CHAPTER TWO

Voltage- and Current-Clamp Methods for Determination of Planar Lipid Bilayer Properties

Peter Kramar,[1] Damijan Miklavčič,[1] Malgorzata Kotulska,[2] *and* Alenka Maček Lebar[1,*]

Contents

Abstract

Biological membranes, the barriers that envelope the cell and its inner organelles, play a crucial role in the normal functioning of cells. The simplest model of these biological membranes is the planar lipid bilayer. Because its geometry allows chemical and electrical access to both sides of the bilayer, the physical properties of this model membrane can be easily measured. Usually, a thin bimolecular film composed of specified phospholipids and organic solvent is formed on a small aperture in a hydrophobic partition separating two compartments containing aqueous solutions. From the electrical point of view, a planar lipid bilayer can be considered as an imperfect capacitor; therefore, two electrical properties,

* Corresponding author: Tel.: +386 1 4768 770; Fax: +386 1 4264 658
E-mail address: alenka.macek.lebar@fe.uni-lj.si

[1] Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia
[2] Institute of Biomedical Engineering and Instrumentation, Wroclaw University of Technology, Wroclaw, Poland

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capacitance (*C*) and resistance (*R*), determine most of its behavior. Electrodes placed in the aqueous compartments on each side of the planar lipid bilayer permit the measurement of current and voltage across the model membrane. The two measuring techniques most commonly used to measure the properties of planar lipid bilayers are voltage-clamp methods and current-clamp methods.

The focus of this chapter is to review measurement systems and methods for the determination of the physical properties of planar lipid bilayers.

1. Introduction

Biological membranes, the barriers that envelope the cell and its inner organelles, play an important role in normal functioning of the cells. The membranes maintain crucial concentration gradients by acting as a selective filter for water-soluble ions and molecules [1]. Although biological membranes are composed of lipids, proteins, and small amounts of carbohydrates, the barrier function is assured by the thin layer of amphipathic phospholipids, which in polar liquid environments spontaneously arranges in various forms of lipid bilayers. A basic understanding of the properties and functioning of biological membranes can be obtained by investigating model systems, such as artificial liposomes or vesicles, which mimic the geometry and size of cell membranes, but are void of ion channels and the multitude of other embedded components commonly present in cells. The artificial planar bilayer lipid membrane (BLM) is the simplest model of a lipid system. It is usually formed across a small hole in a hydrophobic partition that separates two compartments filled with aqueous solutions. The advantage of the BLM is that both sides of the membrane can be easily altered and probed by electrodes.

Two methods of BLM formation are in common use. In one technique, the BLM is created by spreading a solution of lipids dissolved in an organic solvent. This method was introduced by Mueller and colleagues [2] and is named the painting technique. Both compartments of the chamber are filled with salt solutions and a dispersion of lipids is drawn across the hole in the partition separating them using a small paintbrush or a plastic rod. The cluster of lipids thins out in the center of the hole spontaneously forming a bilayer (Fig. 1). In the other procedure, the bilayer is formed from the apposition of two lipid monolayers [3]. A lipid solution in a volatile solvent is spread on the water–air interface of each compartment. Evaporation of the solvent creates a monolayer on the surface of the aqueous solution. When the monolayer formation is completed the water level in both compartments is raised above the hole and the bilayer is formed (Fig. 2).

A number of techniques have been developed to allow investigations of the functions and physical properties of these thin and fragile structures. Electrical measurements are a straightforward way to characterize the barrier

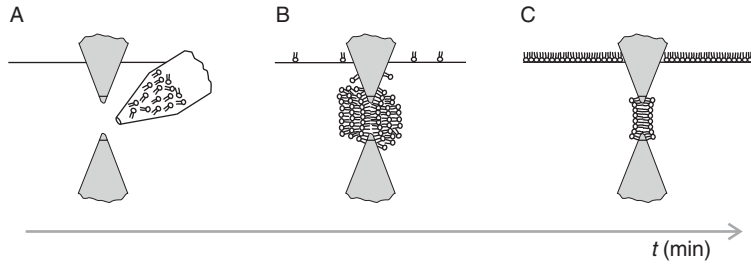

Figure 1 Planar lipid bilayer formation by the painted technique [2]. (A) Lipid molecules are painted on the aperture by pipette or brush. (B) The cluster of lipid molecules on the aperture. Lipid molecules are slowly spreading across the aperture. Nonused lipid molecules flow to the water solution surface. (C) Planar lipid bilayer is formed on the hole by thinning process.

