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Deformation of giant vesicles in AC electric fields —Dependence of the prolate-to-oblate transition frequency on vesicle radius

K. Antonova, V. Vitkova[(a)] and M. D. Mitov

Institute of Solid State Physics, Bulgarian Academy of Sciences - 72, Tzarigradsko Chaussee blvd, 1784 Sofia, Bulgaria, EU

Abstract – The electrodeformation of giant vesicles is studied as a function of their radii and the frequency of the applied AC field. At low frequency the shape is prolate, at sufficiently high frequency it is oblate and at some frequency, f_c, the shape changes from prolate to oblate. A linear dependence of the prolate-to-oblate transition inverse frequency, $1/f_c$, on the vesicle radius is found. The nature of this phenomenon does not change with the variation of both the solution conductivity, σ, and the type of the fluid enclosed by the lipid membrane (water, sucrose or glucose aqueous solution). When σ increases, the value of f_c increases while the slope of the line $1/f_c(r)$ decreases. For vesicles in symmetrical conditions (the same conductivity of the inner and the outer solution) a linear dependence between σ and the critical frequency, f_c, is obtained for conductivities up to $\sigma = 114\,\mu\mathrm{S/cm}$. For vesicles with sizes below a certain minimum radius, depending on the solution conductivity, no shape transition could be observed.

Introduction. – Biological cells in living organisms are widely subjected to various electromagnetic fields whose influence on physicochemical properties on the cells is not completely revealed. Lipid vesicles representing an extremely simplified physical model of procaryotic cells give opportunities to study the related phenomena by controlling important parameters such as vesicle sizes, membrane composition, contents and physicochemical properties of the suspending medium. An external electric field can deform the quasi-spherical shape of phospholipid vesicles in different ways. The type of deformations has been studied for alternating (AC) fields [1–6] and also for strong electric pulses [7,8]. In both cases a frequency-dependent prolate-to-oblate shape transition has been first theoretically predicted [1,6] and later, experimentally observed [7,8]. Vesicle electrodeformations have been studied previously with respect to different parameters such as: the thermal membrane fluctuations [9], the anisotropy of the membrane phospholipid bilayer [10], the conductivity ratio of the inner and outer vesicle solution [1,7,8]. In the low-frequency range (few kHz), the deformed shape is prolate with the rotational axis aligned to the field direction while an increase of the frequency changes the deformation to oblate keeping the rotational axis parallel to the field. Besides, the critical frequency of the shape transition of the ellipsoid adopted by the vesicle does not depend on the field amplitude. The presence of ions in the solution has a drastic effect on the increasing of the transition frequency, which can reach values of the order of MHz [6,9,11] and the vesicle shape is rather cylindrical than prolate [8]. Some of the parameters mentioned are generally dependent on the vesicle radius but few experiments have been carried out with objects which are practically monodisperse in size (in [6] radius $8\,\mu\mathrm{m}$, in [12,13] nanosized vesicles). Very recently, the existence of a relation between the transition frequency and the vesicle size has been announced theoretically [14] but it has not been systematically investigated so far. In the present work an experimental study of giant vesicles deformation in alternating electric field is carried out. These biomimetic objects are chosen in order to clarify the size-dependent effects related to the electric-field action on biological cells. A special attention is dedicated to the influence of the vesicle radius on the critical frequency, at which the prolate-to-oblate transition in the vesicle shape occurs. We studied this dependence at different conductivities of the suspending medium (distilled water or sugar aqueous solutions) and at equal strengths of the
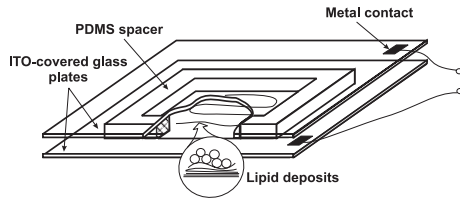
[(a)]E-mail: victoria@issp.bas.bg

Fig. 1: Sketch of the electroformation chamber without contact between the vesicle suspension and the electrical wires.

AC field. In our case, the inner aqueous solution enclosed by the vesicle membrane and the suspending medium are of equal conductivities (symmetrical conditions with respect to the lipid membrane).

