Optical Properties of DNA in Aqueous Solution

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Abstract In the study of DNA electric birefringence, it is usual to use theories that consider that molecules in solution are small in relation to the light wavelength. In this work, we study the DNA electric birefringence using a broken-rod macroion (BRM) model composed of two cylindrical arms which does not restrict the size of the molecules. To achieve this, we include the inhomogeneity effect of the light electric field through the molecule and the interaction between its different parts. To analyze the interaction between a molecule and the incident beam of light, we apply the discrete dipole approximation (DDA), according to which each molecule is described as a finite array of electronic coupled oscillators. The electric birefringence is calculated from the oscillator polarizability. This is obtained from experimental data of electric birefringence saturation and from the increment of the solution refraction index in relation to that of the solvent. Furthermore, the oscillator polarizability is also estimated from DNA absorption spectrum using the Kronig–Kramers relations. This allows us to analyze the contributions of the different absorption bands of DNA to the electric birefringence. We analyze the influence of the inhomogeneity of the light electric field and of the intramolecular interactions in the characterization of DNA optical properties using electric birefringence measurements.

Keywords DNA · Electric birefringence · Discrete dipole approximation · Optical polarizability · UV absorption spectra · Large molecule

1 Introduction

An aqueous solution of DNA fragments shows itself birefringent when an external electric field is applied to it. This occurs because the external electric field orients the molecules in

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solution. Electric birefringence is defined as the difference between the real refraction indexes for incident plane polarized light with its polarization plane parallel and perpendicular to the external electric field.

Experimental data of electrooptical properties of restriction-enzyme-digested DNA and sonicated DNA has been acceptably adjusted using a broken-rod macroion (BRM) model [1] composed of two cylindrical arms. However, the theories for the study of electric birefringence habitually used [2–4] assumes that the molecules are small compared to the light wavelength. We consider it interesting to remove this assumption to estimate the degree of validity of these theories. To do this, we need to consider two effects: the inhomogeneity of the light electric field in the space occupied by the molecule and the interaction between its different parts.

The problem of flow birefringence of molecules whose length is not small compared to the wavelength was analyzed by Taylor and Cramer [5] using the light scattering theory. However, they considered that the molecules in solution are highly orientated to satisfy the necessary conditions for the validity of the Rayleigh–Gans approximation. In the case of electric birefringence, this consideration is satisfied only for very high electric field strength, i.e., in the saturation state. There are some other works that studied the optical anisotropy of large molecules based on their scattering properties. The anisotropy of the optical polarizability in the Rayleigh–Gans approximation was analyzed by Stoylov and Stoimenova [6]. The same approximation was used by Ridgeway [7] in his study of transient electric birefringence of asymmetric ellipsoids. Orientational birefringence in the approximations of Stevenson and Ikeda were analyzed by Stoimenova and Stoylov [8]. A good discussion about electric birefringence from light scattering theories is given in Stoylov's book [9]. It is important to note that when the light scattering approximations are used to study the electric birefringence, the effect of the interaction between the different parts of the molecules is not included.

An alternative study of electric birefringence including the retardation effects of the light electric field and the interaction between the different parts of the molecule can be made using the discrete dipole approximation (DDA) to describe the optical properties of the molecules in solution. DDA, also known as the coupled dipole approximation (CDA), was developed initially by DeVoe [10] to study the optical properties of molecular small aggregates including the intramolecular interactions. Later, Purcell and Pennypacker [11] removed the restriction of small molecules including the retardation effects of the light electric field in their study of light scattering and absorption by nonspherical dielectric grains. Basically, DDA describes the optical properties of the molecules with a finite array of electronic coupled oscillators having complex polarizabilities that represent the electronic transitions in the molecule. One application of DeVoe's theory to the study of optical properties of DNA molecules was done by Self and Moore [12, 13] who studied the absorption and dichroism of DNA in the infrared region.

In the present work, we use the DDA to study the electric birefringence of DNA fragments like BRM with comparable dimensions to the light wavelength including the inhomogeneity of the light electric field and the intramolecular interactions. Electric birefringence is expressed as function of the oscillators' optical polarizability at visible wavelengths. This is obtained from experimental data of saturation electric birefringence and increment of refraction index of solution with respect to the solvent. We also use the Kronig–Kramers relations to analyze the contribution of the different absorption bands to the electric birefringence. Although the calculation of electric birefringence is done for a BRM model, the developed study can be applied to molecules with more complex geometries.

