Alignment and Opening of Giant Lecithin Vesicles by Electric Fields

W. Harbich

Institut für Atom- und Festkörperphysik, Freie Universität Berlin

W. Helfrich

Institut für Theorie der kondensierten Materie, Freie Universität Berlin

Z. Naturforsch. **34 a,** 1063 – 1065 (1979) ; received July 12, 1979 *

AC electric fields ware applied to suspensions of giant lecithin vesicles observed under a phase contrast microscope. Elongated vesicles were found to align parallel to the field. In sufficiently strong fields the vesicles opened at both ends and became cylinders which in some cases were immutable under further increase of the field. The cylinder parameters can be used to calculate the edge energy of the cylinder wall. For the single bilayer we obtain $2 \cdot 10^{-6}$ erg cm^{-1}.

I. Introduction

It is a well-known phenomenon that nonconducting bodies in water, e. g. cylinders or elongated ellipsoids, align their long axes parallel to an applied electric field. The same type of electric alignment may be expected for elongated lecithin vesicles in aqueous solution if the electric resistance of their wall is high enough to squeeze the field lines out of the enclosed volume. Apart from confirming the alignment, we wish to report here on the opening of vesicles by electric fields and on the shapes of opened vesicles. The final shape at high enough fields, which we found to be cylindrical, will be used to calculate the energy per unit length of the wall edge. The calculation requires, besides the parameters of the cylinder, knowledge of the curvature-elastic modulus of the wall [1].

II. Experimental

In our experiments egg lecithin purchased from Merck was used without further purification. Giant vesicles were prepared by simple swelling of lecithin in water as described previously [2]. Viewing our preparations under a phase contrast microscope we found vesicular structures of many different shapes, often thin-walled and ranging in size up to 100 μm. From the dispersion some vesicles were brought by pipetting into a sealable microchamber consisting of two parallel glass slides and two parallel platinum wires. The wires served as spacers and electrodes over a length of 1 cm, they were 200 μm thick and 0.5 cm apart.

Continuous electric fields produced the undesired effect of electrophoresis, i. e. a more or less rapid movement of the vesicles. Therefore, we used in our experiments an alternating electric field of constant frequency $\nu = 2 \cdot 10^3\ \mathrm{s}^{-1}$. At root-mean-square fields $E < 10\ \mathrm{Vcm}^{-1}$ no orienting effect could be observed. The first significant alignment of large nonspherical vesicles was seen at $E \approx 15\ \mathrm{Vcm}^{-1}$. Thin vesicle walls continued to show wave-like fluctuations up to a field $E \lesssim 30\ \mathrm{Vcm}^{-1}$. With still higher fields the shape fluctuations vanished and the walls became very smooth. The speed at which elongated vesicles aligned parallel to the field increased with the strength of the applied field.

The origin of the orienting force may be assigned to the Maxwell stresses acting just outside the vesicle. We note that the total (static) force or torque exerted on the vesicle must be transmitted by the external field.

We may write for the torque

$$\oint (\boldsymbol{r} \times \boldsymbol{\sigma}\, \mathrm{d}\boldsymbol{A})$$

where the integral can in principle be taken over any closed surface around the vesicle and inside the aqueous medium. $\boldsymbol{\sigma}$ is the Maxwell stress tensor and $\mathrm{d}\boldsymbol{A}$ a normal vector pointing outwards. The electric conductance of bilayers is known to be of the order of $10^{-9}\ \Omega^{-1}\ \mathrm{cm}^{-2}$ [3]. As the conductivity of the aqueous medium was about $10^{-6}\ \Omega^{-1}\ \mathrm{cm}^{-1}$ the current through the vesicles could be neglected in our experiments. Any insulating body in a conducting medium is surrounded by the external field lines despite the existence of internal fields (there will be

---

* Eingang der 1. Fassung am 28. 5. 1979.

Sonderdruckanforderungen an Dipl.-Phys. W. Harbich, Institut für Atom- und Festkörperphysik, Fachbereich Physik, Freie Universität Berlin, Königin-Luise-Straße 28 – 30, D-1000 Berlin 33.