Experimental. –

Sample preparation. The lipid vesicles were prepared from 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) (Avanti Polar Lipids, Inc., Alabama, USA) either by electroformation [15] or using the gentle hydration method [16] in distilled water or 0.2 M of sucrose or glucose aqueous solutions (both sugars were purchased from Sigma-Aldrich Chemie GmbH, Germany). All substances were used without further purification. For spontaneous swelling a lipid film was formed in the following way. A small amount of $200\,\mu l$ of the lipid solution (1 g/l of SOPC in chloroform-methanol mixture with volume ratio 9 : 1) was dried for six hours under vacuum on the bottom of a glass flask in order to remove any traces of organic solvents. After the formation of the lipid film it was gently hydrated with 5 ml of sugar aqueous solution. In order to study the impact of the preparation conditions on the studied phenomena different geometries of electroformation chambers, comprising of two parallel transparent plates covered by a thin (20 nm) conductive layer of indium-tin oxide (ITO) (MERCK, Germany), were used to obtain giant unilamellar vesicles. In most of the chambers the aqueous solution was in direct contact with the electrodes. A specially designed electroformation cell was also applied in which the ITO-covered plates were separated by a 7 mm spacer of polydimethylsiloxane (PDMS) (Sylgard 184 silicone elastomer kit, Dow Corning, Germany) in a way to avoid any contact between the water and the wires, connecting the chamber to the generator (see fig. 1). After their formation, vesicles were taken out of the cell and kept in a glass flask up to a day. The conductivities σ of all solutions were measured by a conductivity meter (Hydromat, LM302, Dresden, Germany). The suspension with highest conductivity has been obtained by electroformation in a chamber sealed by fresh silicon paste and in which the solution was in direct contacts with the ITO electrodes and metal wires. We suppose a drastic influence of the fresh silicon paste enriching the solution with free ions during its drying. Between these two extreme cases the vesicles were obtained by electroformation in water and sucrose in different chambers.
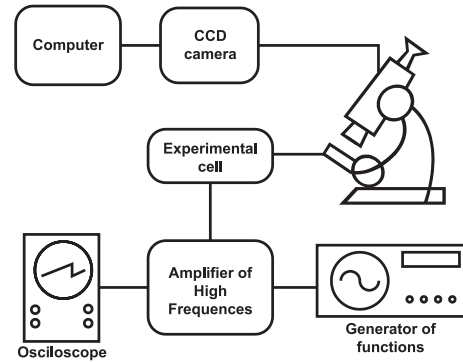

Fig. 2: Scheme of the experimental set-up.

Observation and registration of lipid vesicles in AC fields. The study of the vesicle deformation under an external electric field was carried out using a simple setup, described in detail in [17], specially adapted to avoid the sample electrolysis and to minimize the measurement artifacts due to charge migration, solution evaporation, etc. The samples were filled and flame-sealed in flat glass capillaries (VitroCom (New Jersey, USA) with inner dimensions of $0.05 \times 1 \times 100$ mm and wall thickness of 0.05 mm). The electric field, parallel to the capillary long axis, was applied between external electrodes of aluminium foil fixed at the capillary outer wall at a distance of 1 mm. Sinusoidal AC voltage with amplitude U up to 200 V and frequency varying from 0.5 kHz up to 1 MHz was formed using a function generator and high-frequency amplifier (Krohn-Hite 7602M). Numerical calculations of the electric field penetrating in the sample have shown that the field in the inter-electrode area was rather uniform (less than 5% of variation) and the effective (rms) amplitude was $E = 7 \times 10^4$ V/m (at frequencies > 10 kHz) [17]. The experimental set-up is depicted in fig. 2. All observations were performed on an inverted microscope Axiovert 100 (Zeiss, Germany) in phase contrast using a long-working distance objective $63\times$ (NA 0.75). Image recording was effectuated by a CCD camera with C2400-60 (Hamamatsu, Japan) control unit. The video signal from the camera was fed to a frame grabber board (DT3155, Datatranslation, USA) mounted in a computer for proper digitization (768×5768-bit pixels). For fluctuation analysis, an image was acquired once per second and recorded on the PC to obtain an image sequence of at least 100 frames for each studied vesicle in absence of electric field [18].

