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NON-LINEAR CAPACITIVE EFFECTS AT PLANAR LIPID BILAYERS

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The current-voltage characteristics of planar lipid bilayers clearly deviate from linearity at high field strengths. Models for conductivity in bilayers neglect the influence of the electric field on the macroscopic shape and microscopic structure of the membrane. Changes in the macroscopic shape and microscopic structure of the bilayer can be detected by capacitance measurements. Usual methods [1,2] for measuring capacitance are slow and cannot reveal changes in the bilayer produced by a rapidly varying electric field. A technique that has been used for measuring hysteresis in ferroelectrics is suited for this purpose [3]. By means of this method a particular capacitance voltage behaviour of membranes is observed which seems to be closely related to the breakdown of the membrane.

Fig. 1 shows the measuring circuit used in this method. A constant capacitor C_c is placed in series with the bilayer capacitance C_{BLM} which depends upon the applied voltage. The voltage across the bilayer is displayed on the x-axis of the oscilloscope while the y-axis shows the voltage drop across C_c which, of course, depends upon the polarization of C_{BLM}. When C_c is much greater than C_{BLM}, the y-axis voltage is directly proportional to the value of C_{BLM}.

The oscilloscope traces in Fig. 2 show a distinct hysteresis-like effect which appears over the observation range of 1 Hz to 1 kHz. These traces

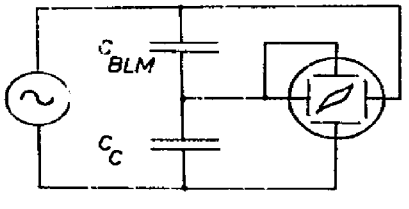

Fig. 1. The X and Y signals are amplified by a Tektronix dual channel operational amplifier type O with adjustable input resistance and phase. Function Generator, Hewlett-Packard 3311A; Oscilloscope, Tektronix 502; C_c, 1 μF.

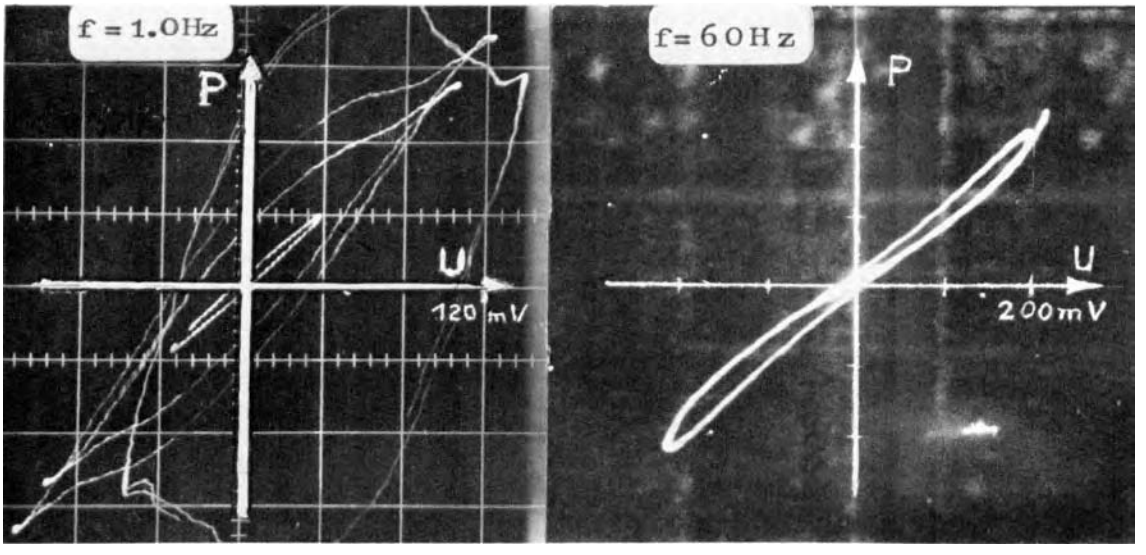

Fig. 2. Displays of the capacitance-voltage behavior of black lipid bilayers at 28°C. The bilayers were prepared from a 2% solution of lecithin in *n*-decane. Electrolyte, 0.1 m KCl, silver/silver chloride electrodes; diameter of the bilayer, 2 mm; bilayer capacitance ($U \approx 0$), 9.7 nF; bilayer resistance (U = 10 mV), $1.2 \cdot 10^9$ Ω.

were obtained from lecithin bilayers that were prepared from 1,2-diisostearyl-3-*sn*-phosphatidylcholine, a synthetic fully saturated lecithin [4], by a conventional method [5] where a lecithin/decane solution is spread over a hole in a teflon foil, which separates two compartments filled with electrolytes.

