

## Electro-optical Analysis of Macromolecular Structure and Dynamics

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### Abstract

Electro-optical effects are induced by external electric field pulses applied to solutions or suspensions and are recorded by various optical techniques. These effects are very useful for the characterization of macromolecular structures and their dynamics in solution. One of the field-induced effects is alignment of molecular dipoles, which can be detected at a very high sensitivity by measurements of the dichroism or the birefringence. Stationary values of these optical parameters recorded at different electric field strengths can be used to characterize dipole moments and to determine the orientation of chromophores with respect to the dipole vector. The transients reflect rotational diffusion, providing a particularly accurate measure of size and shape. The internal flexibility is also reflected in these transients. Another type of field-induced effect is chemical relaxation, which can be detected selectively and is very useful for the characterization of reactions, like ligand binding and conformation changes. The techniques based on electric field effects are unique in the sense that problems can be solved, which are difficult or even impossible to be sorted out by other techniques.

**Key words:** Electric dichroism, Electric birefringence, Rotational diffusion, Chemical relaxation, Stopped flow

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### 1. Introduction

Electrostatic interactions are known to be essential for most biological structures and their functions. This is simply due to the large number of charged residues in biological macromolecules and the fact that electrostatic interactions operate over long distances. Electrostatics is not only important for internal interactions but also for the molecular identity exposed to the environment, i.e., for interactions with other molecules. The electrostatic “character” is reflected by the response of these molecules to external electric fields. Studies of molecules by measurements of the response to

external electric field have a long history. One of the pioneers was Peter Debye, the “master of molecules,” who provided the basis for characterization of molecules by dipole moments (1). The response of molecules to an external electric field by a sensitive optical procedure was first demonstrated by John Kerr (2). This approach has been developed to a powerful method (3–6) providing electrical, optical, and hydrodynamic parameters at a high accuracy. Another closely related technique was developed by Manfred Eigen and coworkers, who used electric field pulses for perturbation of molecular interactions and established chemical relaxation for studies of fast reactions (7).

The methods based on effects induced by external electric fields are simple in principle, but not always simple in the technical implementation. Some of the instruments were developed to a high degree of sophistication. Unfortunately, the special instruments are currently not available commercially as a whole and must be assembled from components. The experiments described below require two separate functions: a generator for the electric field pulses and a spectro-photometric detection system. The details are very much dependent on the goals of the experiments.

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## 2. General

### **2.1. Orientation and Reaction Effects Induced by Electric Fields**

As indicated already above, electric fields may induce two different types of effects:

1. Orientation corresponding to alignment of the molecules in the direction of the field vector, determined by an induced, permanent, or other dipole moment associated with the molecules. When the electric field is applied, the molecules rotate from a random distribution in space toward a state with their dipoles being partially aligned in the direction of the field vector (cf. Fig. 1). Upon pulse termination, the molecules turn back to the random distribution by rotational diffusion with a characteristic time constant or a spectrum of time constants. Because rotational diffusion is very much dependent on the size and the shape, the decay of the orientation provides accurate information on the structure. Moreover, the optical anisotropy may be studied at different field strengths corresponding to different degrees of orientation. Such data can be used to evaluate the nature and the magnitude of the electric dipole. Furthermore, these data tell the orientation of the chromophore(s) used for the measurements with respect to the dipole vector. The orientation of molecules does not change their conformation or state of aggregation and thus may be classified as a “physical” effect.

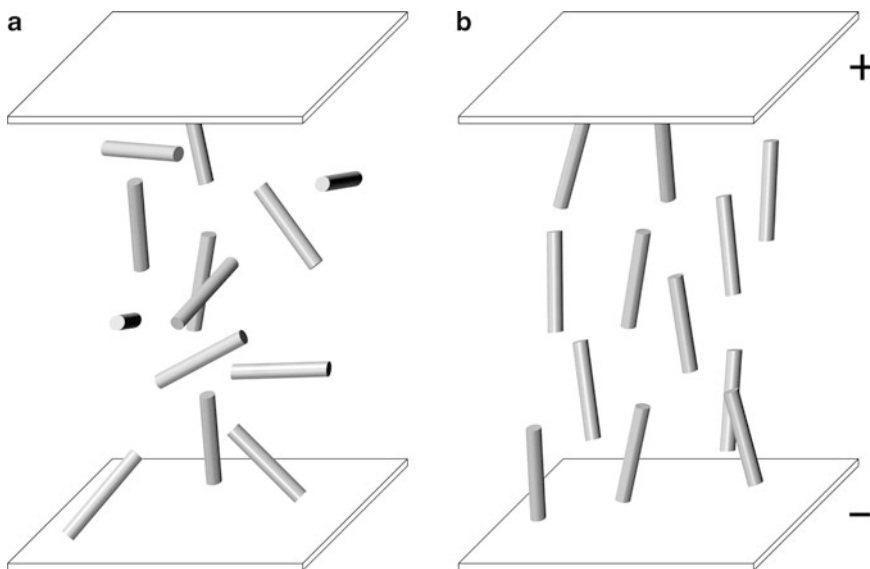


Fig. 1. Scheme of the alignment of rod-like molecules under the action of an electric field. (a) Random orientation in the absence of an external field; (b) partial alignment in the presence of an external electric field.

- Examples for reactions induced by electric field pulses are changes of conformation, changes of ligand binding, or changes in the degree of association. In most cases, changes of noncovalent interactions are induced, whereas chemical bonds usually are not affected. Thus, field-induced reactions usually are in the domain of “soft” chemistry. These effects are very useful to study the dynamics of the molecules under investigation. When the perturbations induced by the field remain small, the transients are described by relaxation time constants. Measurements at different concentrations can be used to derive rate constants and also equilibrium constants.