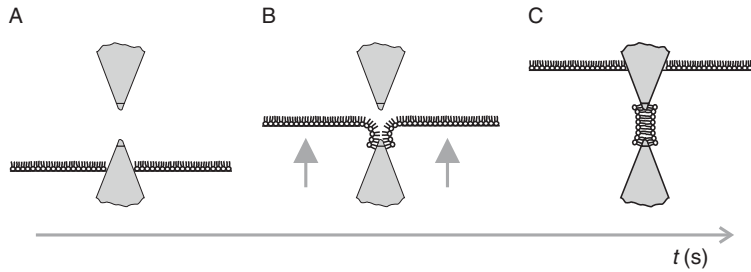

Figure 2 The folding method [3]. (A) Layer of lipid molecules on the salt solution. (B) The levels of the salt solution are slowly raised above the hole. (C) Planar lipid bilayer is formed on the hole.

function of a bilayer—its ability to prevent the flow of ions. From an electrical point of view, a planar lipid bilayer can be easily imagined as an imperfect capacitor, which means that the capacitance (C) has a finite parallel resistance (R). The typical resistance is very high since the hydrophobic core is impermeable to any charged species, and it is called a gigaseal. But the resistance drops dramatically even if a few nanometer-sized holes are present in a lipid bilayer. Formation of pores can be induced by a strong electric field externally applied to the BLM, and electrical measurements permit determination of BLM breakdown voltage (U_{br}). The electrical properties of the BLM are dependent on the physical properties of the lipids that compose the bilayer. Elasticity modulus and surface tension, for example, can be calculated from the electrical characteristics of the BLM.

Two electrical measurement methods are common (Fig. 3): the voltage-clamp method and the current-clamp method. When the voltage-clamp method is used, a voltage signal is applied to the planar lipid bilayer: a step change [4], pulse [5–7], linear rising [8], or some other shape of the

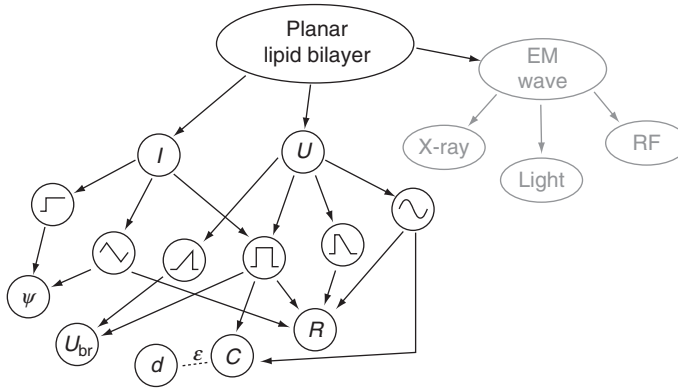

Figure 3 Measuring concepts and electrical properties of planar lipid bilayers. Resistance (R), capacitance (C), thickness (d), voltage breakdown (U_c), and mass fluctuation (Ψ) are measured with application of current (I) or voltage (U) signals of various shapes.

voltage signal. When the current-clamp method is used, a current is applied to the lipid bilayer [9]. Although these two methods are interchangeable when applied to objects of constant impedance, the situation changes when the electrical properties of a measurement subject are transient and related to the electrical signal. Separation of these two methods is useful, for example, in BLMs when electrically induced pores are studied. The shape of these pores is very unstable and their conductance is constantly changing throughout experiments.

A combination of electrical recording techniques with different kinds of high-frequency electromagnetic fields offers additional possibilities of investigating the structure–function relationships of planar lipid bilayer and of membrane interacting peptides [10–13].

