

Electroporation of planar lipid bilayers

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Duration of the experiments: 120 min

Max. number of participants: 4

Location: Laboratory for skin and planar lipid bilayers

Level: Basic

PREREQUISITES

Participants should be familiar with Laboratory safety (S1). No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

A planar lipid bilayer can be considered as a small fraction of total cell membrane. As such has often been used to investigate basic aspects of electroporation; especially because of its geometric advantage allowing chemical and electrical access to both sides of the lipid bilayer. Usually a thin bi-molecular film composed of specific phospholipids and organic solvent is formed on a small aperture separating two aqueous compartments. Electrodes immersed in these two aqueous compartments allow to measure current and voltage across the lipid bilayer (Figure 1).

Two different measurement principles of planar lipid bilayer's properties can be used: voltage or current clamp method. Planar lipid bilayer from an electrical point of view can be considered as imperfect capacitor, therefore two electrical properties, capacitance and resistance, mostly determine its behaviour.

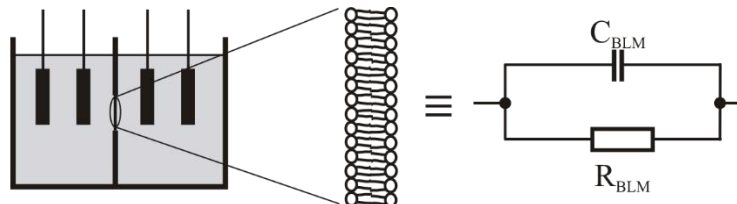


Figure 1: Equivalent circuit of a planar lipid bilayer.

The aim of this laboratory practice is to build a planar lipid bilayer by painting method (Muller - Rudin method) or/and foldig method (Montal – Mueller) and to determine capacitance and resistance of the planar lipid bilayer using LCR meter. Basic aspects of planar lipid bilayer electroporation will be given by observing formation of the pores and determining its breakdown voltage.

EXPERIMENT

Protocol:

Muller-Rudin method

Form a planar lipid bilayer by covering the surface of the aperture in a barrier separating two compartments of a measuring vessel with a lecithin solution (20 mg/ml of hexane). After evaporation of hexane, fill compartments with solution consisting of 0.1 mol KCl, 0.01 mol of HEPES, at pH=7.4. Connect the electrodes and apply a drop of lecithin dissolved in decane (20 mg/ml) to the aperture by

the micropipette or paint it by a teflon brush. Measure capacitance and determine if the formation planar lipid bilayer is appropriate.

Montal – Mueller method

Cover the surface of the aperture in a barrier separating two compartments of a measuring vessel with 1 μl lecithin solution (10 mg/ml of hexane and ethanol absolute in ratio 9:1). After evaporation of hexane and ethanol, add on the aperture 1,5 μl solution of pentan and hexadecane in ratio 7:3. Fill compartments with solution consisting of 0.1 mol NaCl, 0.01 mol of HEPES, at pH=7.4. On the solution surface apply 2 μl of lecithin solution in each compartment. Wait approximately 15 minutes that lipid molecules are equally spread on the solution surface. Then rise the solution surface above aperture synchronously in both compartments by pumps. Measure capacitance and determine if the formation planar lipid bilayer is appropriate.

Measuring methods: When the planar lipid layer is formed, we apply the current or voltage to the planar lipid bilayer. In the current clamp method the current is applied to the planar lipid bilayer and we measure voltage across the bilayer. Apply a linearly increasing current and record a voltage across the bilayer. During the experiment you will obtain the time course of the voltage across the bilayer and the plot of the programmed current flowing between two current electrodes. In the voltage clamp method the voltage across the planar lipid bilayer is applied and current, which flows through planar lipid bilayer, is measured. To the planar lipid bilayer apply a linearly increasing voltage and record a flowing current. Like at the current clamp method you will obtain the time course of the flowing current and the plot of the programmed voltage across the planar lipid bilayer.