## 2.2. Electric Dichroism

The field-induced orientation is reflected by changes in the absorbance of polarized light. The absorbance change  $\Delta A(\varphi)$  at an angle  $\varphi$  of the polarization plane with respect to the vector of a given external electric field is dependent on  $\varphi$  according to

$$\Delta A(\varphi) = \Delta A_{\parallel} (3 \times \cos^2 \varphi - 1) / 2. \quad (1)$$

The maximal change of absorbance,  $\Delta A_{\parallel}$ , is observed, when the plane of polarization and the field vector are parallel ( $\varphi = 0$ ). For perpendicular alignment of polarization plane and field vector ( $\varphi = 90$ ), an effect in the opposite direction,  $\Delta A_{\perp}$ , appears as described by

$$\Delta A_{\parallel} = -2\Delta A_{\perp}. \quad (2)$$

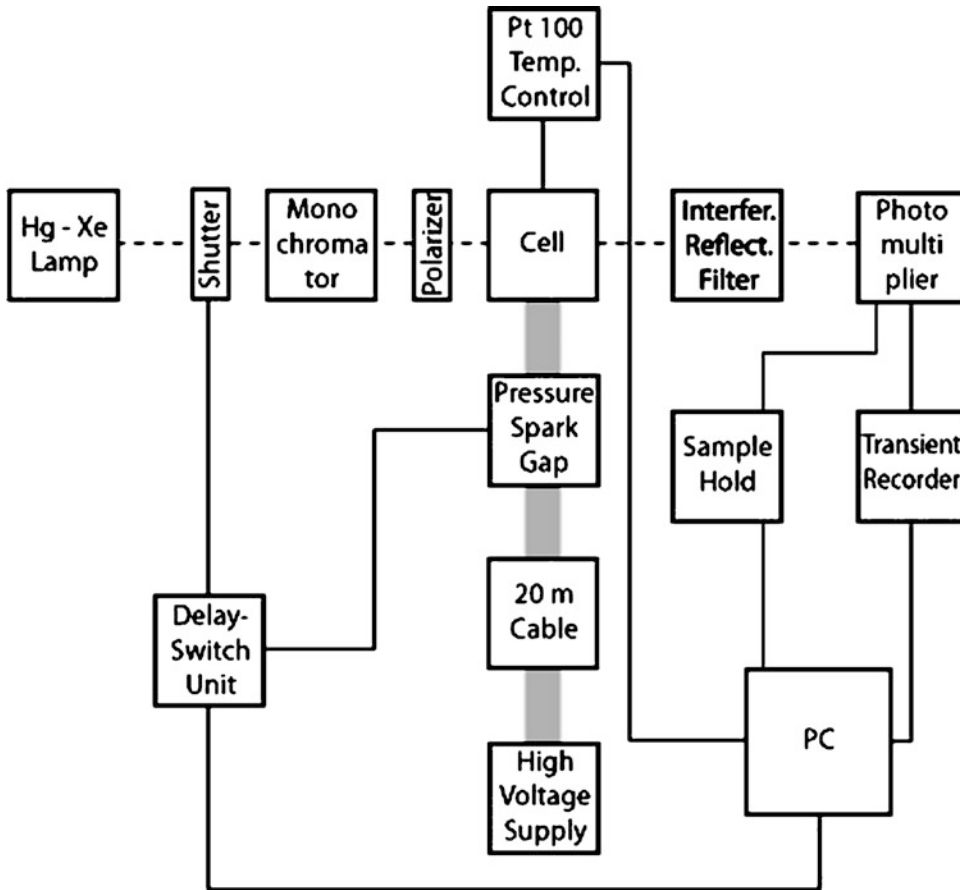


Fig. 2. Scheme of an instrument for automatic measurements of the electric dichroism. A high-pressure Hg–Xe lamp is used as a light source. The interference reflection filter is used to suppress electro-luminescence. Electric field pulses are generated by discharge of a high voltage cable, using a pressure spark gap as a fast high voltage switch. The instrument is used for automatic data collection: at fixed time intervals and provided that the temperature in the cell is within preset limits, an initiation signal is sent by the PC, which opens the shutter and activates the pressure spark gap. A “Sample Hold” records the total light intensity before pulse application. The time sequence of these events is optimized to reduce photo-damage as much as possible and to apply the field pulse as soon as the detection system is stationary. Most of the high voltage energy is dissipated in a compensation cell parallel to the measuring cell (Figure adapted from ref. (13)), reprinted with permission from American Institute of Physics.

The relation between  $\Delta A_{\parallel}$  and  $\Delta A_{\perp}$  is useful as a control (cf. below). The optical effect is presented in most cases in the form of the reduced dichroism

$$\xi = (\Delta A_{\parallel} - \Delta A_{\perp}) / \bar{A}, \quad (3)$$

where  $\bar{A}$  is the isotropic absorbance (in the absence of the external electric field). A scheme including the optical components required for measurements of the electric dichroism is shown in Fig. 2: a high intensity light source, a monochromator, a polarizer, and a photomultiplier detector (for more details, cf. Subheading 2.6).