2 Theory

2.1 Theoretical Background

Let us consider an aqueous solution of DNA fragments orientated due to the presence of an external electric field. In this case, the solution behaves as a uniaxial crystal with its optical axis parallel to the external electric field. Let us suppose that a plane polarized beam of light that propagates perpendicularly to the optical axis falls upon the solution. The laboratory coordinate system (LCS) is chosen with its x - and z -axes coincident with the propagation direction of light and the external electric field direction, respectively. The light will propagate through the solution with its electric field vector given by:

$$\mathbf{E}(x) = \begin{bmatrix} 0 \\ E_{oy}\exp(-in_y\omega x/c) \\ E_{oz}\exp(-in_z\omega x/c) \end{bmatrix} \quad (1)$$

where n_y and n_z are the complex refraction indexes of the solution in the directions y and z , respectively, ω is the angular frequency of the light, and c is the light velocity in vacuum.

The interaction between the light electric field and the molecules leads to the electronic polarization of the system. The polarization vector is important because it allows connecting the macroscopic properties of the solution, such as the refraction indexes, with the microscopic properties of the individual molecules. From a macroscopic view point, the polarization vector is given by:

$$\mathbf{P}(x) = \begin{bmatrix} 0 \\ P_{oy}\exp(-in_y\omega x/c) \\ P_{oz}\exp(-in_z\omega x/c) \end{bmatrix} \quad (2)$$

where P_{oy} and P_{oz} are components not dependent on x . Vectors $\mathbf{P}$ and $\mathbf{E}$ are connected through the relation $\mathbf{P} = \epsilon_o[\chi]\mathbf{E}$, where ϵ_o is the electric permittivity of vacuum and $[\chi]$ is the electric susceptibility of the solution. In the LCS, the electric susceptibility is represented by a diagonal tensor whose nonzero components determine the refraction indexes n_y and n_z through the relation:

$$n_k^2 = 1 + \chi_{kk} \quad k = y, z \quad (3)$$

where we use the well-known relations $n_k^2 = \kappa_{kk}$ and $\kappa_{kk} = 1 + \chi_{kk}$, where κ_{kk} ($k=y, z$) are the diagonal components of the relative electric permittivity of the solution.

Electric birefringence is defined as the difference between the real refraction indexes in the parallel and perpendicular directions to the optical axis:

$$\Delta n_r = n_{rz} - n_{ry}. \quad (4)$$

2.2 Molecular Model

We use the BRM model to represent the geometry of DNA fragments. The BRM model is composed of two cylindrical arms of length $L/2$ having an angle β between them. The BRM is placed in the plane $x'z'$ of the molecular coordinate system (MCS) as shown in Fig. 1. The BRM model includes as a special case ($\beta=180^\circ$) the straight-rod macroion (SRM) model. Unless the opposite is indicated, in the present work, we will use the BRM model ($\beta \neq 180^\circ$).

Fig. 1 The BRM is placed in a molecular coordinate system $x'y'z'$. The electronic oscillators are placed along each one of its arms. The $x''y''z''$ system is the principal coordinate system for the oscillators belonging to the upper arm

The optical properties of DNA molecules are described using the DDA. In this approximation, each molecule is equivalent to an array of M electronic oscillators that interact among themselves and with the incident light beam. We place the oscillators along each arm of the BRM separated in such a way that the distance D between two any adjacent oscillators is always the same (see Fig. 1). Let us consider an oscillator belonging to the upper arm of the BRM. A system of axes x'', y'', z'' chosen with the z'' -axis coincident with the symmetry axis of the upper arm constitutes the principal coordinate system for this oscillator. In such a system, the optical polarizability of this oscillator is represented by a diagonal tensor:

$$[\alpha] = \begin{bmatrix} \alpha_{\perp} & 0 & 0 \\ 0 & \alpha_{\perp} & 0 \\ 0 & 0 & \alpha_{//} \end{bmatrix} \quad (5)$$

where $\alpha_{//}$ and $\alpha_{\perp}$ are the components of the optical polarizability tensor in the parallel and perpendicular directions to the DNA helix, respectively. The optical polarizability tensor of an oscillator that belongs to the upper arm expressed in the MCS is given by:

$$[\alpha_{\text{up}}] = \begin{bmatrix} \alpha_{//} \cos^2(\beta/2) + \alpha_{\perp} \sin^2(\beta/2) & 0 & \sin(\beta/2) \cos(\beta/2) (\alpha_{//} - \alpha_{\perp}) \\ 0 & \alpha_{\perp} & 0 \\ \sin(\beta/2) \cos(\beta/2) (\alpha_{//} - \alpha_{\perp}) & 0 & \alpha_{//} \sin^2(\beta/2) + \alpha_{\perp} \cos^2(\beta/2) \end{bmatrix}. \quad (6)$$

In a similar way, we obtain the optical polarizability tensor of an oscillator that belongs to the lower arm in the MCS:

$$[\alpha_{\text{low}}] = \begin{bmatrix} \alpha_{//} \cos^2(\beta/2) + \alpha_{\perp} \sin^2(\beta/2) & 0 & -\sin(\beta/2) \cos(\beta/2) (\alpha_{//} - \alpha_{\perp}) \\ 0 & \alpha_{\perp} & 0 \\ -\sin(\beta/2) \cos(\beta/2) (\alpha_{//} - \alpha_{\perp}) & 0 & \alpha_{//} \sin^2(\beta/2) + \alpha_{\perp} \cos^2(\beta/2) \end{bmatrix}. \quad (7)$$

