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A QUARTZ CELL FOR STUDYING PLANAR LIPID BILAYER MEMBRANES

JOAQUIM PROCOPIO [a], WAMBERTO A. VARANDA [a] and JOSÉ A. FORNES [a,*]

[a] *Departamento de Fisiologia e Farmacologia, Instituto de Ciencias Biomédicas, Universidade de São Paulo, 05508 São Paulo, S.P. and*
[b] *Instituto de Matemática e Física, Universidade Federal de Goiás, Campus Universitário, Bloco IMF-2, 74000 Goiania (Brasil)*

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A quartz chamber is proposed for use in experiments with planar lipid bilayer membranes. Membranes are formed in a hole made on the lateral wall of a fused quartz test tube, immersed in an electrolyte solution. The quartz cell is easy to clean, chemically inert and easily made. Membranes formed in this chamber had specific resistances higher than 10^8 $\Omega\cdot cm^2$ and excellent mechanical stability.

The usual set-up for studying lipid bilayer membranes consists of two aqueous compartments separated by a partition and communicating through a small hole in the partition, where the membrane is formed. Critical points in such a system are:
(1) Electrical insulation between the aqueous compartments. (2) Construction of the hole. (3) Cleaning the chamber and its final degree of cleanliness.

The partition is usually made of polyethylene, Teflon, or Saran wrap sheet (0.1–0.5 mm thick) and supported by a plastic body (Teflon, lucite, etc...) which includes the reservoirs for solutions. Electrical insulation between partition and chamber body is usually accomplished with vaseline, silicone grease or gaskets of thin silicone rubber [1]. These substances, particularly vaseline and silicone grease are potential sources of contaminants.

Another assembly for studying planar bilayers is the so called 'Teflon cup'. The difficulties with this system are constructing the hole and the general cleaning procedures for Teflon.

An alternative for the above cells is an all-glass chamber. However, glass cells are not indicated for most membrane studies because of the low resistivity of glass as compared to lipid bilayer membranes. Even the most resistive glasses (Corning 8870, for exemple) have resistivities well below that of Teflon which is about 10^{18} ohm $\cdot$ cm^2 [2]. Quartz on the other hand, has a resistivity equal or higher than Teflon [4]. The two main problems with quartz are: making the hole for supporting the membrane and the possible leak pathways at the interface between quartz and bulk lipid torus.

We report here a simple design of a quartz cell for use in lipid bilayer experiments. Its cleaning is easy and efficient: we have used as cleaning procedure a sequence of rinsing in water, ethanol, Folch, ethanol, water and finally flaming in a Bunsen burner (blue flame) for about 2 min. Total cleaning time is about 5 min. The last cleaning step probably removes all organic impurities on the surface. The hole for supporting the membrane is made on the lateral wall of a quartz tube (internal diameter about 1 cm; wall thickness 0.5–0.8 mm). The open end of the tube is closed with a stopper and then a small region (around 5×5 cm) of the lateral wall is heated with a propane/oxygen blowtorch having sufficient temperature to soften

* To whom correspondence should be addressed.

quartz. The increase in internal pressure and the simultaneous softening of the wall produce spontaneous formation of a bubble which upon further heating explodes, leaving a star shaped aperture. Fire polishing of this region with the same flame produces a round hole with a regular and thin border (0.16 mm thick) suitable to support membranes. A typical quartz cell is shown in Fig. 1.

Membranes were formed by the 'brush technique' [5] after prepainting the hole rim with the lipid mixture to be used in forming the membrane. Electrical parameters were measured using an operational amplifier in the current to voltage converter configuration (42K Analog Devices OP-AMP). Electrodes were Ag/AgCl throughout. Resistances were measured by applying a voltage step and recording the current through the membrane. Capacitances were measured as the steady-state

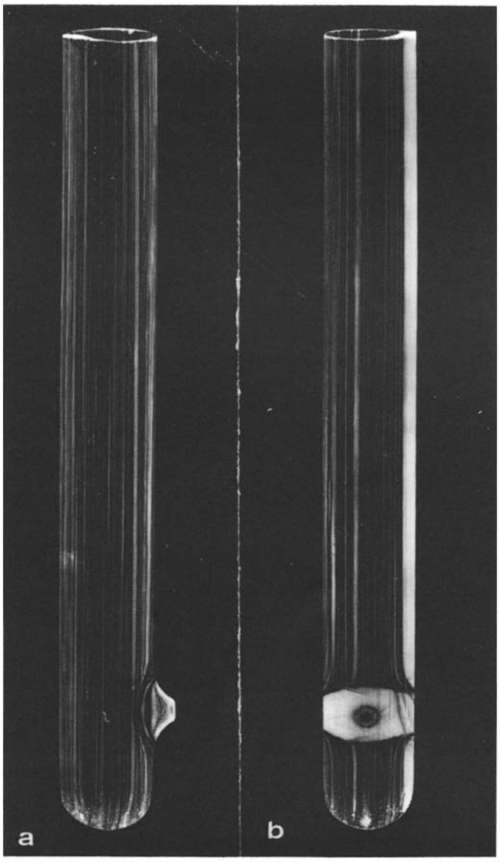

Fig. 1. Lateral (a) and front (b) views of the quartz cell showing the hole for supporting the membrane. Actual size.

TABLE I

ELECTRICAL PARAMETERS OF MEMBRANES FORMED IN THE QUARTZ CELL

Hole area: $1.62 \cdot 10^{-2}$ cm^2. Effective membrane area: $(1.41 \pm 0.11) \cdot 10^{-2}$ cm^2 ($n = 32$). Lipids used: Egg lecithin (PC) and diphytanoylphosphatidylcholine (DPPC) were obtained from Avanti Biochemicals Inc. and mixed soybean phospholipids (Asolectin) from Sigma Chemical Co. Asolectin was washed according to Kagawa and Racker [3]. Cholesterol (Chol) (99+%) and n-decane (99+%) were also from Sigma Chemical Co. Membranes were formed from an n-decane solution containing 2% of the indicated lipid. Numbers are mean ± S.D.; n is the number of experiments.

Lipid	Effective membrane resistivity ($\Omega \cdot cm^2$) ($\times 10^{-8}$)	Membrane capacitance ($\mu F \cdot cm^{-2}$) ($\times 10^7$)
PC/Chol (1:1, w/w)	3.35 ± 1.30 ($n = 14$)	5.59 ± 0.76 ($n = 8$)
DPPC	1.86 ± 0.65 (n-8)	3.57 ± 0.62 ($n = 6$)
Asolectin	2.23 ± 1.38 ($n = 6$)	26.2 ± 19.1 ($n = 6$)

current responses to a voltage triangular waveform (5 Hz, 10 mV p-p), and using the equation $C = I_c/(dV/dt)$, were I_c is the capacitive current, V is the applied potential and t is time. Table I shows results of specific resistance and capacitance determinations in membranes formed in 0.1 M NaCl solutions. All membranes studied showed a specific resistance higher than 10^8 ohm $\cdot$ cm^2 which is an upper limit for membranes formed in the Teflon cup assembly [2,6]. This suggests that electrical leaks at the lipid/quartz interface are not larger than in the lipid/Teflon interface. Membrane stability with the quartz cell was found to be equal or better than with conventional Teflon chambers.

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