In the following, we review electrical measuring principles and methods that have been applied for determination of planar lipid bilayer properties. According to the fact that each planar lipid bilayer property can be measured in many ways (Fig. 3) and therefore by different measuring systems, the main goal of the chapter is only to describe the existing measuring principles and to point out their experimental abilities. The choice of the most appropriate measuring system should be determined by combination of planar lipid bilayer properties that has to be followed.

2. Measurement Systems

The first stable planar lipid bilayer membranes were reported by Mueller and coworkers in 1962 [14]. Since then, a variety of measuring systems have been designed for studying planar lipid bilayer properties.

Measuring principles have been improved during the years, as well as the lipid chambers and measurement instrument accuracies. This review of measuring systems is divided into two parts according to the nature of the stimulus—voltage or current. Some basic characteristics of the systems, such as type of stimulating signal, number and material of electrodes volume of the chamber, etc., are given in Appendix A.

2.1. Voltage Clamp

2.1.1. System Hanai–Haydon–Taylor

The system was published in 1964 [15]. Within this system, DC and AC signals were applied to a planar lipid bilayer (Fig. 4). The DC signal was supplied by an accumulator. The voltage was controlled by a potentiometer. The current and resistance measurements were taken by electrometer. In the AC measurements, the capacitance and conductance were measured by two bridges, the Universal Bridge and Radio Frequency Bridge, which were designed on the basis of the transformer ratio-arm principle. The accuracy of the measurements was generally better than 1%. Two signal generators covering the ranges 50 Hz–100 kHz and 100 kHz–5 MHz were used, calibrated against standard frequencies. The bridge balance was detected with an oscilloscope with a preamplifier and a communications receiver. Two Ag–AgCl electrodes for DC measurements and two black platinum electrodes for AC measurements were immersed in salt solution.

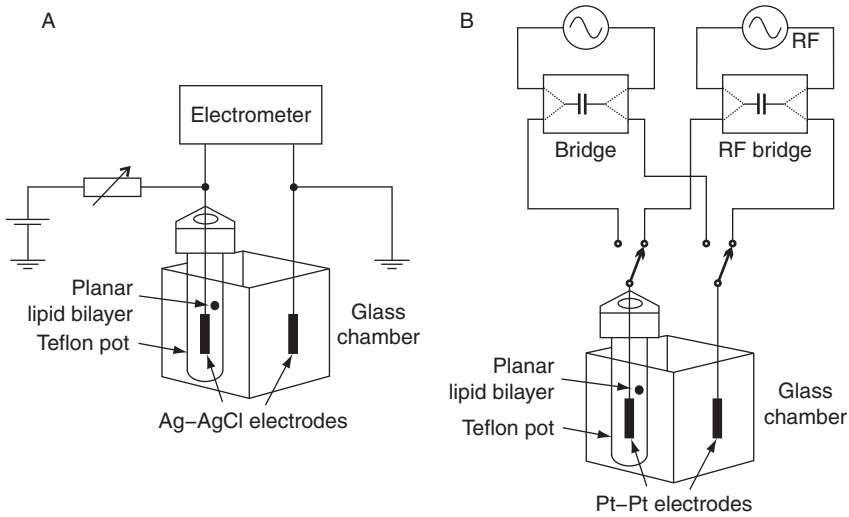

Figure 4 System Hanai–Haydon–Taylor. The figure was drawn according to description in Ref. [15].

The chamber had outer and inner parts. The outer part was made of glass cell from tubing of 4 cm × 4 cm bore. The inner part was made out of Teflon rod. The aperture for planar lipid bilayer was about 0.141 cm in diameter made by punching. The thickness of Teflon around the aperture was about 0.05 cm. The whole chamber was enclosed in a double-walled box for temperature stabilization by a water shell. The temperature during experiments was controlled with an accuracy of ±0.5 °C. Planar lipid bilayer was formed by the painted technique with a small brush.

The system was applied to quantitative assessment of the BLM molecular composition. Membrane thickness was obtained from the capacitance measurements that were obtained by a capacitance to voltage conversion method.

