant, $\Delta\epsilon$ is the dielectric strength, τ_0 the mean relaxation time and $1 - \alpha$ the symmetric broadening of the relaxation time distribution function of the LF and HF dielectric mode [10, 14, 15].

HF mode (MHz region) In the HF mode and dilute regime, for short DNA fragments (146 bp long) characteristic length scale decreases with increasing the concetration of DNA with the power law $c_{DNA}^{-0.33}$ [12]. The observed characteristic length scale is smaller than the chain contour length of 50nm which is explained by the reduced average distance between the chains (Figure 14) [14].

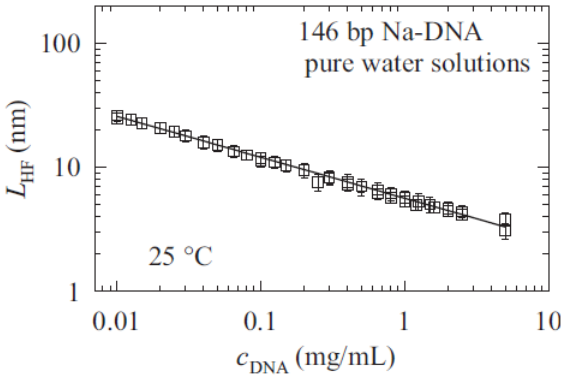


Figure 14: Characteristic length of the HF mode for pure water 146 bp DNA solutions in dilute regime as a function of DNA concentration [14]

This agrees with the theory for polyelectrolytes in dilute regime where the long-range repulsive interactions between the chains exist and they are positioned as far away from one another as possible. In this model, the polymer is placed at the center of a cell that is subdivided into two zones: a smaller cylindrical one and a larger spherical zone. Inside the small cylinder the electrostatic interaction energy is large and in the large sphere variations of the electrostatic potential are small and the counterions are distributed almost uniformly. The response of DNA counterions to an applied alternating current signal is localized at the smaller cylindrical volume. Their oscillation reflects the cylinder size instead of the size of the sphere which corresponds to the average distance between the chains [14].

For the long DNA chains in semidilute regime, the characteristic length decreases as the DNA concentration increases and follows the power law $c_{DNA}^{-0.5}$ (Figure 15) [11, 12]. This dependence corresponds to the solution mesh size or the de Gennes-Pfeuty-Dobrynin (dGPD) correlation length ξ. It is the length scale which limits the space around each polyion in the solution from which other polyions are strongly expelled. The scaling dependence of the correlation length on the polyelectrolyte density can be derived by minimization of the total interaction energy which consists of the electrostatic part and the elastic contribution due to the stretching of the chain. This means that for volumes smaller than ξ^3, the polyelectrolyte chain is stiffened by electrostatic interactions. For scales larger than ξ it behaves as a free-

flight chain or random walk. It can be said that the chain is a random walk of correlation blobs [14].

In semidilute regime of long DNA and its low concentrations, the correlation length scales as $c^{-0.33}$(Figure 15). This means that conformational fluctuations (DNA denaturation bubbles) partially expose the hydrophobic core of DNA. When more salt is added, the Debye screening length becomes relevant length scale for HF mode. This occurs when sufficient salt is added so that the corresponding Debye screening length becomes comparable to and eventually smaller than the dGPD correlation length [14].

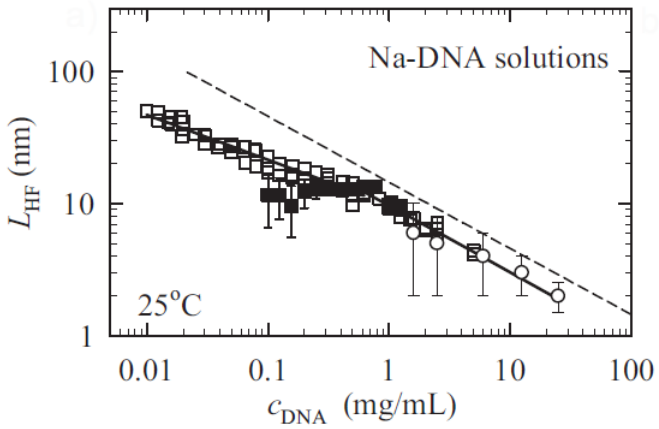


Figure 15: Characteristic length of the HF mode for DNA solutions with added salt (full squares) in semidilute regime as a function of DNA concentration. The full line is a fit to power laws $L_{HF} \sim c_{DNA}^{-0.33}$ and $\sim c_{DNA}^{-0.5}$ [14]