0340-4811 / 79 / 0900-1063 $ 01.00/0

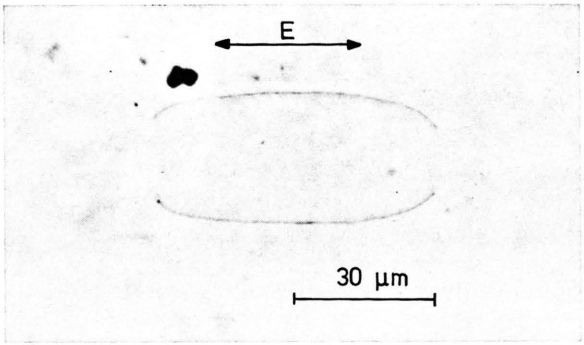

Fig. 1. Photographed contour of a vesicle with fairly wide openings at $E = 35\ \mathrm{Vcm}^{-1}$.

very high fields in the vesicle membrane, especially near the "poles"). Inspection of the external field and the associated Maxwell stresses explaines the well-known fact that elongated insulators align parallel to the field [4].

Long and thin-walled vesicles were ruptured at both ends by fairly low fields. Assuming for the break-down voltage ΔV the approximative relationship

$$E\,L = 2\,\Delta V\,,$$

where L is the length of the vesicle, we computed for the thinnest walls $\Delta V \approx 100\ \mathrm{mV}$. This value is in good agreement with other results [3]. The openings in the wall widened when the applied field was raised further and the shape became more and more cylindrical. An intermediate shape is shown in Figure 1. The length of the cylinders usually grew with rising field strength. However, length and radius were not simply a function of wall area and electric field but appeared to depend also on the number of bilayers forming the wall. The wall area always remained constant during deformations. In a few special cases no further changes of the cylinder with field strength could be discerned at sufficiently high fields. (The upper limit imposed by our equipment was $E = 70\ \mathrm{Vcm}^{-1}$.) As is to be shown next, this phenomenon permitted us to evaluate the edge energy of the egg-lecithin bilayer in water.

III. Theory

To derive a formula for the edge energy of a cylinder wall we start from the two contributions to the mechanical energy of a perfect hollow cylinder of radius r. Its edge energy is given by

$$E_\gamma = 2 \cdot 2\,\pi\,r\,\gamma \tag{1}$$

where γ is the energy per unit length of edge. The elastic energy of curvature residing in a fluid membrane may be expressed by [5]

$$E_\varkappa = \frac{1}{2}\,\varkappa\,(c_1 + c_2 - c_0)^2\,A \tag{2}$$

where c_1 and c_2 denote the two principal curvatures, $\varkappa$ a curvature-elastic modulus, and A the membrane area. The spontaneous curvature c_0 allows for possible differences between inside and outside of the fluid wall including the aqueous media. It was previously found not to be quite zero for most closed vesicles [6]. One of the two principal curvatures of a cylinder is zero, the other equal to the reciprocal of the cylinder radius. If the wall is a single bilayer, the two monolayers are most likely joined at the membrane edge by forming a half circle. There should be no difference between the aqueous medium inside and outside the open cylinder. Therefore, the spontaneous curvature is expected to be zero. Accordingly we may write for the elastic energy

$$E_\varkappa = \frac{1}{2}\,\varkappa\,\frac{1}{r^2}\,A \tag{3}$$

and for the total mechanical energy

$$E_{\mathrm{mech}} = 4\,\pi\,r\,\gamma + \varkappa\,A/2\,r^2\,. \tag{4}$$

Now we look for the cylindrical shape of minimum energy. Variation of E_{mech} as a function of r only leads to

$$\mathrm{d}E_{\mathrm{mech}}/\mathrm{d}r = 4\,\pi\,r - \varkappa\,A/r^3 = 0\,. \tag{5}$$

Making use of

$$A = 2\,\pi\,r\,L = \mathrm{const} \tag{6}$$

we obtain a formula of the edge energy,

$$\gamma = L\,\varkappa/2\,r^2\,. \tag{7}$$

The principal role of the electric field is to enforce a cylindrical shape. As the vesicle wall has an electric permittivity different from that of water we could introduce a dielectric energy of the wall. However, this energy should not depend on r if the wall is strictly parallel to the field and of constant area. There is also an electric contribution to the edge energy. An estimate showed it to be negligible in the case of a single bilayer, which conforms with the experimental observation of a final shape no longer dependent on field strength.