From collected data determine the breakdown voltage (U_{br}) and the lifetime (t_{br}) of planar lipid bilayer.

FURTHER READING:

Kalinowski S., Figaszewski Z., A new system for bilayer lipid membrane capacitance measurements: method, apparatus and applications, *Biochim. Biophys. Acta* 1112:57-66, 1992.

Pavlin M, Kotnik T, Miklavcic D, Kramar P, Macek-Lebar A. Electroporation of planar lipid bilayers and membranes. In Leitmanova Liu A (ed.), *Advances in Planar Lipid Bilayers and Liposomes, Volume 6*, Elsevier, Amsterdam, pp. 165-226, 2008.

Koronkiewicz S., Kalinowski S., Bryl K., Programmable chronopotentiometry as a tool for the study of electroporation and resealing of pores in bilayer lipid membranes. *Biochim. Biophys. Acta*, 1561:222–229, 2002.

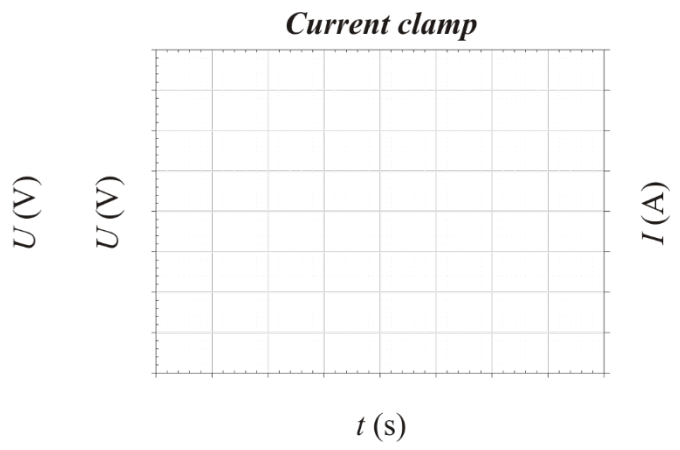
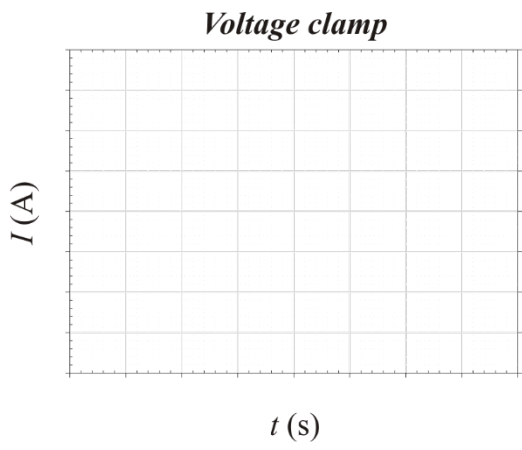
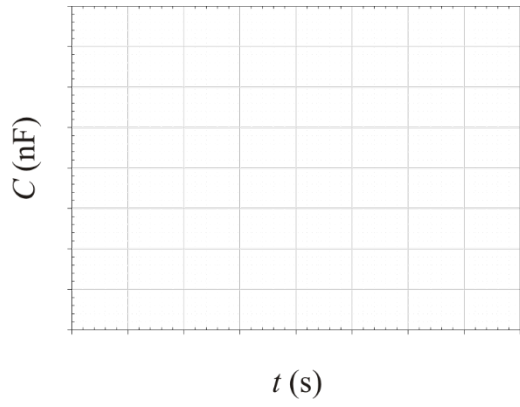
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Montal M., Mueller, P., Formation Of Bimolecular Membranes From Lipid Monolayers And A Study of their Electrical Properties, *PNAS*, 69:3561-3566, 1972.

Kramar P, Miklavčič D, Maček-Lebar A. Determination of the lipid bilayer breakdown voltage by means of a linear rising signal. *Bioelectrochemistry* 70: 23-27, 2007.

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