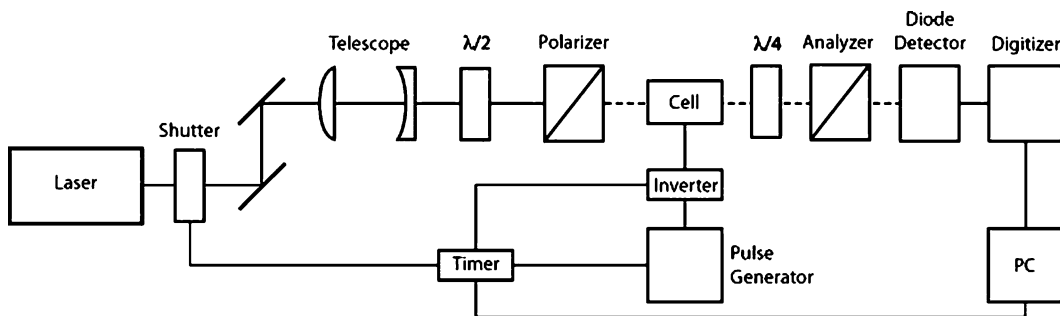


Fig. 3. Scheme of an instrument for measurements of the electric birefringence. The telescope is used to reduce the beam diameter. The PC controls automatic data accumulation: (1) The digitizer is armed; (2) The timer is activated and gives signals to open the shutter, to invert the pulse polarity, and finally to trigger the pulse generator.

### 2.3. Electric Birefringence

The orientation of molecules under electric field pulses is not only reflected by changes of absorbance but also by changes in the refraction index. These optical signals are complementary. The electric birefringence is the difference in the refraction index for light polarized parallel and perpendicular to the field vector. A standard setup for measurements of the electric birefringence (3) has the following optical components (Fig. 3): (1) Lasers are used as a convenient light source. (2) The light must be polarized at an angle of  $45^\circ$  with respect to the vector of the applied external electric field. If the laser light is polarized, the plane of polarization may be rotated to the required  $45^\circ$  by a  $\lambda/2$  plate, and finally the degree of polarization is increased by a polarizer. The polarized light passes the cell, a  $\lambda/4$  plate with its slow axis parallel to the polarization plane of the polarizer, an analyzer in almost crossed position and finally arrives at the diode detector (see Fig. 3). A procedure for proper alignment is presented below (Subheading 4.3.1).

The change of light intensity  $\Delta I_\delta$  induced by an electric field pulse is given (3) by

$$\Delta I_\delta / I_\alpha = [\sin^2(\alpha + \delta/2) - \sin^2(\alpha)] / \sin^2(\alpha). \quad (4)$$

$I_\alpha$  is the total light signal measured in the absence of an external electric field at the analyzer position rotated by an angle  $\alpha$  from the crossed position.  $\delta$  is the phase shift or optical retardation:

$$\delta = 2\pi\ell\Delta n/\lambda, \quad (5)$$

where  $\Delta n$  is the difference in the refractive index,  $\ell$  is the optical path length, and  $\lambda$  is the wavelength of light used for the measurement.

#### **2.4. Electric Birefringence Versus Electric Dichroism: Pros and Cons**

The electric birefringence offers some advantages compared to electric dichroism:

1. Birefringence can be measured outside the range of light absorption and, thus, photoreactions are avoided.
2. Lasers with their high light intensity and narrow light beams are very useful for increased sensitivity. Narrow light beams are essential for construction of cells with low electrode distance and long optical pathways.

However, the electric dichroism also has some advantages:

1. Transients of the electric dichroism are not perturbed by contributions of the solvent. (In aqueous solutions the birefringence of water may be relatively large.)
2. Field-induced reactions can be detected more easily: measurements at the magic angle demonstrate directly and selectively the existence of reactions (see Subheadings 2.5 and 4.2.3).
3. Calculations of the electric dichroism from known structures or from models are more simple and straightforward.

#### **2.5. Selective Study of Reaction Effects in the Presence of Orientation Effects**

In many polymer systems, electric fields induce orientation of molecules without changes of the conformation or other reaction effects, but it is always advisable to check for the existence of any field-induced reaction. Fortunately, there is a simple approach for selective detection of field-induced reactions by measurements of the absorbance or fluorescence under magic angle conditions (8). This approach is based on equation (1), which implies that the absorbance change due to orientation disappears at the “magic angle”  $\varphi = 55.74^\circ$ . Thus, any change of absorbance observed at this orientation of the polarized light must be due to some reaction. A corresponding relation holds for fluorescence (9).

The theoretical basis of the magic angle technique is relatively simple, whereas clean experiments under magic angle conditions require special caution. This is due to the fact that absorbance changes resulting from orientation are usually very large, whereas the changes from reaction effects often are much smaller. Thus, the magic angle has to be adjusted very carefully. Furthermore, optical windows of standard observation chambers may change the plane of polarization, whenever these windows experience some strain. For this reason, the optical windows must be absolutely strain-free and must be inserted into the cell without inducing any strain.

In most cases, electric field pulses induce only relatively small changes of chemical reaction equilibria. Under these conditions, the approximations used for the analysis of chemical relaxation are valid: rate equations may be simplified by linearization (7, 10, 11) and, thus, the evaluation of data is facilitated considerably. An obvious drawback of small perturbations is small amplitudes of

observed effects. However, this problem may be overcome by repeating the relaxation experiment many times with signal averaging, until the signal-to-noise ratio is sufficiently large.

### **2.6. Components for Instruments**

Studies of electric field effects require special instruments, which are not available commercially but must be assembled from components. An extensive choice of optical components is provided by various companies. Currently, it is more difficult to find suitable generators for high voltage pulses. A wide range of different generators has been used for applications starting from relatively low field strengths in the range around 1 kV/cm up to very high field strengths close to the limit of breakdown voltages around 100 kV/cm. The required technical efforts are not only dependent on the voltage range but also on the limit of time resolution. Among the advantages of electric field techniques is the possibility of a high time resolution. Pulse generators based on the cable discharge technique with a time resolution of a few nanoseconds have been described (12, 13). An example of an instrument for automatic collection of dichroism data with continuous supervision of operating conditions is illustrated in Fig. 2. Fast analog-digital converters and automatic sampling together with signal averaging for increased signal-to-noise ratios are required for optimal processing of experimental data.