IV. Results and Discussion

Although open cylinders were seen fairly often, many preparations had to be studied to determine the edge energy of the single bilayer. From the cylinders we selected those showing lowest optical contrast. In this case we were reasonably sure from experience that the wall was just one bilayer. Another restriction was given by the very large ratio between cylinder length and radius characteristic of thin-walled specimens. The whole cylinder had to be viewed and photographed at a magnification sufficient to measure the radius. Radii of the order of 1 μm or less could not be reliably resolved. These limitations may explain why in a long series of experiments we found only three suitable cylinders. The data are listed in the Table 1.

Table 1

$r/\mu m$	$L/\mu m$	γ/dyn
1.4	360	$2.1 \cdot 10^{-6}$
1.5	292	$1.5 \cdot 10^{-6}$
1.6	295	$1.4 \cdot 10^{-6}$

The value of $\varkappa$ used in calculating γ is $2.3 \cdot 10^{-12}$ erg [1]. The first value for γ differs markedly from the two others which were measured three months later. During this period the egg lecithin had visibly darkened. The decrease of γ with time might thus be due to an accumulation of degradation products. Generally speaking, the chemical composition of the edge is likely to differ from that of the rest of the bilayer if more than one chemical compound is involved or impurities are present. As the edges contain very little material most of the lipid exchange should be very fast. Reassuringly, no particular time lag was noticed in the experiments.

It may be noted that the three cylinders were observed for about half an hour and found to be stable. Figure 2 shows one of the cylinders as seen in the phase contrast microscope. The cylinders were photographed and the radii photometrically determined from the negatives.

The fact that the cylinders could always be brought into focus over their whole length indicates the homogeneity of the applied field.

The mean value for the edge energy is larger by about a factor of two than an earlier estimation given by de Gennes [7] who equated it to the typical value of the Frank elastic constant of nematic liquid crystals [8], which is $1 \cdot 10^{-6}$ dyn.

Most of the cylinders we saw had a smaller ratio of length to radius than those listed in the table. The edge energies calculated from (7), using the $\varkappa$ quoted, were two or one powers of ten smaller than the above values. Incidentally, we found them to increase linearly with the applied field. We think that the walls of these cylinders consisted of more than one bilayer, a conclusion which in most cases could also be clearly drawn from the optical contrast. The edges might have been of a complicated form, such as turning loops of bilayers. Their compression by an increasing electric field could explain the apparent increase of the edge energy.

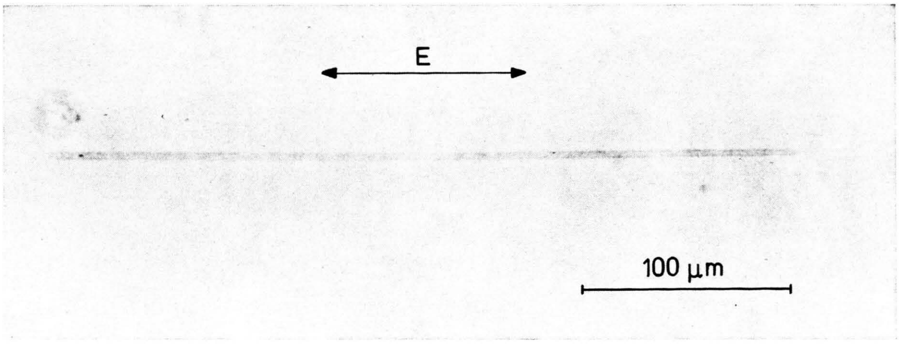

Fig. 2. Photograph of an open cylinder in its final shape.

[1] R. M. Servuss, E. Harbich, and W. Helfrich, Biochim. Biophys. Acta **436,** 900 (1976).
[2] W. Harbich, R. M. Servuss, and W. Helfrich, Phys. Lett. **57 A,** 294 (1976).
[3] See for instance H. Ti Tien in Bilayer Lipid Membranes, Marcel Dekker Inc., New York 1974.
[4] G. Schwarz, M. Saito, and H. P. Schwan, J. Chem. Phys. **43,** 3562 (1965).
[5] W. Helfrich, Z. Naturforsch. **28 c,** 693 (1973).
[6] W. Harbich, H. J. Deuling, and W. Helfrich, J. Physique **38,** 727 (1977).
[7] P. G. de Gennes, Private communication, 1974.
[8] P. G. de Gennes, The Physics of Liquid Crystals, Clarendon Press, Oxford 1974.