Results and discussion. – We have shown an unambiguous dependence of the shape transition frequency f_c on the vesicle radius r. In fig. 3 we represent a set of photos demonstrating this dependence. The bigger the vesicle radius r the lower f_c.

We consider the suspension of floating vesicles as an aqueous solution with a certain conductivity in which vesicles represent closed quasispherical lipid bilayers,
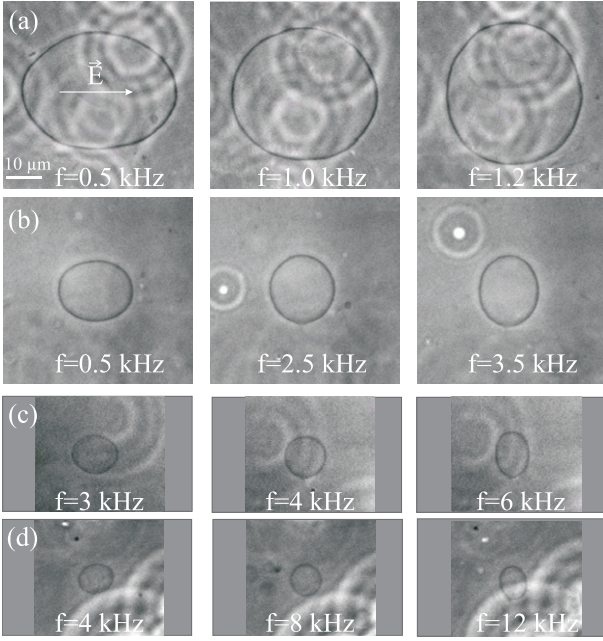
Fig. 3: Phase contrast images of vesicles in AC electric fields with strength $E = 0.7\,\text{kV/cm}$ and $\sigma = 35\,\mu\text{S/cm}$ —the critical frequency of the prolate-to-oblate transitions of their shapes depends on the vesicle radius: (a) $R = 22.8\,\mu\text{m}$, $f_c = 1.1\,\text{kHz}$; (b) $R = 11.35\,\mu\text{m}$, $f_c = 2.5\,\text{kHz}$; (c) $R = 7.2\,\mu\text{m}$, $f_c = 3.8\,\text{kHz}$; (d) $R = 5.4\,\mu\text{m}$, $f_c = 7.8\,\text{kHz}$.

Fig. 4: Dependence of the axes lengths, a, on the electric-field frequency for vesicles with different radii and membrane tensions. The conductivity of the suspending medium is $\sigma = 35\,\mu\text{S/cm}$. Full symbols stand for the axis along the field and open symbols give the length of the axis perpendicular to the field.