2.3 Microscopic Analysis of Polarization

In this section, we present a microscopic analysis of the polarization process induced in the solution by the electric field of the incident light. We base our analysis on the use of the DDA to describe the optical properties of the DNA molecules by means of an array of oscillators as indicated in the previous section. The electric field on the i oscillator of a molecule is obtained by adding to the electric field of the incident light the electric field of other oscillators in the molecule and the electric field of the distant molecules treated as continuous by means of the Lorentz–Lorenz approximation. Thus, the electric dipole moment induced in the i oscillator is:

$$\mu_i = ([R]^{-1} [\alpha_i] [R]) \left[\mathbf{E}(x_i) + \frac{1}{3\epsilon_o} \mathbf{P}(x_i) - \sum_j ([R]^{-1} [T_{ij}] [R]) \mu_j \right] \quad (8)$$

where $[R]$ is the Euler angles transformation matrix from the LCS to the MCS and $[T_{ij}]$ is the dipole interaction tensor between the dipoles i and j expressed in the MCS:

$$[T_{ij}] = \frac{1}{4\pi\epsilon_o} \left[k^2 ([I] - \mathbf{e}_{ij} \mathbf{e}_{ij}^t) \frac{1}{r_{ij}} - ([I] - 3 \mathbf{e}_{ij} \mathbf{e}_{ij}^t) \left(\frac{1}{r_{ij}^3} - \frac{ik}{r_{ij}^2} \right) \right] \exp[ikr_{ij}] \quad (9)$$

where $\mathbf{e}_{ij}$ is a unit vector directed from the position of dipole j to the position of dipole i , r_{ij} is the distance between them, and k is the wave vector in the medium. It is necessary to mention that we represented $\mathbf{e}_{ij}$ by a column matrix. Therefore, the product $\mathbf{e}_{ij} \mathbf{e}_{ij}^t$ in the last equation is given by a matrix with dimension 3×3 .

Solving (8) for μ_i , we obtain:

$$\mu_i = \sum_j ([R]^{-1} [A_{ij}] [R]) \left[\mathbf{E}(x_j) + \frac{1}{3\epsilon_o} \mathbf{P}(x_j) \right] \quad (10)$$

where $[A_{ij}]$ (with $i, j = 1, 2, \dots, M$) are blocks of dimension 3×3 of the hypermatrix $[A]$ given by:

$$\begin{bmatrix} [A_{11}] & [A_{12}] & \cdots & [A_{1M}] \\ [A_{21}] & [A_{22}] & \cdots & [A_{2M}] \\ \vdots & \vdots & \ddots & \vdots \\ [A_{M1}] & [A_{M2}] & \cdots & [A_{MM}] \end{bmatrix} = \begin{bmatrix} [B_{11}] & [B_{12}] & \cdots & [B_{1M}] \\ [B_{21}] & [B_{22}] & \cdots & [B_{2M}] \\ \vdots & \vdots & \ddots & \vdots \\ [B_{M1}] & [B_{M2}] & \cdots & [B_{MM}] \end{bmatrix}^{-1} \quad (11)$$

where

$$[B_{ij}] = \delta_{ij} [\alpha_i]^{-1} + [T_{ij}]. \quad (12)$$

The dipole moments given by (10) are directly related to the microscopic expression of the polarization vector. In other words, they are related to the electric dipole moment per unit volume average over a macroscopically small volume V_m which nevertheless contains a large number of molecules. The microscopic expression of $\mathbf{P}$ must be compatible with (2) in the sense that it must lead to amplitudes independent of x multiplied by the corresponding factors of phase. We use the same microscopic expression of $\mathbf{P}$ used by DeVoe [10] extended to anisotropic systems:

$$\mathbf{P}(x) = \frac{N_m}{V_m} \sum_i \left\langle \begin{bmatrix} 0 \\ \mu_{iy} \exp[-in_y \omega (x - x_i)/c] \\ \mu_{iz} \exp[-in_z \omega (x - x_i)/c] \end{bmatrix} \right\rangle \quad (13)$$

where N_m is the number of molecules in the volume V_m , μ_{iy} and μ_{iz} are the components of the electric dipole moment μ_i induced on the oscillator i , x_i the x coordinate of this oscillator, and where the sum is extended over all oscillators in a molecule. The angular brackets indicate an average over all orientations.