2.1.2. System Rosen–Sutton

The system was first published in 1968 [16]. Its main part was an AC signal generator with amplitude of 5 mV and frequency range from 100 Hz to 2 MHz (Fig. 5). Electrodes were connected to the transformer ratio-arm bridge. The bridge was initially balanced at a given planar lipid bilayer capacitance, which allowed DC potential to be applied between the electrodes during the AC measurements. The amplitudes of the DC potentials were up to 200 mV. An oscilloscope monitored Lassajous figures to permit observation of the conductance and capacitance contribution. The AC signal was applied through the bridge to planar lipid bilayer and to X channel of the oscilloscope. The planar lipid bilayer response was traced on Y channel. Four electrodes were immersed in salt solution. Two Calomel electrodes were used for measurement of transmembrane voltage by electrometer while voltage signal was delivered by two platinum electrodes. The planar lipid bilayer was painted across a round hole of about 1 mm in diameter.

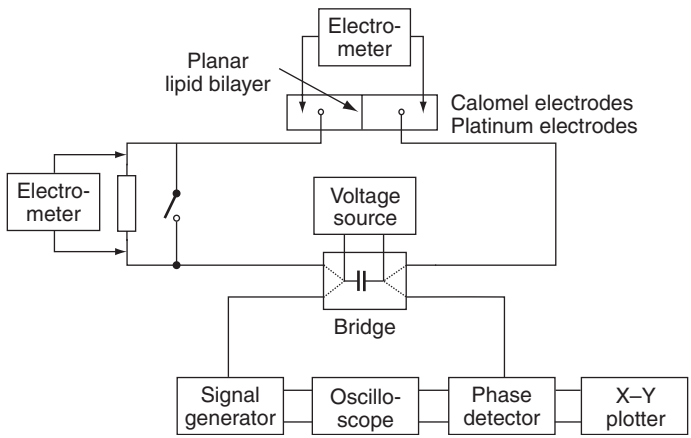

Figure 5 System Rosen–Sutton [16].

The system was applied to study an influence of the temperature and concentration of the salt solution on the electrical parameters of planar lipid bilayer [16].

2.1.3. System White

The system was first published in 1970 [17]. The basic idea follows the construction of the Rosen–Sutton system. The planar lipid bilayer capacitance was obtained from impedance measurements with an AC Winston bridge (Fig. 6). The chamber was made of Plexiglas with Teflon partition. Four platinized Ag–AgCl electrodes were used. A planar lipid bilayer was painted across round aperture of about 1 mm in diameter [17,18].

The authors studied the influence of the BLM thickness changes on BLM capacitance. They also investigated the planar lipid bilayer capacitance in dependence of the transmembrane voltage. The capacitance measurements were done by a capacitance to voltage conversion method.

2.1.4. System Wobschall

The system was published in 1971 [19,20]. The basic idea follows the construction of the Rosen–Sutton system but it was upgraded for planar lipid bilayer elasticity measurements (Fig. 7). The volume of one compartment of the chamber could be regulated by a flexible diaphragm with an acoustic oscillator. Pressure changes were measured with a pressure-sensitive transistor. The AC voltage signal was supplied by a function generator and a DC source. Both were regulated by a feedback circuit. The bridge excitation voltage with a frequency of 4 kHz varied from 5 to 15 mV. Four Ag–AgCl electrodes were immersed in a salt solution. The planar lipid bilayer was formed by the painted technique.

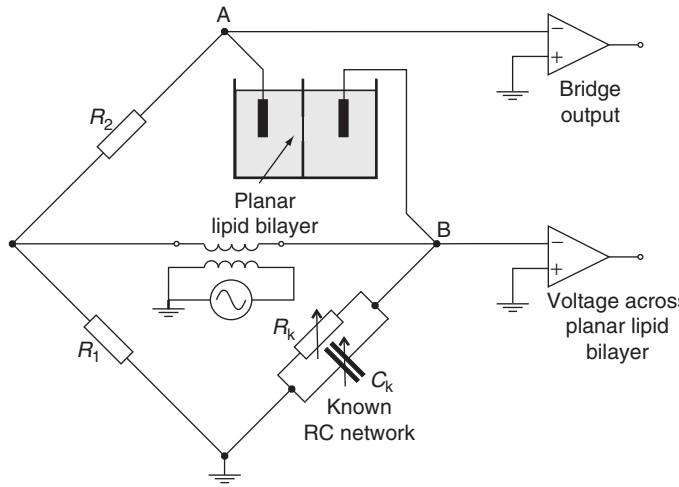

Figure 6 System White [17].

