A Cell for Simultaneous Measurements of Optical and Electrical Properties of Black Lipid Membranes

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Black Lipid Membranes, Simultaneous Measurements, Optical Properties, Electrical Properties, Recombination Experiments

A universal cell was constructed, which allows measurements of the resistance (d. c.), capacity, optical reflectivity, and spectral absorption of black lipid films simultaneously. These measurements reflect different properties of the films, supplementary to each other. The purpose is to eliminate differences of experimental conditions especially in recombination experiments with proteins. The cell is totally symmetric, can be completely disassembled, and the parts are exchangeable. It allows application of several methods for film production, and is held together simply by mechanical pressure, without need of any sealing agent. The inner parts are exclusively of glass and teflon. The fluid volume of the cell is 7–15 ml and the insulation between the two cell compartments is better than 10^{14} Ohms.

1. Introduction

Since about 15 years ago, black lipid membranes have been used to examine the interaction between the lipids and proteins of membranes. Measuring the thickness of these artificial membranes is an important means of characterising them. Measurements of d. c. and a. c. properties [1], as well as optical measurements (reflectivity [2] and spectral absorption [3]) were employed to determine this quantity. However, the results of these measurements are not equivalent, but rather supplementary to each other.

The d. c. properties, as well as the capacity essentially reflect the thickness of the apolar central layer consisting of the fatty acid chains of these virtually bimolecular lipid membranes. Although the resistance can be greatly influenced by pores of negligible area in the membrane [4], the presence of these pores does not necessarily cause a change of capacity.

Optical reflectivity measurements, on the other hand, give us information about the overall thickness of these black membranes including outer polar layers [2]. In experiments where proteins are attached to the membrane, the optical reflectivity only gives us the entire membrane thickness when its surfaces are densely packed with proteins [5]. With spectrophotometric measurements one can determine if this is actually the case [6].

Therefore, in recombination experiments with lipids and proteins, it is not only useful, but necessary to be able to make as many of these measurements as possible simultaneously on the same membrane.

2. Cell

The cell, which allows each of the above mentioned measurements to be made on the same film simultaneously, is built on the following principle (Fig. 1) *: The central part is a 3 mm thick partition of teflon. This partition has a central hole for the application of the lipid membrane. The diameter of the hole can be varied up to 10 mm, although a mean value of 5 mm has been found to be most convenient. The wall thickness of the partition immediately around the hole is only a few tenths of a millimeter.

One way of producing the lipid film is by application of the appropriate lipid solution with a small brush after filling the cell with aqueous solution. Another possibility is to apply the lipid solution on the surface of the salt solution in the cell, on both sides of the partition, and then raise the surface of the solution from below to above the hole so that a film is formed within.

In order to eliminate disturbances by scattered light, the teflon used for the partition is blackened

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* Figs 1–4 see Plate on page 40 a.

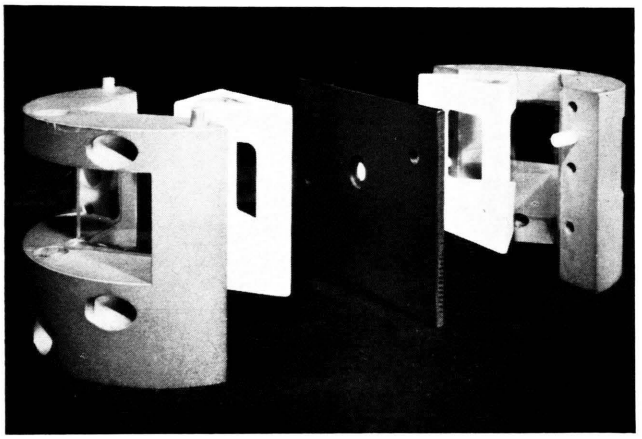

Fig. 1. Disassembled cell.

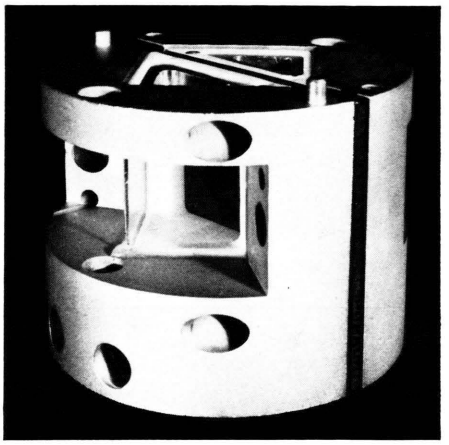

Fig. 3. Assembled cell.