In the case of solutions, it is necessary to consider the solute and solvent's contributions to the polarization of the system. Using (10) and (13), we obtain the polarization vector:

$$\mathbf{P}(x) = [N_s \langle \alpha_s \rangle [I] + N \langle [\alpha_m] \rangle] \left[\mathbf{E}(x) + \frac{1}{3\varepsilon_o} \mathbf{P}(x) \right] \quad (14)$$

where N_s and N are the number of solvent molecules and solute molecules per unit volume, respectively, $\langle \alpha_s \rangle [I]$ is the average optical polarizability tensor of a solvent molecule (which we suppose isotropic and transparent) and $\langle [\alpha_m] \rangle$ is the average optical polarizability tensor of a solute molecule whose components are defined in the following way:

$$\langle [\alpha_m] \rangle_{kl} = \delta_{kl} \sum_{ij} \left\langle (R^{-1} [A_{ij}] R)_{kl} \exp[-in_k \omega (x_j - x_i)/c] \right\rangle \quad k, l = x, y, z \quad (15)$$

where we approximate the solution refraction index with the solvent refraction index in the argument of the complex exponential. The Kronecker delta δ_{kl} is included because the numeric calculations demonstrate that the nondiagonal elements of $\langle [\alpha_m] \rangle$ are negligible with respect to the diagonals, at least for the analyzed cases in the present work. That is consistent with the diagonality of the electric susceptibility tensor in the LCS.

Solving (14) for polarization and using the relations $\mathbf{P} = \varepsilon_o [\chi] \mathbf{E}$ and $n_k^2 = 1 + \chi_{kk}$, we find that the refraction indexes of the solution satisfies:

$$\frac{n_k^2 - 1}{n_k^2 + 2} = \frac{1}{3\varepsilon_o} (N_s \langle \alpha_s \rangle + N \langle [\alpha_m] \rangle_{kk}). \quad (16)$$

Separating the refraction indexes of the solution into its real and imaginary parts according to $n = n_{rk} + in_{ik}$ and since, for dilute solutions, $n_{ik} \ll n_{rk}$, the real and imaginary parts of (16) gives:

$$\frac{n_{rk}^2 - 1}{n_{rk}^2 + 2} = \frac{1}{3\varepsilon_o} (N_s \langle \alpha_s \rangle + N \operatorname{Re} \langle [\alpha_m] \rangle_{kk}) \quad (17)$$

$$n_{ik} = \frac{N}{2\varepsilon_o} A(\lambda) \operatorname{Im} \langle [\alpha_m] \rangle_{kk} \quad (18)$$

where $A(\lambda) = (n_s^2 + 2)^2 / (9n_s)$ is an internal field factor or an environment coefficient dependent on the solvent refraction index n_s .

2.4 Electric Birefringence

Electric birefringence can be expressed as a function of the molecules' microscopic properties applying (17) in the parallel and perpendicular directions to the optical axis:

$$\frac{n_{rz}^2 - 1}{n_{rz}^2 + 2} - \frac{n_{ry}^2 - 1}{n_{ry}^2 + 2} = \frac{N}{3\varepsilon_o} \left(\text{Re}\langle [\alpha_m] \rangle_{zz} - \text{Re}\langle [\alpha_m] \rangle_{yy} \right). \quad (19)$$

Making the approximation $(n_{rz}^2 + 2) \left(n_{ry}^2 + 2 \right) \cong (n_s^2 + 2)^2$ valid for dilute solutions, we obtain the following expression for the electric birefringence:

$$\Delta n_r = \frac{N}{2\varepsilon_o} A(\lambda) \left(\text{Re}\langle [\alpha_m] \rangle_{zz} - \text{Re}\langle [\alpha_m] \rangle_{yy} \right). \quad (20)$$

2.5 Light Absorption and Optical Polarizability

There exists a range of wavelengths for which the incident light intensity is attenuated while it passes through the solution. We characterize the solution absorption with the molar extinction coefficient in the absence of an external electric field. Due to the influence of the thermal motions, this case corresponds to a solution of molecules randomly orientated and, therefore, to a solution optically isotropic with a single refraction index which the imaginary part is given by:

$$n_i = \frac{N}{2\varepsilon_o} A(\lambda) \text{Im}\langle \alpha_m \rangle \quad (21)$$

where we use the nomenclature $\text{Im}\langle \alpha_m \rangle$ to represent any of the elements $\text{Im}\langle \alpha_m \rangle_{yy}$ or $\text{Im}\langle \alpha_m \rangle_{zz}$ because they are equal for random orientation.

