Experimental study of the electrically induced optical birefringence in suspension of densely packed giant lipid vesicles

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Abstract. Optical birefringence $\Delta n \sim 10^{-7}$, induced in suspension of giant lipid vesicles by high-frequency (500 kHz) AC electric field was measured. The liquid phases inside and outside the vesicles were iso-osmolar solutions of sucrose and glucose (0.22 mol/L). The two solutions have different optical densities and different specific weights. The estimated volume fraction of the vesicles in the studied suspension was 48 v%. The amplitude of the applied electric field was varied in the interval $5 \div 30$ V/mm. The observed effect was supposed to be a result from the electro-deformation of the giant vesicles leading to an optical anisotropy. The measured value of $\Delta n$ in our case is in the same range as for suspensions of large unilamellar vesicles with considerably higher lipid concentrations, as reported in the literature.

1. Introduction

Upon application of a high frequency AC electric field spherical vesicles change their shape to one of a prolate rotational ellipsoid with its short axis of symmetry parallel to the electric field. Such deformations have been observed in isolated vesicles [1] as well as in suspensions of giant (~20 µm) vesicles [2] and have been supposed to proceed in a suspension of small (~100 nm) vesicles [3]. The question whether the system of deformed vesicles implies an optical anisotropy has been discussed in a few publications [4-6] considering undetectable shape-transformation in vesicular suspensions of small dimension objects ($d \sim 100$ nm). Very weak optical anisotropy of the order of $\Delta n \sim 10^{-7}$ has been measured [3] and attributed to the vesicle deformation (anisotropy of the form) and to the possible vesicle pearling into relatively long chains due to the interaction of the electric field with the vesicles.

In the present work we carried out measurements of the optical response of a suspension of giant unilamellar vesicles subjected to a high frequency AC electric field. The process of the vesicle deformation was clearly visible by a microscope camera. So far, there are no publications on electro-optics for this kind of vesicular suspensions. In our experiments we applied a technique to detect the very weak effect of the field-induced optical anisotropy, as described in [7].

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2. Materials and methods

2.1. Preparation of giant vesicles suspension
In our experiments giant unilamellar vesicles (GUV) were prepared by electroformation [8] from 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-choline (SOPC), cat. No 850467 (Avanti Polar Lipids Inc., AL, USA). Double-distilled water was obtained from a quartz distiller. Sucrose (cat. No S7903, Sigma Ultra ®) was purchased from Sigma-Aldrich Chemie (Germany) and D(+)-Glucose monohydrate (cat. No 108346) – from Merck (Germany). Methanol and chloroform (“for analysis” grade) were purchased from Fluka Inc. (Germany). For GUV electroformation lipid depositions were made by the careful spreading of 50 µL of SOPC solution with concentration of 1 g/L in chloroform-methanol (9:1 volume parts) organic solvent on each ITO-coated glass electrode. We used freshly prepared organic solution of the lipid (previously lyophilized and kept under vacuum at - 20°C). After the complete drying of the lipid deposits for at least 2 hours under vacuum, the electroformation cell was assembled and completely filled with 0.2 mol/L of sucrose solution avoiding air bubbles. Then AC electric field with low frequency (10 Hz) was applied and its amplitude was successively increased up to $E_{pp} = 0.3$ V/mm (where $E_{pp}$ denotes the peak-to-peak amplitude of the electric field applied). Usually, high yield of unilamellar vesicles without any visible defects was obtained after four hours accordant with the observations of other authors [9]. The initial vesicle suspension was diluted 20 times in 0.22 mol/L of glucose and left to sediment in a glass flask with conical bottom. The glucose concentration in the water solution was taken slightly higher than the sucrose one in order to obtain flaccid vesicles after the equilibration of the partial pressures of the two sugars. The difference in densities for the two sugar solutions was $\Delta \rho = 10^{-2}$ kg/m$^3$ and of the refractive indexes $\Delta n = 3 \cdot 10^{-3}$. In this way, the relative weight of vesicles, which are slightly heavier than the suspending solution, allowed to concentrate the vesicles at the lowest conical part of the flask. After their complete sedimentation (approx. 6 hours) the supernatant was removed and the sediment of vesicles was collected and ready for manipulation. The precipitation continued in the sample cell. As a result, closely packed vesicular suspension (lower part of the cell) in contact with free of vesicles water solution of glucose (upper part of the cell) was obtained. Volume fraction of vesicles in the initial suspension filling the cell was determined to be 48% via vesicle counting performed on an inverted phase contrast microscope Axiovert 100 (Zeiss, Germany) with a dry x63 objective. The size distribution curve of the vesicle diameters had its main maximum at 9.7 µm and its width was 3.0 µm.

2.2. Experimental equipment and measurements of the electro-induced optical anisotropy
The technique for detection of very weak electro-induced optical anisotropy [7] is based on a measurement of the optical path difference between two directions in the cell (parallel and perpendicular to the electric field $\mathbf{E}$) as a function of the applied voltage. In figure 1 the experimental set-up is represented schematically.