A very sensitive detection of field-induced orientation effects is possible by birefringence measurements (cf. Fig. 3). Using lasers with a high light intensity and a sufficiently high stability, the sensitivity of birefringence measurements could be extended beyond the standard level used in the past. Because of narrow light beams, it is possible to construct cells with short electrode distances and high optical pathlengths. A major challenge remains the construction of cells with strain-free optical windows. A simple cell construction used at low field strengths is given in Fig. 4a. A novel type of cell window construction (Fig. 4b) proved to be very useful for minimization of strain and reduction of cell window birefringence (27).

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## **3. Sample Preparation**

### **3.1. Dialysis**

The signals of most biological macromolecules induced by electric fields are very strongly dependent on the salt concentration. This is mainly due to ion binding at various sites on these macromolecules, affecting their electric parameters. In order to avoid problems with reproducibility, the samples should be transferred into a well-defined ionic environment by extensive dialysis against the buffer used for measurements. Bivalent ions like  $Mg^{2+}$  or  $Ca^{2+}$  often have a particularly high affinity. Thus, measurements to be conducted in the absence of these ligands require extensive dialysis of solutions

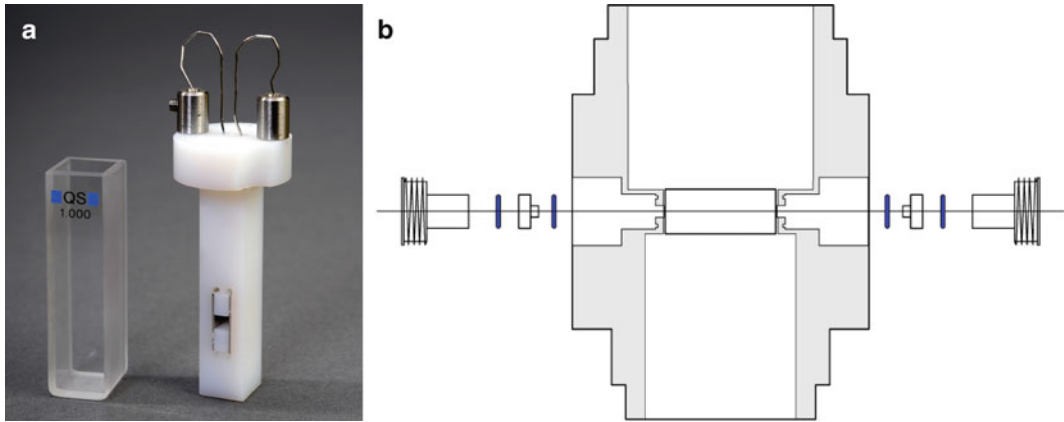


Fig. 4. Cells for electro-optical measurements. (a) The quartz cuvette (with an optical pathlength of 1 cm) is selected for minimal strain. The insert, made from Teflon, holds the Pt-electrodes at a given distance and the connection wires for pulse application. (b) Construction of optical windows inserted in cell bodies machined from dynal, polyacrylate, or any other synthetic polymer: silicon O-rings hold the quartz windows in a state with minimal strain and, thus, birefringence of the windows is reduced to a minimum (27). Pt-electrodes are inserted from the bottom and from the top.

against buffers containing EDTA; for complete exclusion of bivalent ions, some initial dialysis cycles in the presence of high concentrations of monovalent salt are recommended.

1. Select dialysis tubing with a pore size smaller than the size of the macromolecule.
2. Boil the tubing in water for ~5 min; exchange the water and boil again—during this procedure impurities are washed out, the tubing material is converted to a swollen and sterile state.
3. Seal one end of the tubing by a double knot—use gloves whenever handling the tubing.
4. Fill in the solution of your macromolecule and seal the open end of the tubing by a double knot.
5. Put the tubing into a reservoir of buffer with a volume approximately 100× larger than the volume inside the tubing. The buffer in the reservoir should be stirred. Removing of multivalent ions is supported by adding a high concentration of monovalent salt (e.g., 1 M NaCl) and by adding EDTA during initial dialysis cycles. The dialysis time depends on the pore size—usually a few hours for one equilibration. The number of repetitions depends on the required purity—usually several times. If the sample requires the presence of multivalent ions for remaining in its native state, the dialysis against EDTA and probably also against high salt should be omitted.
6. The procedure is completed by extensive dialysis against the final buffer—with at least several exchange cycles.



For safety of the sample, the dialysis should be conducted in a cold room. If ions like  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  must be removed and ETDA cannot be a component of the final dialysis buffer (e.g., for studies of bivalent ion binding), dialysis and storage of the samples should be in vessels from, e.g., polypropylene or quartz.

### **3.2. Degassing**

Application of electric field pulses may cause cavitation effects, perturbing the measurements and even leading to cell destruction in the worst case. This type of problem may be avoided by careful degassing of solutions within the measuring cell. A standard procedure involves filling the solution into the measuring cell, putting the cell into a desiccator, which is then evacuated by a simple pump (water jet or one stage oil pump). The final pressure should be only slightly below the boiling pressure, in order to avoid loss of solution by boiling retardation. It is advisable to reduce the pressure slowly and watch carefully the appearance of bubbles. Gentle mechanical impacts may be applied to support detachment of bubbles from surfaces. When the solution is at its boiling point, bubbles tend to appear in pulses. It is recommended to return to standard pressure quickly after appearance of such pulse by stripping the vacuum tube from the desiccator.