We use the Lambert–Beer law [14] to relate the molar extinction coefficient per mole of molecule $\gamma(\lambda)$ with the imaginary part of the refraction index:

$$\gamma(\lambda) = -\frac{4\pi N_0}{2.3\lambda N} n_i. \quad (22)$$

Replacing (21) in (22), the molar extinction coefficient results:

$$\gamma(\lambda) = -\frac{2\pi N_0}{2.3\lambda \varepsilon_o} A(\lambda) \text{Im}\langle \alpha_m \rangle. \quad (23)$$

Equation (23) is useful to determine the imaginary optical polarizability of each oscillator from the experimental data of the absorption spectrum of DNA. At this point, we can determine the real optical polarizability of each oscillator by applying one of the Kronig–Kramers relations [15]:

$$\text{Re } \alpha(\lambda) = -\frac{2}{\pi} P \int_0^\infty \frac{\text{Im } \alpha(\lambda')}{\lambda' \left[1 - (\lambda'/\lambda)^2 \right]} d\lambda' \quad (24)$$

where the letter P means the principal values of the integral. The last relation is applied to each of the elements of the optical polarizability tensor.

2.6 Increment of the Solution Refraction Index with Respect to that of the Solvent

Let us consider again the case in which a light beam falls upon a solution of DNA molecules in the absence of the external electric field. Due to the random orientation of the molecules, the solution is characterized by only one refraction index whose real part n_r satisfies:

$$\frac{n_r^2 - 1}{n_r^2 + 2} = \frac{1}{3\varepsilon_o} (N_s \langle \alpha_s \rangle + N \operatorname{Re} \langle \alpha_m \rangle) \quad (25)$$

where $\operatorname{Re} \langle \alpha_m \rangle$ is the average real optical polarizability of a solute molecule calculated for random orientation. For the solvent refraction index, we have a similar equation to (25):

$$\frac{n_s^2 - 1}{n_s^2 + 2} = \frac{N_s}{3\varepsilon_o} \langle \alpha_s \rangle. \quad (26)$$

Subtracting (25) and (26) and making the approximations $(n_r^2 + 2)(n_s^2 + 2) \cong (n_s^2 + 2)^2$ and $n_r + n_s \cong 2n_s$ valid for dilute solutions, we obtain:

$$\delta n_r = \frac{N}{2\varepsilon_o} A(\lambda) \operatorname{Re} \langle \alpha_m \rangle \quad (27)$$

where $\delta n_r = n_r - n_s$ is the increment of the refraction index of the solution with respect to that of the solvent.

For DNA molecules like SRM, without considering the retardation effects of the incident light electric field, the optical polarizability of the oscillators in the visible region can be obtained using experimental measurements of δn_r and of the saturation electric birefringence. In that particular case, the molecule's real optical polarizability in the MCS is represented by a diagonal tensor with elements $\operatorname{Re} \alpha_{m//}$ and $\operatorname{Re} \alpha_{m\perp}$ in the parallel and perpendicular directions to the DNA helix, respectively. The average real optical polarizability of a solute molecule, randomly averaged over all orientations, is given by:

$$\operatorname{Re} \langle \alpha_m \rangle = \frac{1}{3} (2 \operatorname{Re} \alpha_{m\perp} + \operatorname{Re} \alpha_{m//}). \quad (28)$$

Therefore, the increment of the refraction index of the solution with respect to the solvent results:

$$\delta n_r = \frac{N}{6\varepsilon_o} A(\lambda) (2 \operatorname{Re} \alpha_{m\perp} + \operatorname{Re} \alpha_{m//}). \quad (29)$$

3 Results and Discussion

All the calculations in the present work were made in the MATLAB 6.0 environment. The parameters that characterize the used molecular model are: $D=23.8 \text{ \AA}$, $\beta=165^\circ$ for the BRM model, and $\beta=180^\circ$ for the SRM model. The dispersion of the water refraction index has been included using the values reported by Segelstein [16].

3.1 Oscillators' Optical Polarizability from Absorption Spectrum Values

In this section, we determine the real optical polarizability that we must assign to the oscillators to represent the optical properties of DNA in the visible region ($\lambda=589$ nm). The procedure for that consists in integrating the Kronig–Kramers relation given by (24) along all wavelengths where the absorption of DNA is appreciable. We have previously determined the integrand of the Kronig–Kramers relation using the experimental data of the DNA absorption spectrum. In the present work, we use the absorption spectrum shown in Fig. 2. This has been measured by Yamaoka and Matsuda [17] in the region $\lambda>215$ nm for DNA fragments in aqueous solution. For $\lambda<215$ nm, water absorption begins to be significant and, therefore, the data of DNA absorption in aqueous solution for smaller wavelength has not been reported. Nevertheless, we complete the spectrum for $\lambda<215$ nm with measurements made by Sontag and Weibezahn [18] for DNA film. This could be done because both spectra present an identical shape in the region $\lambda>215$ nm. Thus, we could determine a scale factor for the two spectra to coincide in the abovementioned region and we use the same scale factor to complete the spectrum of DNA in aqueous solution with the spectrum of DNA film for $\lambda<215$ nm (see Fig. 2).