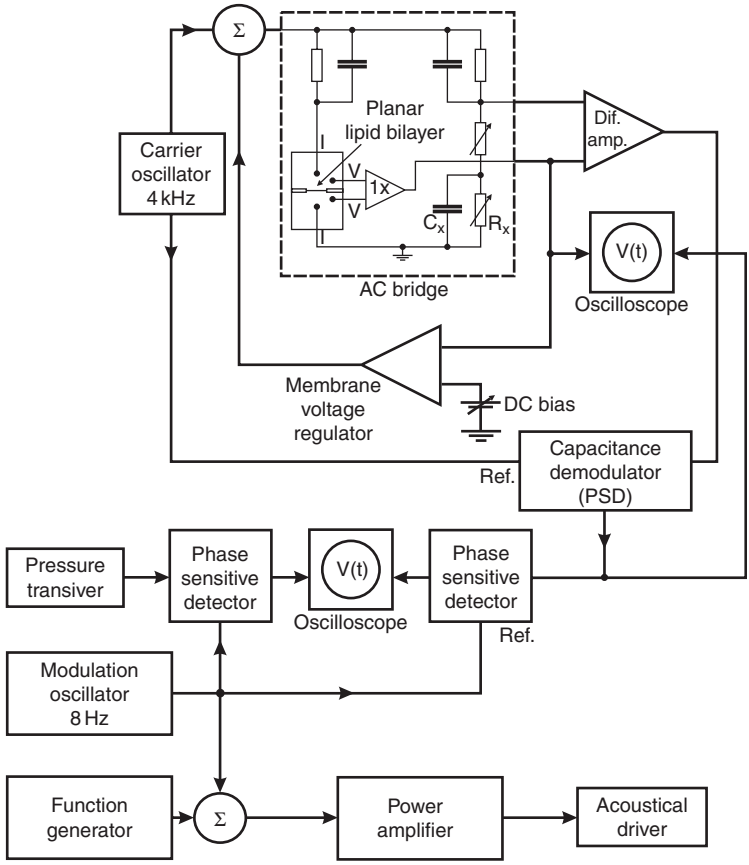

Figure 7 System Wobshall [19,20].

The capacitance of planar lipid bilayer in dependence of voltage and frequency was measured by a capacitance to voltage conversion method.

The construction of the chamber had a possibility of concaving the planar lipid bilayer in the shape of lens, which extended the area of BLM. This idea was employed to study the BLM capacitance in relation to its surface [20]. The elasticity of planar lipid bilayer at its breakdown was determined [19].

In the latter versions of this experimental system, the bridge was replaced by an impedance meter [21].

2.1.5. System Montal–Mueller

Montal and Mueller published the design of their measuring system in 1972 [3]. It was one of the first measuring systems that combined concepts of both measuring principles: voltage clamp and current clamp. The measuring

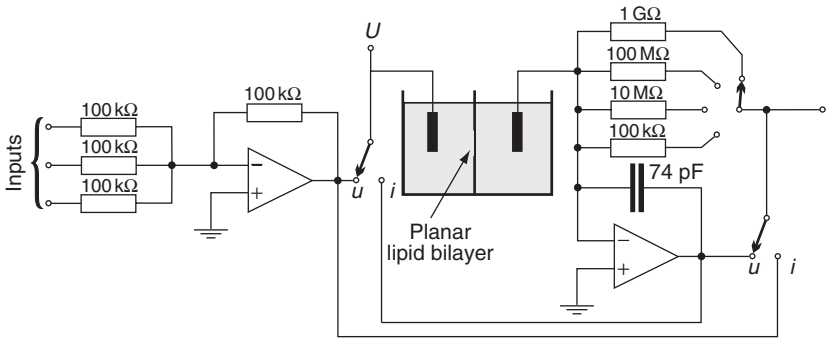

Figure 8 System Montal–Mueller [3].