The electrolyte was 0.1 M KCl. The series resistance of the electrolyte-electrode produced no observable phase shift even at the highest frequency, 1 kHz. Consequently at low voltages the hysteresis loop is nearly closed because C_{BLM} is nearly constant and there is no resistance phase shift.

The area enclosed by one cycle of voltage is proportional to the electrical power loss in the bilayer and hence related to the conversion of electrical energy into thermal and mechanical energy. Consequently the particular capacitance voltage behaviour of these curves and especially the magnitude of the enclosed area, may serve as a criterion for the stability of the bilayer.

Actually, two different frequency-dependent breakdown mechanisms are observed. At low frequency (1 Hz) and voltages above 120 mV the ordinary elastic behaviour of the bilayer is lost, as shown at the left hand side of Fig. 2. The capacitance increases as the applied voltage decreases. For electric fields E satisfying the inequality*

$$E^2 > \frac{8\pi\sigma}{d\epsilon}$$

*Helfrich, W., personal communication.

the membrane bulges spontaneously, by increasing the bimolecular area. σ denotes the normal surface tension of the membrane, $\approx$ 1 dyne/cm*, d is the thickness of the bilayer and ϵ the dielectric constant. This "negative capacitance voltage" relation can only be observed within a small voltage range. At slightly increased voltages the membrane always ruptures at the beginning of the negative capacitance region.

At higher frequencies (60 Hz) as shown at the right hand of Fig. 2, a less pronouced hysteresis curve but no negative capacitance voltage dependence is observed. The breakdown voltage of approx. 250 mV is in good agreement with the model of a bilayer treated as an elastic dielectric, where breakdown occurs in the bimolecular area [6].

There are some reasons for the frequency-dependent breakdown and hysteresis effects. (1) The bilayers are in equilibrium with a large torus volume. The time constant for the field-induced material transport between bimolecular area and torus is 100 ms [7]. At frequencies which are low compared to the time constant of material transport, bulging* affects the material in the bilayer/torus interface yielding an electrical breakdown. Measurements with different ratios of aperture diameter and thickness of the teflon separator foil according to ref. 8 show, that the breakdown voltage decreases and the hysteresis area increases strongly with the reduction of this geometric parameter.

At frequencies which are high compared to the 100 ms time constant the material transport is inactive and the breakdown occurs somewhere in the bimolecular region. It seems as though the breakdown of the bilayer in the low frequency case is associated with the transport of material. Electro compression and bulging effects where the bilayer thickness decreases and the bilayer area increases mainly contribute to the voltage dependence of the capacitance.

(2) In addition, enclosures of solvent by the bilayer (microlenses) can be distorted by the electric field producing changes in capacitance [9], but this effect is probably too small to explain the capacitance changes.

(3) The field can produce changes in the microscopic structure of the bilayer. Lecithin molecules can be displayed from their equilibrium position thereby changing the ionic permeability of the membrane which increases the conductivity and electrical energy loss of the bilayer.

With the measurement of the hysteresis of the capacitance as a function of voltage and frequency a new parameter for the stability of bilayers in electric fields is introduced. The technique utilized here is an alternative to the voltage clamp technique [10] for monitoring rapid changes in capacitance. The method proposed in this paper allows the elimination of the influence of slow effects, e.g. thinning or bulging of the membrane due to different evaporation of the electrolytes in the compartments or other microscopic changes.

Additional measurements are necessary to elucidate the mechanism responsible for the changes in capacitance reported here. For example, a laser heterodyne interferometer [11] can be used to measure directly the thickness

*See footnote, opposite page.

of the bilayer. The bilayer under a periodical external field can modulate the phase of laser radiation, and this modulation can be detected as a beat signal from a photo detector with a quadratic response [11] and related to the membrane thickness (Braun, H.P., in preparation).

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