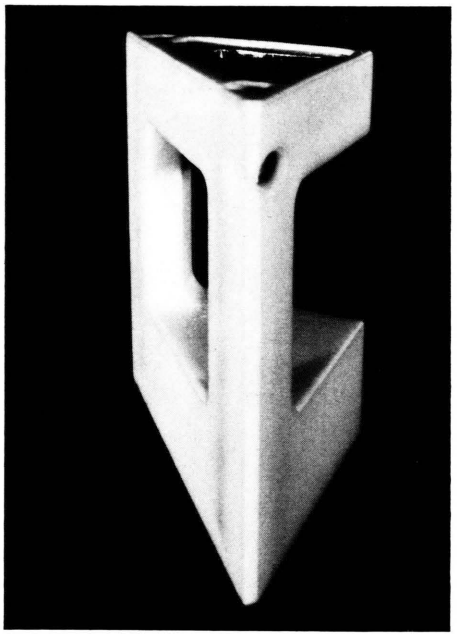

Fig. 2. Teflon compartment body.

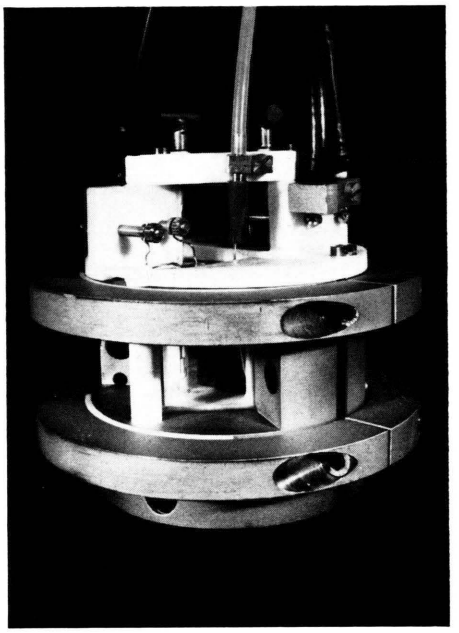

Fig. 4. Cell with electrodes and connections.

by a content of 5% graphite. The effective cell volume is determined by two prismatic compartments of teflon (Fig. 2), which are attached on both sides of the partition. In order that these parts retain their shape, they have been reinforced internally with stainless steel (visible in Fig. 2 at the top part of the component and through the partially translucent teflon). Furthermore, the teflon components were heated at 250 °C for several hours between the various stages of their manufacture.

The cell is electrically screened by two symmetrical half cylindrical metal blocks. In order to make the cell optically accessible from outside, there are rectangular openings in the blocks with glass windows.

All five parts of the cell are held together simply by pressure and pegs with corresponding holes, without need of a sealing agent. This requires however, that the joining teflon surfaces must be worked very precisely, with the highest possible smoothness. This is best accomplished with a tooth milling cutter at a high milling speed and low feed. After it is finished, the surface should not show any interference colours, which would indicate the presence of grooves. With a partition which has been counterbored, but not drilled through, an isolation resistance can be obtained of better than 2×10^{14} Ohms, which is certainly sufficient for d. c. measurements.

The completely assembled cell essentially represents a glass cuvette with a quadratic cross section and a diagonal partition wall which contains the hole for the membrane (Fig. 3). The only materials in contact with the salt solution in the cell are glass and teflon. The use of these practically inert materials, as well as the ability to be completely disassembled, allows the cell to be thoroughly cleaned. The exchangeability of the different components, furthermore, offers the possibility to use partitions with holes of varied diameters and types.

Through openings at the tops of the two prismatic compartments (Figs 2, 3) electrodes and magnetically driven stirring rods can be passed, as well as canulae for controlling the bulging of the membrane or for exchange of the liquid in the cell compartments (Fig. 4). For application of the lipid membrane by the brush technique, these holes may also be used.

In case the isolation resistance of the glass windows is not high enough, the two metal blocks must not be connected electrically and a further isolation of the cell against the outer metal screening is required, since otherwise parasitic voltages between the salt solution in the cell and the metal blocks may build up. Although these voltages have a very high internal resistance they cannot be neglected since the lipid membranes also have a comparable high resistance.

3. Electrodes

For d. c. and a. c. measurements different types of electrodes were used: Ag/AgCl electrodes for d. c.; and metallic platinum plates for a. c. The d. c. electrodes were made mainly according to the method of Brown [7]. They consist of wire electrodes with a length of about 40 mm and a diameter of 0.6 mm dipping into glass tubes with an inner diameter of about 5 mm, which are closed with a silicone stopper and protected against light by a teflon band wound around the outside (Fig. 4). The amount of electrode surface exposed to the KCl solution inside the test tube is limited by the application of lacquer. The connection between the KCl solution and the respective salt solution in the cell was not made, as usual, by Agar bridges, but instead by bridges filled with polyacrylamide gel to which KCl is added to in various molarities. The durability of these bridges is practically unlimited. Interelectrode voltage can be minimized to 0.05 mV by selecting appropriate pairs of electrodes.

Platinized platinum electrodes at low frequencies would show a much smaller a. c. impedance than bare platinum plates [8], but are very delicate to handle, not stable over longer periods of time, and hard to clean. Therefore bare platinum plate electrodes with a surface area of about 120 mm² were used.