principle was selected by two switches (Fig. 8). In the scheme, two amplifier circuits are present; the circuit on the left is voltage amplifier while the circuit on the right is current to voltage converter in the voltage-clamp mode (the switches are in position u) and voltage to current converter in the current-clamp mode (the switches are in position i). In voltage-clamp mode, the applied voltage is measured at connecting point U and the voltage that corresponds to current flowing through the planar lipid bilayer is measured at connecting point I. In the current-clamp mode, the applied current is recorded at connecting point I and the transmembrane voltage is measured at connecting point U. For planar lipid bilayer stimulation and corresponding measurements, two Calomel electrodes immersed in salt solution were used. The salt solution filled the Teflon chamber, which was divided into two compartments by 25 μm thick Teflon foil with an aperture of about 0.25 mm in diameter. The dimension of each compartment was (18 × 12) mm^2. The planar lipid bilayer was formed by the folding method [3].

The system was used for measurement of BLM capacitance and resistance. The capacitance was measured by charging method.

2.1.6. System Benz

The system was published in 1976 [6,7]. It is one of the simplest systems for observing planar lipid bilayers (Fig. 9). It consists of DC signal generator with the amplitude range from 10 mV to 5.4 V, switch, and battery supplied charge generator. The function of the switch was accomplished with FET transistor 2N5653. The output signal was a square pulse with duration from 500 μs to 500 ms. Oscilloscope Tektronix 7633 was used to measure the voltage response on planar lipid bilayer. The signal was filtered to the band of 80 Hz to 40 MHz. Two Ag–AgCl electrodes were immersed in a salt solution. The volume of each compartment of the Teflon chamber was of about 3 cm^3. In most cases, the area of the aperture was of about 2 mm^2. The planar lipid bilayer was formed by painted technique.

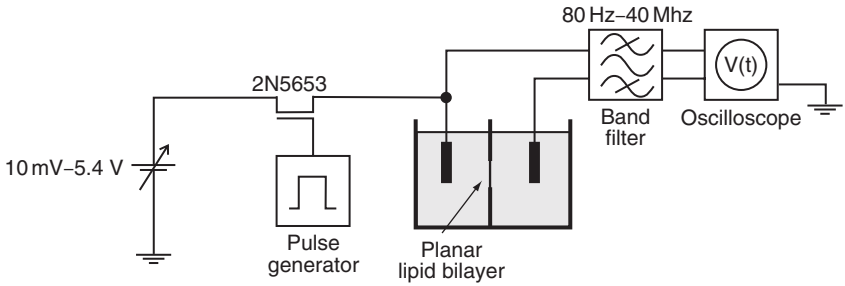

Figure 9 System Benz. The figure was drawn according to description in Ref. [6].

Benz *et al.* studied the capacitance of planar lipid bilayers using a discharge method. Most of the phospholipids used in their studies were synthesized in their own laboratory. In most cases, they used 0.1 M NaCl as the salt solution. They estimated thickness of the planar lipid bilayers and observed the thinning process of the planar lipid bilayers by capacitance measurement. The same system was used to investigate voltage breakdown as a function of salt concentration and pH [7].

The system was applied by the group of Chernomordik, whose experimental and theoretical studies made a great impact on understanding of the planar lipid bilayer breakdown process and related phenomena [4,22,23]. They observed fluctuations of the current with amplitude of about 10^{-11} A as a consequence of applying the voltage (100 mV–1 V) to the BLM. The membrane lifetime at a given voltage was also tested, defined as the time corresponding to the onset of an irreversible growth of current.