The resulting absorption spectrum is divided into five absorption bands indicated by the dashed lines in Figure 2 in which each band is approximated with the Gaussian function:

$$\gamma_i(\lambda) = \gamma_{oi} \exp \left[-\frac{(\lambda - \lambda_i)^2}{2\sigma_i^2} \right] \quad (30)$$

where the peak position λ_i , the maximum molar extinction coefficient γ_{oi} , and the corresponding standard deviation of the i band are given in Table 1.

The optical polarizability tensor of each oscillator is expressed by (5). The subscripts // and $\perp$ are used to represent the anisotropy in the optical properties in the parallel and perpendicular directions to the DNA helix, respectively. To include this anisotropy, we separate the contributions to the optical polarizability of the five absorption Gaussian bands in two different groups depending on its origin. We assigned the absorption bands 1 and 2 to the bases' plane in an isotropic manner to represent the π - π^* transitions predominant at

Fig. 2 DNA absorption spectrum. Reported data for $\lambda>215$ nm by Yamaoka and Matsuda for DNA in aqueous solution. Data for $\lambda<215$ nm are proportional to the ones reported by Sontag and Weibezahn for DNA in film

Table 1 Resolution of DNA absorption spectrum into Gaussian components

Band	λ_i (nm)	$\gamma_{oi} \times 10^{-5}$ (m ² mol ⁻¹)	σ_i (nm)
1	260.85	4.38	16.45
2	195.85	6.27	19.18
3	142.95	7.81	20.02
4	108.58	6.01	17.64
5	60.55	9.51	27.60

these wavelengths. Its contribution to the real optical polarizability tensor is expressed in the following way:

$$\text{Re}[\alpha_1]_{589\text{ nm}} = \begin{bmatrix} \text{Re } \alpha_1 & 0 & 0 \\ 0 & \text{Re } \alpha_1 & 0 \\ 0 & 0 & 0 \end{bmatrix}_{589\text{ nm}} \quad (31)$$

where $\text{Re } \alpha_1(589\text{ nm})$ is calculated using the Kronig–Kramers relation:

$$\text{Re } \alpha_1(589\text{ nm}) = -\frac{2}{\pi} P \int_0^{\infty} \frac{\text{Im } \alpha_1(\lambda')}{\lambda' [1 - (\lambda'/589\text{ nm})^2]} d\lambda'. \quad (32)$$

The function $\text{Im } \alpha_1(\lambda')/\lambda'$, necessary to the integration, is adjusted in such a way that (23) reproduces the experimental results of absorption bands $\gamma_1(\lambda)$ and $\gamma_2(\lambda)$ for a discrete set of wavelengths λ_i . Figure 3 shows the obtained values (open circles) for $\text{Im } \alpha_1(\lambda_i)/\lambda_i$ and their corresponding theoretical fits (indicated by the dashed and solid lines).

Kiseleva et al. [19] have reported experimental studies of the absorption of sugar and of phosphate groups for wavelengths shorter than 160 nm. We include this absorption considering the absorption bands $\gamma_3(\lambda)$, $\gamma_4(\lambda)$, and $\gamma_5(\lambda)$ isotropic in the space. The

Fig. 3 Imaginary optical polarizability corresponding to the Gaussian bands 1 and 2

contribution of these three Gaussian bands to the real optical polarizability tensor is expressed in the following way:

$$\text{Re}[\alpha_2]_{589\text{ nm}} = \begin{bmatrix} \text{Re } \alpha_2 & 0 & 0 \\ 0 & \text{Re } \alpha_2 & 0 \\ 0 & 0 & \text{Re } \alpha_2 \end{bmatrix}_{589\text{ nm}} \quad (33)$$

where

$$\text{Re}[\alpha_2]_{589\text{ nm}} = -\frac{2}{\pi} P \int_0^{\infty} \frac{\text{Im } \alpha_2(\lambda')}{\lambda' [1 - (\lambda'/589\text{ nm})^2]} d\lambda'. \quad (34)$$

In this case, the function $\text{Im } \alpha_2(\lambda')/\lambda'$, necessary for the integration, is adjusted in such a way that (23) reproduces the experimental results of absorption bands $\gamma_3(\lambda)$, $\gamma_4(\lambda)$, and $\gamma_5(\lambda)$ for a discrete set of wavelengths λ_i . Figure 4 shows the obtained values (circles) for $\text{Im } \alpha_2(\lambda_i)/\lambda_i$ and their corresponding theoretical fitting.

The contributions of the absorption bands 1–2 and 3–4–5 to the real optical polarizability calculated through (32) and (34) are: $\text{Re } \alpha_1(589\text{ nm}) = 4.89 \times 10^{-39} \text{ F m}^2$ and $\text{Re } \alpha_2(589\text{ nm}) = 8.22 \times 10^{-39} \text{ F m}^2$. We considered both contributions to determine the real optical polarizabilities in the parallel and perpendicular directions to the DNA helix adding (31) and (33). Thus, we obtain the values $\text{Re } \alpha_{//}(589\text{ nm}) = 8.22 \times 10^{-39} \text{ F m}^2$ and $\text{Re } \alpha_{\perp}(589\text{ nm}) = 1.31 \times 10^{-38} \text{ F m}^2$.