LF mode (kHz range) This mode probes single-chain properties of the polyelectrolyte solution [13, 14]. In dilute regime, short DNA fragments behave as nonuniformly stretched chains. This is due to the fact that interchain interactions are negligible compared to intra-chain interactions. The contour length is concentration-independent and equals 50 nm. It is the fundamental length scale for short DNA fragments in dilute regime (Figure 16) . If added salt concentration is larger then the DNA concentration, this result isn't observed. Instead, the single-chain length scale shrinks in size, and becomes two times smaller than the nominal contour length of the chain [13, 14].

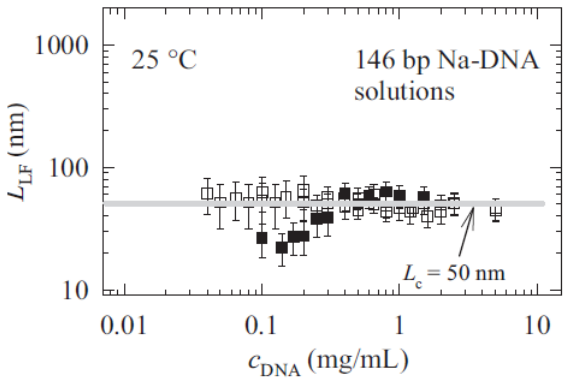


Figure 16: Characteristic length of the LF mode for pure water 146 bp DNA solutions (open squares) and for DNA solutions with added salt (full squares) in dilute regime as a function of DNA concentration [14]

In the semidilute regime with long DNA chains which are strongly charged and semiflexible, the characteristic length L_{LF} decreases when DNA concentration increases. This follows the power law $L_{LF} \sim c_{DNA}^{-0.25}$ (Figure **17**a) [12, 14]. This corresponds to the average size of the chain R in the limit of low added salt. In this regime, DNA acts as its own salt and polyelectrolyte is expected to behave as a random walk of correlation blobs [12, 14, 15]. In the limit of high added salt the characteristic length L_{LF} behaves as the persistence length $L_p = L_0 + aI_S^{-1}$ (Figure 17b) where $L_0 = 50\,nm$ is the structural persistence length and aI_S^{-1} is Odijk-Skolnick-Fixman electrostatic persistence length contribution .

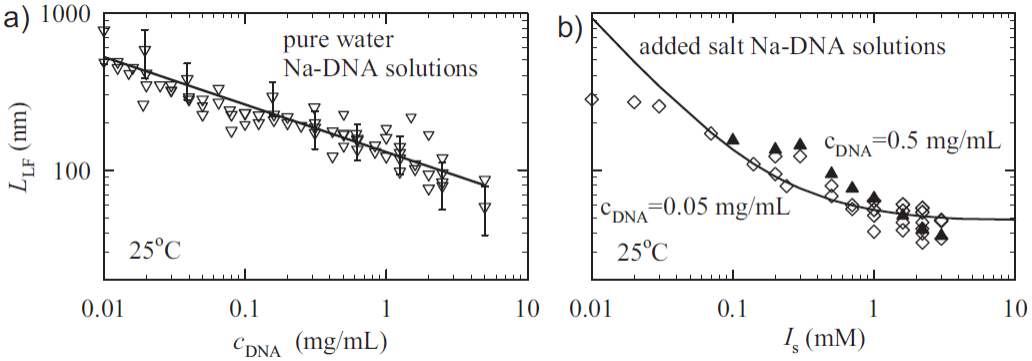


Figure 17: a) Characteristic length of the LF mode for pure water DNA solutions as a function of DNA concentration. The full line is a fit to the power law $L_{LF} \sim c_{DNA}^{-0.25}$ b) Characteristic length of the LF mode for DNA solutions with varying ionic strength of added salt for different DNA concentrations [14]

It is proportional to the inverse of the added salt ionic strength I_S , which is a quadratic function of the Debye length [14].

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