Some protein solutions tend to precipitate upon degassing. In such cases, the cell may be filled with buffer and degassed separately. Subsequently, the buffer is removed from the cell, keeping a thin layer of the solvent at the surfaces and in the corners. The protein solution is degassed in another vessel separately and then filtrated directly into the cell.

It is always useful to check the concentration and the state of the sample by taking a spectrum in the measuring cell. Any changes of the sample during the measurements may be detected by a comparison of spectra before and after measurements.

#### *3.2.1. Resistance*

Whenever electric fields are applied to samples, the resistance of the sample in the measuring cell is essential. Thus, a control of the resistance of each sample before start of the measurements is important—using an instrument for recording of ionic conductivities. The resistance may also be used to determine the increase of the temperature induced during pulse application.

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## **4. Measurements**

### **4.1. General**

#### *4.1.1. Temperature Control*

Measurements of the electric dichroism/birefringence may provide rotational time constants at a very high accuracy. For quantitative evaluations, the temperature of the solutions must be under careful control, because the rate of rotational diffusion is directly

dependent on the viscosity and the viscosity of water is strongly dependent on the temperature. A change of the temperature from 20 to 19°C induces a change of the water viscosity by 2.5%, which is a very large effect in view of the high accuracy routinely accessible by the electric dichroism/birefringence technique ( $\pm 1\%$  for the time constants of rigid rods like DNA fragments).

#### 4.1.2. Field Strengths

For various reasons, it is useful to measure experimental data for a range of electric field strengths. Any change of, e.g., rotational time constants with the field strength may indicate some special effect, e.g., a field-induced change of the conformation. However, there may be also other special phenomena from superposition of different relaxation effects associated with opposite amplitudes, resulting in an unusual relaxation effect at a given field strength. As demonstrated by recent model calculations (14), singular points with a relaxation effect suggesting an unusual conformation may appear. Erroneous conclusions from this type of effect may be avoided easily by measurements over a sufficiently wide range of electric field strengths.

#### 4.1.3. Control of Dichroism

For convenience, the electric dichroism is often measured only at parallel orientation of the polarized light with respect to the field vector, because the effect is maximal under these conditions. It is very useful, however, to check the magnitude of the dichroism also for perpendicular orientation, because deviations from the expected ratio  $\Delta A_{\parallel}/\Delta A_{\perp} = -2$  may indicate field-induced reaction effects. A direct control for the existence of field-induced reaction effects is possible by measurements at the magic angle (cf. Subheading 4.2.3).

#### 4.1.4. Spectroscopic Parameters

Selection of the optimal wavelength is crucial for any spectroscopy investigation. Usually, a wavelength is selected close to the maximum of the absorbance of the molecules under investigation. A major advantage of this choice is the fact that measurements can be conducted at concentrations where aggregation effects are minimal. However, there may be other criteria for selection. One of them is a maximal light intensity for optimal signal-to-noise ratios. Among the available light sources, mercury-xenon arc lamps provide particularly high light intensities at special mercury emission lines: 248.2, 265.2, 280.4, 289.4, 302.2, 313.2, 334.2, 366.3, 404.7, 435.8, and 546.1. Because of the relatively close spacing, useful lines may be found for most applications.

In some cases, a wavelength outside the range of maximal absorbance is favorable. The electric dichroism strongly depends on the orientation of the transition dipole: usually ( $\pi^* \leftarrow \pi$ ) transitions are polarized in the plane of aromatic chromophores, whereas ( $\pi^* \leftarrow n$ ) transitions are usually polarized in perpendicular

direction to the aromatic plane. The difference in orientation leads to large changes in the electric dichroism and, thus, it is very useful to do test measurements in different wavelength ranges. In close analogy, it is crucial to check spectral changes before starting measurements of chemical relaxation. Here, the relative change may be more important than the absolute one. In some cases, these relative changes are much higher at spectral shoulders than at the absorbance center.

#### 4.1.5. Time Resolution and Signal-to-Noise Ratio

The quality of relaxation transients is determined by the signal-to-noise ratio, which is affected by many different factors. This ratio is increased by increasing the light intensity and also by limiting the bandwidth, i.e., the time constant of the detector. In both cases, there are practical limits given by the photo-stability of the sample and the required time resolution. The photo-stability must be checked experimentally. A simple rule of thumb for the time resolution indicates that the time constant of the detector should be adjusted to a value smaller by a factor of  $\sim 10$  than the time constant of the fastest process to be detected. When this rule cannot be implemented, the experimental data may be evaluated by numerical deconvolution procedures. Because there are quite reliable deconvolution procedures (15), it may be even useful to limit the bandwidth of the detector beyond the usual rule of thumb with the benefit of an increased signal-to-noise ratio and subsequently eliminate the detector effect on the transients by deconvolution.

#### 4.1.6. Averaging

When all the spectroscopic parameters are optimized, the quality of the experimental data may still be improved by signal averaging. Because the signal-to-noise ratio increases with the square root of the number of shots included in the average, an increase of the signal quality by a factor of 10 requires 100 shots. Thus, there are practical limits in averaging. Constraints may also be given by insufficient sample stability. This stability may be checked by taking spectra before and after measurements.

### 4.2. Dichroism

#### 4.2.1. Dichroism Amplitude

1. Adjust polarization plane parallel to field vector.
2. Adjust wavelength and slit width of monochromator and bandwidth of detector.
3. Measure the total light intensity  $I_{\parallel}^0$  for light polarized parallel to the field vector in the absence of an external electric field; take as much light as possible, but ensure that all parts of your transients are recorded in the linear range of your detector.
4. Apply an electric field pulse and record the transient; the electric field pulse should be long enough to induce a stationary level of the light intensity at the end of the pulse, i.e., the intensity should not change anymore, when the pulse length

is increased. For evaluations of accurate time constants, it is important to record signals for a sufficiently long time period such that the final level of the signal is defined precisely.