A polarizing microscope was used. The light from the incandescent lamp passed through an interference filter ($\lambda = 515$ nm, $\Delta \lambda = 2.5$ nm). The symmetry axis of the sample cell (coinciding with the electric field direction) was placed under 45 degrees towards both the crossed polarizer and analyzer, thus introducing a path difference of $\Delta l(U) = l_0 - l(U)$, where $U$ is the applied voltage and $l_0$ is the initial path difference at $U = 0$ V. A Bräce-Köhler compensator giving a variable retardation $-l_r$ enabled a total phase shift variation $\Delta l_r(U) = I(U) - I_0$. The transmitted light intensity $I(U) = I_0 \sin^2 \left( \pi l_r(U) / \lambda \right)$ was detected by a photo-multiplier (PM). Using the compensator, $I_0$ was calibrated between the minimum (at $I_0$) and maximum (at $l_r = \lambda / 2$) transmission, then the compensator was fixed in position $I_0 = I = \lambda / 4$ to obtain a linear response of the transmitted intensity change $\Delta I(U) \approx I_0 \pi \Delta l_r(U) / \lambda$. To measure $\Delta I(U)$ avoiding any parasitic ionic effects in the...
suspension, sine voltage (500 kHz) with an amplitude from $U = 5$ V up to $U = 30$ V was applied to the cell.

The amplitude of the applied voltage was modulated to almost 100% by a low frequency (0.04 Hz) sine signal from a second generator. A differential amplifier was used to subtract the DC background from the PM output signal before further amplification. Finally, both the modulation signal (used as reference) and of the optical response signal (output of the differential amplifier) were transmitted to a PC by analogue-to-digital converters (ADC) after proper filtering to avoid aliasing effects.

**Figure 1.** Experimental set-up

**Figure 2.** Photographs of the vesicular suspension at: a) $U = 0$ V; b) $U = 30$ V.
The homogeneity of the field \( E \) was achieved by the construction of the experimental cell containing the suspension. A pair of ITO electrodes was evaporated at a distance \( \alpha = 1 \) mm on each of the two glasses of the cell. An O-ring (with diameter 8 mm and cross-section 0.5mm) was used as spacer. The voltage was applied to the respective “left” and “right” electrodes on the two glasses. Thus a homogeneous lateral electric field was exerted on the vesicular suspension. Its amplitude \( E \) was assumed to be \( E=U/\alpha \).

3. Results and discussion
Photographs of the vesicular suspension at \( U = 0 \) V and at voltage \( U = 30 \) V are presented in figure 2. It can be clearly seen that upon electric field the vesicles deformed into oblate rotational ellipsoids with their short axis of symmetry parallel to the electric vector \( E \).

Figure 3 shows an example of the part of the modulation signal and the optical response signal as measured for 300 s. The measured magnitude of the induced optical anisotropy changed with the frequency of the modulating signal. There was slight retardation of the optical response of the suspension with respect to the electric signal possibly due to viscous effects. The period of the low frequency modulation signal was chosen long enough (25 s) to assure that the viscous effects are negligible.

The optical response of the system was decomposed into its Fourier series and the amplitude of the term corresponding to the modulating frequency was determined. A second harmonics of the optical signal was also observed (see figure 4), possibly due to the strong nonlinearity of the induced anisotropy dependence on the electric field.

The dependence of the optical anisotropy, determined from the Fourier amplitudes corresponding to the frequency of the modulating signal, on the applied field intensity is shown in figure 5. At low values of the field, the optical anisotropy \( \phi_\epsilon - \phi_0 = \Delta I / (I_0 G) \) (where \( G \) is the gain of the differential

![Figure 3](image-url)
amplifier) is proportional to the square of the field. At high enough fields, the anisotropy attains some maximal value and does not depend on the field any more.

One possible explanation of this phenomenon is that the eccentricity of the ellipsoid approaches its maximal value determined by the excess area of the spherical vesicle [10].

**Figure 4.** Fourier spectra of the optical response of the vesicular suspension to applied electric field. A peak corresponding to the frequency of the modulating electric signal (f=0.04 Hz) is clearly seen. Second harmonic appears as well, possibly due to the strong nonlinearity of the induced anisotropy dependence on the electric field.

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**Figure 5.** Dependence of the induced anisotropy on the squared effective electric field amplitude. The experimental data are fitted reliably with a function \( f(E) = f_0 \left(1 - \exp\left[-\left(E/E_0\right)^2\right]\right) \). The values of the parameters \( f_0 \) and \( E_0 \) calculated from the experimental data are \( f_0 = -0.52 \) rad and \( E_0 = 8.2 \) V/mm.
In order to verify if there is any additional contribution to the anisotropy coming from the possible induced electric reorientation of the solution molecules (water, glucose), reference measurements with distilled water and water solution of glucose (0.22 mol/L) were carried out. No optical response was registered during both the measurements in the frames of the sensitivity of our method \((\Delta n \sim 10^{-8})\).

If the total cell thickness \(d\) is assumed to contribute to the measured optical anisotropy, the maximum effective optical birefringence is estimated to be \(\Delta n = \frac{\lambda (\varphi_2 - \varphi_0)}{2\pi d} \cong -10^{-7}\), which is in the same range as for suspensions of large unilamellar vesicles with considerably higher lipid concentrations, as reported in [3].

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