polydisperse in size and enclosing a part of the same solution. The suspension is subjected to an AC electric field with a varying frequency. The vesicle membrane itself is considered to be an insulator. When an AC electric field is applied, the ions in the inner and the outer solutions execute a directed motion towards the respective electrodes. Inside the vesicles the ions move in a given direction till either the direction of the electric field changes or they reach the vesicle membrane where they accumulate near the vesicle poles. As a result of the ions accumulation, the electric field inside the vesicle becomes zero while the field outside the vesicles is not zero. A detailed model and explanations of the phenomena leading to the prolate deformation (with the long axis of the ellipsoid along the AC field) at low frequencies is given recently by Vlahovska *et al.* [14]. At higher frequencies and/or larger vesicles, the ions inside the vesicles do not reach the membrane for the semiperiod of the AC field, so there is small or no charge accumulation that could compensate the external field. The result is that the electric field inside the vesicle is no more zero (as in the case of low frequencies). The ions just vibrate with the frequency of the AC field around their initial positions creating induced polarization. The conductive medium could be considered as polarizable dielectric. The vesicle membrane itself is another dielectric separating the inside from the outside. In this case the oblate deformation is favorable as obtained in [6].
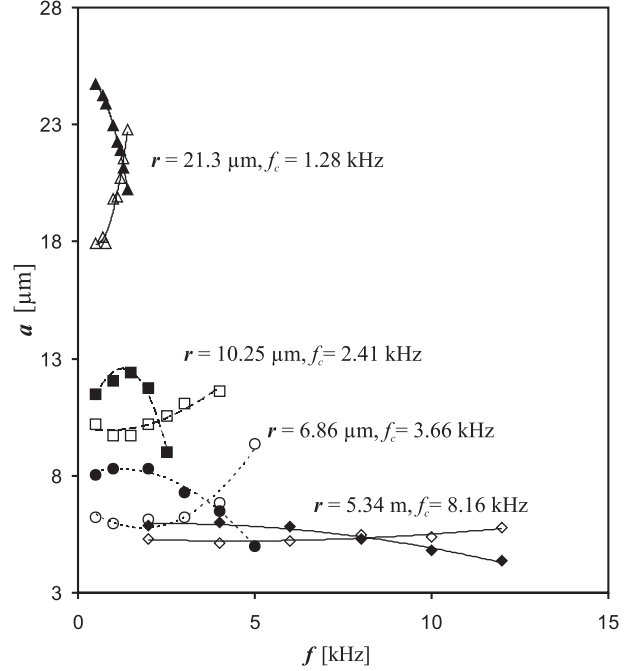
In fig. 4 we present the vesicle axes along the electric field and perpendicular to it as functions of the AC frequency. The experimental points are obtained as an average over an ensemble of vesicles taken from the phase contrast pictures. The cross-point marks the frequency at which the vesicle shape changes from prolate to oblate by passing through a sphere. In the absence of electric field we determined, via independent contour analysis, the radius of every studied vesicle and compared it to the value at the cross-point of the axes. The agreement between both values is excellent (limited by the resolution of the system). The radius dependence of f_c can be clearly distinguished. For a smaller vesicle the two principal axis lengths become equal at a higher frequency. We found a linear increase of $1/f_c$ (the period) with the vesicle radius as shown in fig. 5. During the experimental measurements a significant influence of the solution conductivity was observed as expected [8,14]. The higher induced conductivity could be caused by the metal contacts in some of the electroformation chambers used, the ITO electrodes, ion contamination due to the silicon paste, etc. To reveal the influence of these factors several different types of cells for the vesicle electroformation were made and two samples in water and sugar solutions were prepared using spontaneous swelling. In fig. 5 one can see the increase of f_c with the solution conductivity at $r = \text{const}$ (the values of $1/f_c$ decrease) as previously obtained by other authors [9,11]. The line with the highest slope is for the suspension
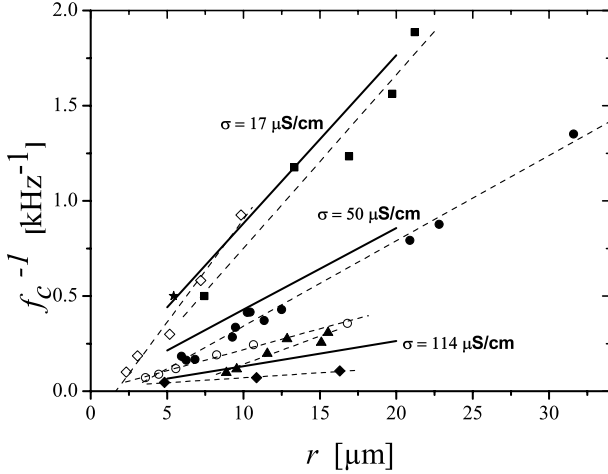
Fig. 5: Experimental results for the radius dependence of the "prolate-to-oblate" transition frequency at different conductivity of the suspending solution. $\diamond$: $\sigma = 17\,\mu\mathrm{S/cm}$ (in $0.2\,\mathrm{M}$ of sucrose); $\star$: $\sigma = 17\,\mu\mathrm{S/cm}$ (in $0.2\,\mathrm{M}$ of glucose); $\blacksquare$: $\sigma = 24\,\mu\mathrm{S/cm}$; $\bullet$: $\sigma = 35\,\mu\mathrm{S/cm}$; $\circ$: $\sigma = 50\,\mu\mathrm{S/cm}$; $\blacktriangle$: $\sigma = 71\,\mu\mathrm{S/cm}$; $\blacklozenge$: $\sigma = 114\,\mu\mathrm{S/cm}$. Solid lines represent the calculated membrane charging time (see text).

prepared by the gentle hydration method in sucrose solution (without any contact to metals). The experimental point ($\star$) is our measurement for a spontaneous swollen vesicle in 0.2M of glucose in water.