We explain in this work some aspects related to the functions $\text{Im } \alpha_1(\lambda')/\lambda'$ and $\text{Im } \alpha_2(\lambda')/\lambda'$. To determine these functions using (23), we need to know, at least approximately, the values of $\text{Re } \alpha_1(\lambda_i)$ and $\text{Re } \alpha_2(\lambda_i)$ in the absorption region. The reason for this is that, for molecules composed of more than one oscillator, there exists a small coupling between the real and imaginary parts of the oscillators' optical polarizability. To approximately estimate the values of $\text{Re } \alpha_1(\lambda_i)$ and $\text{Re } \alpha_2(\lambda_i)$, we first suppose that

Fig. 4 Imaginary optical polarizability corresponding to the Gaussian bands 3, 4, and 5

each molecule is composed of only one oscillator. In this case, the real and imaginary parts of the oscillator's optical polarizability are not coupled. Then, the oscillator's imaginary optical polarizability is determined using (23) and, subsequently, the oscillator's real optical polarizability is estimated using the Kronig–Kramers relation. Finally, we estimate $\text{Re } \alpha_1(\lambda_i)$ and $\text{Re } \alpha_2(\lambda_i)$ for molecules composed of M oscillators, dividing by M the obtained values for molecules composed of one oscillator.

3.1.1 Interaction and Retardation Effects Influence

Figure 5 shows the relative percent difference between the theoretical values of the DNA absorption spectrum calculated including and omitting the interaction between oscillators and the retardation effects. We can observe that the combined influence of both effects leads to the increase and the decrease of the absorption for $\lambda < 175$ nm and $\lambda > 175$ nm, approximately. The difference between both absorption spectra increases with the light wavelength and reaches values of up to approximately 8%. These results are important because they justify the inclusion of the interactions and retardation effects in the theoretical model.

3.2 Oscillators Optical Polarizability from Δn_{rs} and δn_r

We obtain a second estimation of the oscillators' optical polarizability in the visible region using the experimental data of saturation electric birefringence Δn_{rs} and of the increment of the solution refraction index with respect to the solvent δn_r . To do that, we consider a SRM model ($\beta=180^\circ$) and we omit the retardation effects of the incident light through the molecule. This approximation is necessary to remove the coupling between the different components of the optical polarizability tensor. Otherwise, we would not be able to determine its components independently one from another.

The saturation electric birefringence corresponds to a complete orientation of the molecules in the direction of the applied electric field. It is obtained from the electric birefringence experimental data as a function of the orienting electric field and extrapolating

Fig. 5 Relative percent difference between the calculated molar extinction coefficients $\gamma_i(\lambda)$ and $\gamma_{wf}(\lambda)$, including and omitting the interactions and the retardation effects, respectively

for infinite electric field strength. From (20), the saturation electric birefringence is given by:

$$\Delta n_{rs} = \frac{N}{2\varepsilon_o} A(\lambda) (\text{Re } \alpha_{m//} - \text{Re } \alpha_{m\perp}). \quad (35)$$

Solving the system of equations formed by (29) and (35) for the elements of the molecular optical polarizability tensor in the MCS, we obtain:

$$\text{Re } \alpha_{m//} = \frac{2\varepsilon_o}{3NA(\lambda)} (3\delta n_r + 2\Delta n_{rs}) \quad (36a)$$

and

$$\text{Re } \alpha_{m\perp} = \frac{2\varepsilon_o}{3NA(\lambda)} (3\delta n_r - \Delta n_{rs}). \quad (36b)$$