5. Read the difference  $\Delta I_{\parallel}$  between the light intensities before application of the pulse and the stationary level at the end of the pulse.
6. Calculate the absorbance change according to  $\Delta A_{\parallel} = -\log[(I_{\parallel}^{\circ} + \Delta I_{\parallel})/I_{\parallel}^{\circ}]$ .
7. Calculate the reduced dichroism amplitude  $\xi = 1.5 \times \Delta A_{\parallel}/\bar{A}$ , where  $\bar{A}$  is the isotropic absorbance at the given wavelength.

#### 4.2.2. Control of Dichroism Optics

1. Strain in the windows of your cell may be detected by a simple device with a bright light source and two crossed polarizers; put the cell between the crossed polarizers, and look through the windows toward the light source; if there is no strain, light does not pass through the windows; strain is indicated by bright areas appearing in the windows.
2. A solution with a high electric dichroism, e.g., DNA double helices, in a low salt buffer is filled into the cell and the solution is degassed (field-induced denaturation is avoided by, e.g., 100  $\mu\text{M}$   $\text{Mg}^{2+}$  in the buffer).
3. Measure dichroism amplitude  $\Delta A_{\parallel}$  with light parallel to the field vector (see Subheading 4.2.1, steps 1–6).
4. Measure dichroism amplitude  $\Delta A_{\perp}$  with light perpendicular to the field vector (see Subheading 4.2.1, steps 1–6 but polarizer perpendicular to the field vector).
5. The values  $\Delta A_{\parallel}$  and  $\Delta A_{\perp}$  should be consistent with  $\Delta A_{\parallel} = -2\Delta A_{\perp}$  within a few percent.
6. As an extension, the polarizer may be adjusted to the magic angle  $55.7^{\circ}$  with respect to the field vector; the field-induced amplitude  $\Delta A_{55.7}$  should be zero.
7. Deviations may be due to one the following reasons:
  - (a) If the amplitude  $\Delta A_{\parallel}$  is larger than  $\sim 10\%$ , the dichroism relations are usually not fulfilled; then the test should be repeated at a lower electric field strength or at a lower concentration of the polymer.
  - (b) The windows of the measuring cell may be under strain.
  - (c) There may be a field-induced reaction—see next procedure.

#### 4.2.3. Reactions Effects/ Field-Induced Conformation Changes

1. Measure the field-induced changes of light intensity at the polarizer angles  $0$ ,  $55.7$ , and  $90^{\circ}$  and get the absorbance changes  $\Delta A_{\parallel}$ ,  $\Delta A_{55.7}$ , and  $\Delta A_{\perp}$ .
2. Given a significant value  $\Delta A_{55.7}$  check whether  $[\Delta A_{\parallel} - \Delta A_{55.7}] = -2 \times [\Delta A_{\perp} - \Delta A_{55.7}]$ ; this is a test for internal consistency.

3. Additional evidence may be obtained by an analysis of time constants; usually the time constants of orientation are different from those of reactions.

### 4.3. Birefringence

#### 4.3.1. Adjustment of Optics for Birefringence Measurements

1. The polarizer between the light source and the measuring cell should be rotated to an angle of  $45^\circ$  with respect to the field vector in clockwise direction viewed from the direction of the light source.
2. In the absence of the  $\lambda/4$  plate, the analyzer, located between the cell and the detector, should be turned to the crossed position, corresponding to the minimum of light intensity passing from the light source to the detector.
3. The  $\lambda/4$  plate is inserted and rotated until the light intensity arriving at the detector is minimal again; in this angular orientation, the slow axis of the  $\lambda/4$  plate is parallel to the polarization plane of the polarizer.
4. The analyzer is rotated from the crossed position toward the polarization plane of the polarizer by an angle  $\alpha$ ; in this arrangement a negative birefringence of the sample results in a decrease of the light intensity, whereas a positive birefringence induces an increase of the light intensity; the angle  $\alpha$  depends on the available light intensity and the sensitivity of the detector; for measurements of negative birefringence  $\alpha$  is selected such that the total light intensity is close to the upper end of the linear response domain of the detector (e.g., 10% below the maximal value).

#### 4.3.2. Measure Birefringence

1. Read the total light intensity  $I_\alpha$  at the angle  $\alpha$  from the crossed position.
2. Apply an electric field pulse and record the transient induced by the field; read the stationary change of the light intensity  $\Delta I_\delta$  (in the time range where the change arrives at a constant level).
3. The phase shift or optical retardation is obtained by

$$\delta = 2 \times \left( \arcsin \left[ (\Delta I_\delta / I_\alpha) \times \sin^2(\alpha) + \sin^2(\alpha) \right]^{1/2} - \alpha \right). \quad (6)$$

4. The birefringence corresponding to the difference of the refractive index of the solution parallel and perpendicular to the applied field is given by  $\Delta n = \delta \lambda / (2\pi \ell)$ ;  $\lambda$  is the wavelength used for the measurement and  $\ell$  is optical path length of the cell.

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## 5. Data Analysis

### 5.1. Relaxation Components

The field-induced change of the absorbance, dichroism, or birefringence may be composed of several different individual relaxation components, which are identified by a detailed analysis of the transient(s) in terms of exponential functions. There are professional fitting programs (16) for this purpose, but the final decision on the number of contributing exponential terms is not always simple. One of the criteria is of course a direct visual inspection of the quality of the fit(s). A decision may be supported by inspection of plots of the difference between the data and the fit. Moreover, the autocorrelation of this difference may be useful. Finally, the decision must be often based on pragmatic considerations: when admission of an additional exponential does not decrease the error sum by, e.g., more than 20% reproducibly, then there is not enough information on such additional exponential and therefore the parameters of an additional exponential cannot be defined at a sufficient accuracy.