The experimental dependence $1/f_c(r)$ can be described as a linear function of the type: $1/f_c(r) = a \cdot r + b$. Each line crosses the r-axis at a minimum value of 1–$5\,\mu m$ indicating the existence of a threshold for the vesicle size below which there is no prolate-to-oblate shape transition. This observation is consistent with the theoretical evaluations in [1], where for a vesicle with $r \approx 1\,\mu m$ and for the same conductivities inside and outside, the authors have obtained only prolate shape with no shape transitions. At higher frequencies (in the dielectric regime) a minimum vesicle size (about $100\,\mathrm{nm}$) of field penetration has been predicted, leading to an absence of deformation for vesicles with smaller radii. The difference of one order of magnitude between the threshold theoretically obtained and the value extrapolated from our experimental data could be due to the possible effects of the non-negligible (finite) thickness of the space-charge layers, membrane anisotropy and inhomogeneities, which have not been considered in the theory [1].

Introducing the measured values for σ (for the highest, lowest and an intermediate conductivity) into the expression for time constant for membrane charging $\tau_{ch} = 1/f_{ch} = 3r\mathrm{C}/(2\sigma)$, where C is the membrane capacitance (taken to be $1\,\mu\mathrm{F/cm}^2$ [19], we plotted the linear dependence of τ_{ch} on the vesicle radii in fig. 5. The calculated lines go higher than the experimental dependencies, corresponding to the same solution conductivity. This tendency is consistent with the considerations, presented above, that the vesicle prolate-to-oblate transition occurs at a critical frequency $f_c > f_{ch}$.
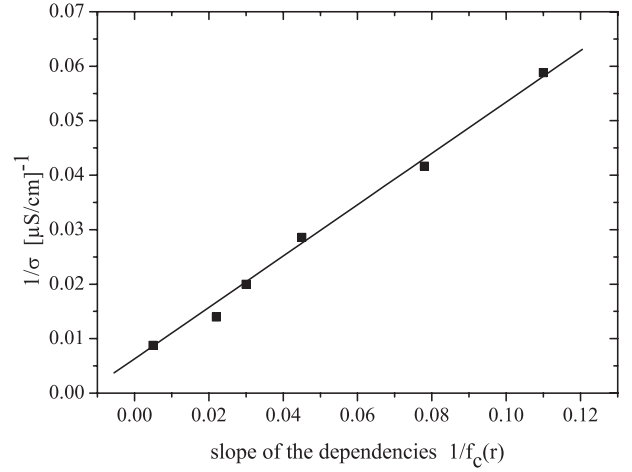

Fig. 6: Dependence between $1/\sigma$ and the slopes of the linear functions $1/f_c$ from fig. 5.

We found experimentally that the reciprocal value of the sample conductivity, $1/\sigma$, depends linearly on the slope, a, of the function $1/f_c(r)$, and can be represented as: $1/\sigma = c \cdot a + d$. On the other hand, $1/f_c(r) = a \cdot r + b$. Thus, the following relation between σ and $f_c(r)$ can be obtained: $\sigma(a,r) = f_c(a,r)/[c/r + (d - cb/r)f_c(a,r)]$. Neglecting b and d ($\sim 10^{-2}$), we obtain $\sigma(a,r)$ proportional to $f_c(r)$ (see fig. 6).

In conclusion, we obtained experimentally that the reciprocal value of the prolate-to-oblate transition frequency of the giant vesicles electrodeformation depended linearly on their radii. At symmetrical conditions with respect to the membrane (equal solution conductivities inside and outside the vesicles) we measured also a linear dependence between σ and f_c up to $\sigma = 114\,\mu\mathrm{S/cm}$. We demonstrated that the linearity of the phenomena does not change with the variation of both the solution conductivity and contents (water, sucrose, glucose). We revealed the existence of a conductivity-dependent threshold for the minimum vesicle radius, for which a shape transition could be observed.

We believe that our results will be useful for understanding the data dispersion due to the size-dependent effects in electrodeformation experiments with vesicles observed by other authors [11]. We hope that this work contributes to the expansion of the existing knowledge of the influence of alternating electric fields on lipid vesicles and will stimulate future research on the effects of electromagnetic fields on biomimetic and biological objects.

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