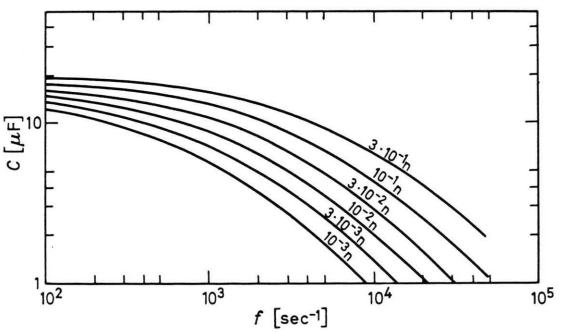

Fig. 5. Series capacity of a metallic platinum plate electrode with a surface area of about 120 mm² dependent on frequency and KCl concentration. Measurements were taken at 200 Hz intervals. Maximum error at higher frequencies of about ±10%.

The impedance values of such electrodes depend on the frequency between 50 Hz and 50 kHz, and the concentrations of the surrounding salt solution and are represented in Figs 5 and 6 (using series equivalent circuit following Schwan [8]). With a membrane

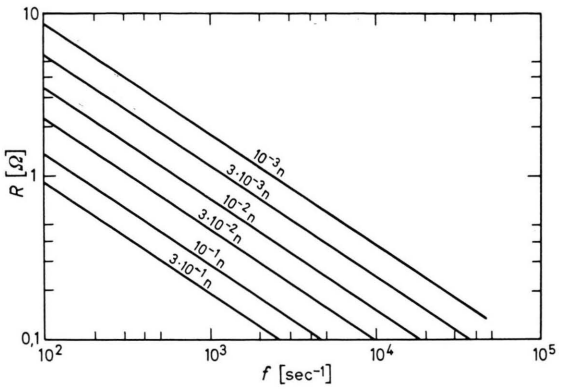

Fig. 6. Series resistance of the same platinum electrode.

diameter of 5 mm, for example, one would expect an influence of the electrode impedance on the measured film capacity only at high frequencies and at low salt concentrations.

Since the d. c. circuit has a much higher resistance than the a. c. circuit, both circuits must be separated from one another. This can be accomplished electronically, or simply by switching the two circuits using highly insulating Reed relays.

4. Optical Measurements

The optical pathway in the measurements of transmission and reflectivity is demonstrated in Fig. 7. The cross section of the laser beam is variable. An image of the lipid membrane is projected onto the cathode of the photomultiplier tube. Built-in diaphragms allow to reach an angle of resolution of up to 20′.

It is necessary to use an adjustable mounting in order to be able to compensate for raising and lowering of the image field and to be able to appropriately position the film and to choose the spot of incidence on the membrane. The direct observation of the reflected image on a focusing screen, which can be used to replace the photomultiplier cathode, facilitates these adjustments.

The mounting device can also be adjusted for temperature, so that measurements at various temperatures can be made.

Because of the perfect symmetry of the cell, it is possible to directly determine the reflectivity of the lipid film by comparison of the reflected and transmitted light intensities. Additional absorption by the salt solution caused by addition of protein in the recombination experiments can be easily eliminated by taking measurements before and after the addition. Most important, the symmetry of the cell permits measurements of reflectivity and spectral transmission to be carried out simultaneously.

The black area of the lipid membrane can be determined by means of a cathetometer, with a telescope, upon which a 35 mm camera is mounted. According to White [9], measurements can be made in this way with an error of only 1%.

In order to protect the system against disturbances from the environment, especially while performing optical measurements, the entire apparatus was freely suspended from 26 springs, just over one meter in length. With an entire mass of nearly 800 kg this system represents mechanically a low-pass filter with a cut-off frequency of only 0.5 Hz.

Reports on measurements with black membranes of lipids from mitochondria, and on the interaction of these membranes with oxidized and reduced cytochrome c will be presented in a subsequent paper.

I wish to thank Prof. Dr. A. Müller-Broich for kindly supporting this work.

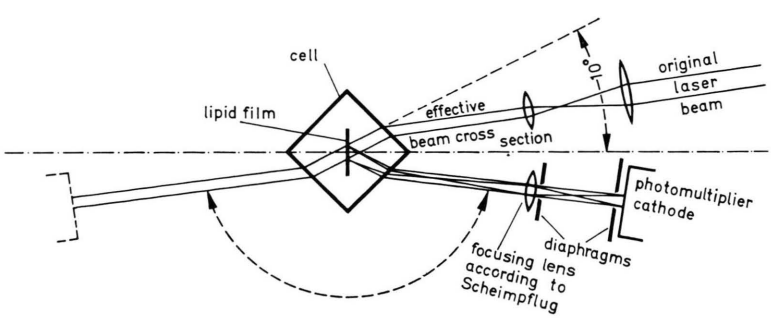

Fig. 7. Measurement of optical reflectivity and transmission of black lipid membranes.

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