2.1.7. System Alvarez–Latorre

The system was published in 1978 [24]. The construction of the system was similar to the measuring systems based on the Winston Bridge [16,17,19]. Since the authors were interested in measuring changes in membrane capacitance rather than the absolute capacitance, they used differential amplifier to subtract the charging current of the membrane from the charging current of the equivalent *RC* network (Fig. 10). The resistance and capacitance of the equivalent *RC* network was set by the planar lipid bilayer capacitance measurement. The method based on 5 kHz, 10 mV peak-to-peak voltage waveform, which was only applied on a planar lipid bilayer. Two Ag–AgCl electrodes, one on each side of planar lipid bilayer, were immersed in the salt solution. The Teflon chamber consisted of two parts; each part had an area of 4 cm^2. A thin Teflon sheet of 19 μm was inserted between the reservoirs. The planar lipid bilayer was formed by the folding method. The output of the differential amplifier was further amplified and recorded with a sampling frequency of 2 MHz.

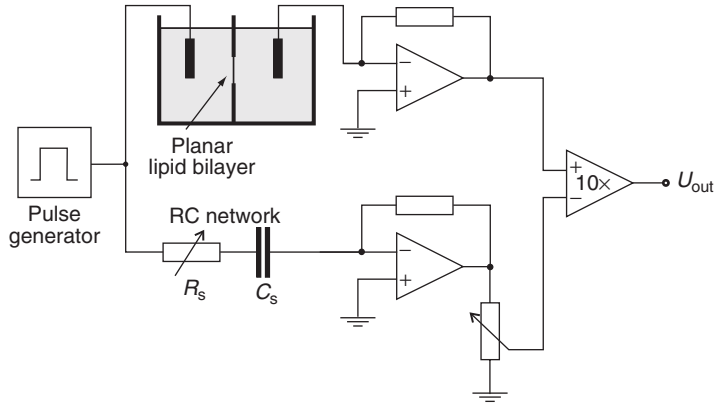

Figure 10 System Alvarez–Latorre [24].

They observed the capacitance of a planar lipid bilayer as a function of transmembrane potential and derived the thickness of planar lipid bilayers.

2.1.8. System Chanturya

The system proposed by Chanturya [25] was based on the system Benz [6]. The only difference in their designs is in a compensating capacitor, placed between the input of the operational amplifier and the inverter of the transmembrane potential (Fig. 11). The capacitor compensated almost all the reactive component of the current. Therefore, the author was able to use high-speed potential changes and obtain high resolution of measurements.

The system was applied for a study on the capacitance and resistance changes due to insertion of channel-forming proteins into the planar lipid bilayer [25].

2.1.9. System Wilhelm–Winterhalter–Zimmermann–Benz

The system was published in 1993 [26]. It was used in many other subsequent studies because it was simple and well defined [26–30]. The planar lipid bilayer was charged by a short voltage pulse with a commercial pulse generator. Instead of the switch, a diode with a reverse resistance $\gg 10^{12}\ \Omega$ was used to discharge the planar lipid bilayer only through the oscilloscope (Fig. 12). The pulse generator produced square pulses with durations from 0.2 to 10.0 μs. Two Ag–AgCl electrodes were immersed in salt solution that filled the Teflon chamber. Authors used different sizes of the apertures in the wall between two compartments—their areas were between 0.3 and 3 mm^2. The planar lipid bilayers were formed by the painted technique. The actual voltage on planar lipid bilayer was amplified with an operational amplifier and recorded with an oscilloscope. The data was processed by a connected computer.

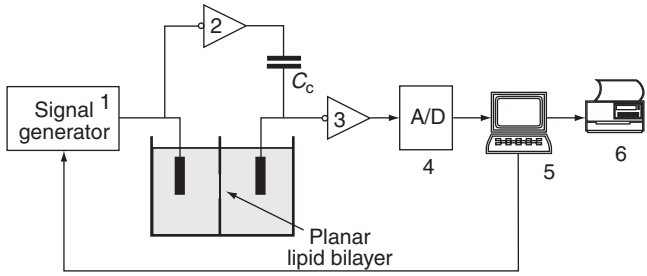

Figure 11 Sistem Chanturya [25]. 1. Signal Generator, 2. and 3. invertors, 4. analog to digital converter, 5. Personal Computer, 6. Printer.