The experimental values of Δn_{rs} and δn_r for a solution of DNA fragments with an average length of 1,350 Å, concentration $c=7$ mg/dl, and visible light $\lambda=589$ nm, have been reported by Bertolotto et al. [20]. We use those experimental data and (36a) and (36b) to calculate the following values of the molecular optical polarizability: $\text{Re } \alpha_{m//} = 9.11 \times 10^{-37}$ F m² and $\text{Re } \alpha_{m\perp} = 1.34 \times 10^{-36}$ F m². Then, we adjusted the oscillator optical polarizability in the parallel and perpendicular directions to the DNA helix in such a way that the values of the above obtained molecular optical polarizability are reproduced. That can be made through the following procedure. First, we transform (15) in order to determine $[\alpha_m]$ in the MCS. As we consider in this section a SRM model, the resulting expression of $[\alpha_m]$ is a diagonal matrix with principal elements $\text{Re } \alpha_{m//}$ and $\text{Re } \alpha_{m\perp}$. The dependences of $\text{Re } \alpha_{m//}$ and $\text{Re } \alpha_{m\perp}$ on the oscillator optical polarizability are given by equations (11) and (12). Because we omit the retardation effects, (12) becomes simplified. Second, the theoretical values of $\text{Re } \alpha_{m//}$ and $\text{Re } \alpha_{m\perp}$ are calculated using the oscillator optical polarizabilities, $\text{Re } \alpha_{//}$ and $\text{Re } \alpha_{\perp}$, as variable parameters. Finally, the obtained values of $\text{Re } \alpha_{m//}$ and $\text{Re } \alpha_{m\perp}$ are plotted versus $\text{Re } \alpha_{//}$ and $\text{Re } \alpha_{\perp}$, respectively, and linear fitting to obtain the values of $\text{Re } \alpha_{//}$ and $\text{Re } \alpha_{\perp}$ that reproduce the molecular optical polarizabilities $\text{Re } \alpha_{m//}$ and $\text{Re } \alpha_{m\perp}$ above obtained from the experimental data of Δn_{rs} and δn_r . Based on the procedure described above, our results are the following: $\text{Re } \alpha_{//} = 1.52 \times 10^{-38}$ F m² and $\text{Re } \alpha_{\perp} = 2.45 \times 10^{-38}$ F m².

Comparing the real optical polarizability in the visible region obtained in this section with the values obtained in the previous section, we can see that the results in this section are smaller than those obtained above by a factor of approximately 1.85. This difference can be a consequence of integrating the Kronig–Kramers relation in a finite interval of wavelengths, omitting all absorption out of the ultraviolet region. Infrared absorption of DNA was not included because its relative intensity is low. Shapiro et al. [21], in their study of light scattering by DNA applying DDA, assigned one polarizability per oscillator in the X-ray region. Nevertheless, this polarizability was not included in the present work because it comes from the absorption data for benzene.

3.3 Interaction Influence

We study the interaction influence on the anisotropy of real molecular optical polarizability $\text{Re } \Delta \alpha_m = \text{Re } \alpha_{m//} - \text{Re } \alpha_{m\perp}$. This is equivalent to analyzing the interaction influence on the saturation electric birefringence (see (35)). The retardation effects are not significant in

this case because in the saturation state the molecules are oriented perpendicularly to the incident light. Figure 6 shows the relative percent difference between the theoretical values of $\text{Re } \Delta\alpha_m$ including and omitting the interactions between oscillators for SRM with different lengths. We can observe an increment of the interaction influence with the molecules' length until it reaches a saturation value of approximately 22%. This means that each oscillator of a molecule significantly interacts only with its nearest neighbors and, therefore, for molecules sufficiently large the edge effects are removed.

3.4 Comparison with Previous Theories

Equation (20) for the electric birefringence has been deduced for molecules like BRM of arbitrary size. This is the usual expression for the electric birefringence, except for the factor $A(\lambda)$ that is usually approximated with $1/n_s$. However, in our case, unlike previous theories [2–4], it has been deduced without supposing that the molecules are small with respect to the light wavelength. The tensor $\langle [\alpha_m] \rangle$ represents the average response of the whole molecule to the incident light considering the inhomogeneity of the light electric field through the molecule and the interactions between its different parts (see (15)). The restriction to molecules like BRM occurs because the diagonality of tensor $\langle [\alpha_m] \rangle$ is a necessary condition to obtain (20), and this has been proved only for the BRM model. However, the tensor $\langle [\alpha_m] \rangle$ should be diagonal because the electric relative permittivity tensor should be also diagonal in the LCS to optically characterize the solution as a uniaxial crystal. Therefore, we expect that the tensor $\langle [\alpha_m] \rangle$ should be diagonal for molecules with more complex geometries, and we are working to prove this point analytically and numerically.

4 Conclusions

DDA is a valid technique to study the electric birefringence of DNA fragments like BRM with comparable dimensions to the light wavelength. Applying DDA, we can include the inhomogeneity of the light electric field through the molecule and the interactions between its different parts. The usually used theories for electric birefringence, and developed supposing that the molecules are small with respect to light wavelength, remains valid to determine the

Fig. 6 Relative percent difference between the anisotropies of the calculated molecular real optical polarizability $\text{Re } \Delta\alpha_{mi}$ and $\text{Re } \Delta\alpha_{mwi}$, including and omitting the interactions between oscillators, respectively

real molecular optical polarizability even for molecules like BRM with arbitrary size. However, to study the contributions of the different parts of the molecule to the molecular optical polarizability, we must include the retardation effects of the light electric field and the intramolecular interactions. For DNA fragments with comparable dimensions to the light wavelength, the molecular optical properties are affected by the retardation effects and the intramolecular interactions up to approximately 22%.

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