### 5.2. Amplitudes

Electro-optical amplitudes corresponding to stationary changes of the dichroism or the birefringence measured over a range of different electric field strengths are useful for the determination of dipole moments and optical anisotropies. For this purpose, the data must be analyzed by orientation functions (3), which are available for the basic cases of induced and permanent dipole moments. Biological macromolecules usually have many charged residues, which may lead to more complex cases of dipolar orientation and require special orientation functions (17). Moreover, electric fields may induce stretching of bent conformations, which has to be considered explicitly (18).

Amplitudes obtained for reaction effects (19) may be used to derive detailed thermodynamic parameters for reactions, provided that the amplitudes are measured over a sufficiently wide range of concentrations.

### 5.3. Time Constants

#### 5.3.1. Electric Dichroism and Electric Birefringence

The time constants derived from decay curves of the electric dichroism or the electric birefringence reflect rotational diffusion of the particles under investigation. These time constants provide detailed information on the size, shape, and also on the flexibility of the particles. The interpretation for particles of simple shape like spheres or rods is based on analytical expressions.

Data for particles of more complex shape can be analyzed by numerical procedures, which proved to be very reliable. Using the procedures of “Quantitative Molecular Electro-Optics”, the electro-optical response of rigid macromolecular structures can be calculated (20). This is very useful not only for comparisons of

structures in crystals and in solutions but also for control of structures designed by molecular modeling (21).

The theory predicts for rigid structures of arbitrary shape the existence of five different exponential terms in the decay curves (22, 23). In practice, the amplitudes for most of these terms are negligible and usually not more than two components can be resolved.

Electro-optical transients have a great potential for analysis of internal mobilities: However, quantitative interpretations require extensive simulations (24, 25).

### 5.3.2. Reaction Effects

In the limit of small perturbations, the interpretation of reaction time constants is relatively simple, because the differential equations can be linearized and the general results obtained for relaxation kinetics can be applied (7, 10, 11). Under these conditions, the number of relaxation effects directly indicates the number of independent reactions for the system under investigation. The concentration dependence of the time constants demonstrates reaction orders and can be used to determine rate constants.

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## 6. Stopped-Flow Electro-Optics

Electro-optical transients provide information on macromolecular structures in a rather compact form. Because these transients can be measured within short time intervals—usually within a few microseconds, it is possible to utilize the electro-optical approach for analysis of macromolecular kinetics by the stopped flow technique. The main requirement for this combination is construction of a simple and reliable stopped-flow electric-field jump instrument. This was enabled by integration of Pt-electrodes into standard quartz cells used for stopped flow measurements (26). Cells with different optical pathlengths have been constructed and proved to be reliable. The information on the reaction is obtained by recording transients measured by application of electric field pulses at different times after the flow is stopped. The method is very sensitive: for the case of ethidium intercalation into DNA double helices (26) the increase in the length of a DNA fragment with 95 base pairs resulting from intercalation of single ethidium molecules could be measured down to reaction times of less than a millisecond. Stopped-flow electro-optics proved to be very useful also for the analysis of structures during the reaction of the cAMP receptor with promoter DNA (28).

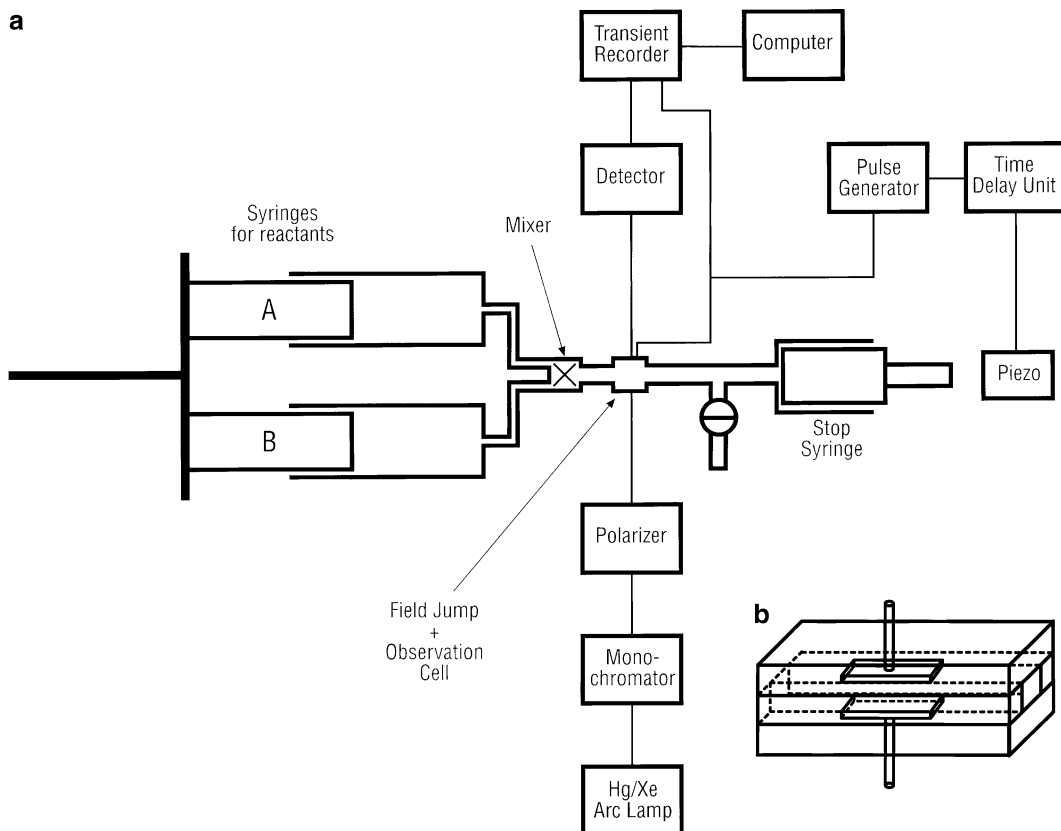


Fig. 5. Stopped flow electric field jump used for measurements of electric dichroism transients. **(a)** Reactants A and B are pushed from syringes through a mixing chamber via the observation cell into a stop syringe. The flow is stopped, when the piston of the stop syringe hits the piezo. The piezo-signal initiates a time delay unit, which triggers after a predefined delay time an electric field pulse applied to the cell. The optical components for measurement of the electric dichroism correspond to those shown in Fig. 2. The information on kinetics is obtained from a sequence of transients measured at different delay times. **(b)** The measuring cell is a quartz channel (cross section  $2 \times 2$  mm) with inserted Pt-electrodes (adapted from ref. (26)), reprinted with permission from Elsevier.

## References

1. Debye P (1929) *Polare Molekeln*. Hirzel, Leipzig
2. Kerr J (1875) A new relation between electricity and light: dielectric media birefringent. *Philos Mag* 50:337–348
3. Fredericq E, Houssier C (1973) *Electric dichroism and electric birefringence*. Clarendon, Oxford
4. O’Konski CT (ed) (1976) *Molecular electro-optics: part 1—theory and methods*. Marcel Dekker, New York
5. O’Konski CT (ed) (1978) *Molecular electro-optics: part 2—applications to biopolymers*. Marcel Dekker, New York
6. Stoylov SP (1991) *Colloid electro-optics: theory, techniques, applications*. Academic, London
7. Eigen M, DeMaeyer L (1963) Relaxation methods. In: Friess SL, Lewis ES, Weissberger A (eds) *Investigation of rates and mechanisms of reactions*, vol. VIII, part II. Interscience, New York, pp 895–1054.
8. Labhart H (1961) Methoden der Zuordnung von Absorptionsbanden von Farbstoffen zu berechneten Übergängen. *Chimia* 15:20–27
9. Meyer-Almes FJ, Porschke D (1993) Mechanism of intercalation into the DNA double helix by ethidium. *Biochemistry* 32:4246–4253



10. Bernasconi CF (1976) Relaxation kinetics. Academic, New York
11. Strehlow H (1995) Rapid reactions in solution. Weinheim, VCH
12. Grunhagen HH (1974) High-power square-wave pulse-generator for investigation of fast electric-field effects in solution. *Messtechnik* 82:19–23
13. Porschke D, Obst A (1991) An electric-field jump apparatus with ns time resolution for electrooptical measurements at physiological salt concentrations. *Rev Sci Instrum* 62:818–820
14. Antosiewicz JM, Porschke D (2009) Effects of hydrodynamic coupling on electro-optical transients. *J Phys Chem B* 113:13988–13992
15. Porschke D, Jung M (1985) The conformation of single stranded oligonucleotides and of oligonucleotide-oligopeptide complexes from their rotation relaxation in the nanosecond time range. *J Biomol Struct Dyn* 2:1173–1184
16. Provencher SW (1976) Fourier method for analysis of exponential decay curves. *Biophys J* 16:27–41
17. Shah MJ (1963) Electric birefringence of bentonite. 2. An extension of saturation birefringence theory. *J Phys Chem* 67:2215–2219
18. Porschke D (1989) Electric dichroism and bending amplitudes of DNA fragments according to a simple orientation function for weakly bent rods. *Biopolymers* 28:1383–1396
19. Winkler-Oswatitsch R, Eigen M (1979) Art of titration—from classical end-points to modern differential and dynamic analysis. *Angew Chem* 18:20–49
20. Porschke D, Antosiewicz JM (2007) Quantitative molecular electro-optics: Macromolecular structures and their dynamics in solution. In: Stoylov SP, Stoimenova MV (eds) *Molecular and colloidal electro-optics*. CRC, Boca Raton, FL, pp 55–1007
21. Porschke D (2010) Allosteric control of promoter DNA bending by cyclic AMP receptor and cyclic AMP. *Biochemistry* 49:5553–5559
22. Wegener WA, Dowben RM, Koester VJ (1979) Time-dependent birefringence, linear dichroism, and optical-rotation resulting from rigid-body rotational diffusion. *J Chem Phys* 70:622–632
23. Wegener WA (1986) Transient electric birefringence of dilute rigid-body suspensions at low field strengths. *J Chem Phys* 84:5989–6004
24. Hagerman PJ, Zimm BH (1981) Monte-Carlo approach to the analysis of the rotational diffusion of wormlike chains. *Biopolymers* 20:1481–1502
25. Antosiewicz J, Nolte G, Porschke D (1992) Modes of rotational motion of wormlike chains and the effect of charges on electrooptical transients. *Macromolecules* 25:6500–6504
26. Porschke D (1998) Time-resolved analysis of macromolecular structures during reactions by stopped-flow electrooptics. *Biophys J* 75:528–537
27. Porschke D (2011) Electric birefringence at small angles from cross position: enhanced sensitivity and special effects. *J Phys Chem* 115:4177–4183
28. Porschke D (2012) Structures during binding of cAMP receptor to promoter DNA: promoter search slowed by non-specific sites. *Eur Biophys J* 41:415–424