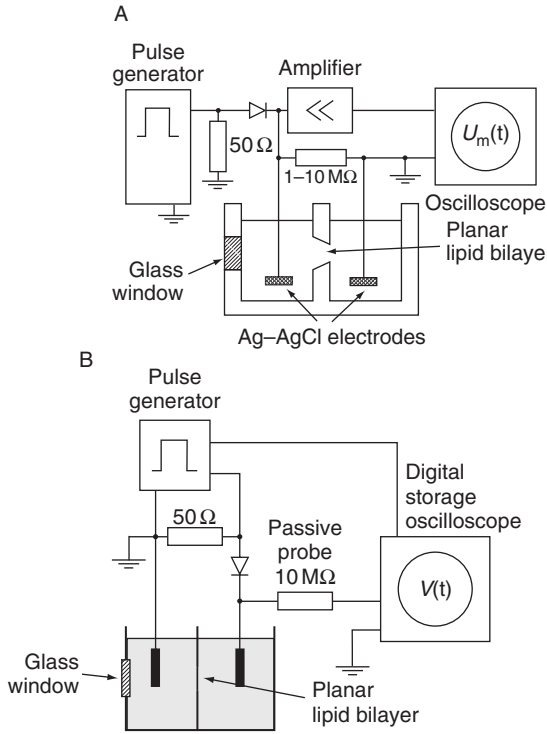

Figure 12 (A) System Wilhelm–Winterhalter–Zimmermann–Benz [26]. (B) Updated version of the system with an Digital Storage Oscilloscope described in Refs. [27–30].

Because many studies were based on this measuring system, a palette of lipids was tested [27–30]. Salt solutions differed from study to study as well as the volume of the lipids. The influence of the planar lipid bilayer composition on the breakdown voltage, capacitance, and rupture kinetics [27–29] was investigated [26,30].

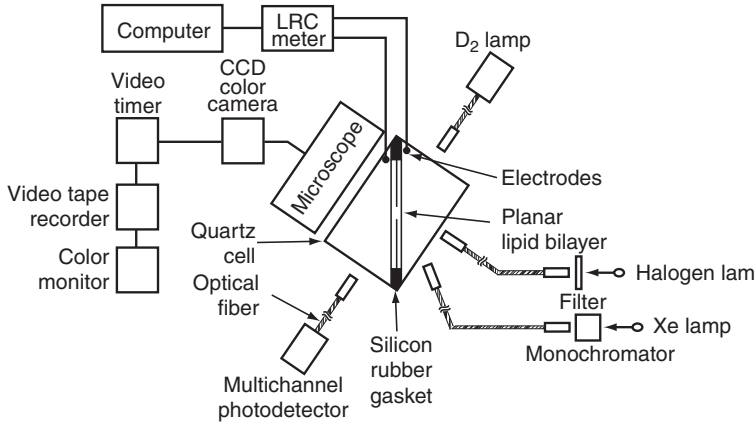

Figure 13 Sistem Yamaguchi–Nakanishi [12].

2.1.10. System Yamaguchi–Nakanishi

The system was published in 1993 [12]. It used combination of electric and optic measurements of planar lipid bilayer properties (Fig. 13). The authors simultaneously measured electrical characteristics and molecular structures of planar lipid bilayer as well as morphological changes. Planar lipid bilayer was exposed to sinus signal 7 mV_{RMS}/1 KHz. The response was measured with LCR meter. The measured data was acquired to the computer. During the electric measurement, the halogen or xenon light was used. Light reflection of planar lipid bilayer was observed and recorded by a color video camera. Two platinum/platinum electrodes were immersed in a salt solution. Chamber consisted of two quartz cells separated by Teflon 0.05 mm thick film, where an aperture of 0.7 mm in diameter was formed. The planar lipid bilayer was formed by the painted technique [12].

The authors measured changes in capacitance and resistance of planar lipid bilayer upon the irradiation by light.

2.1.11. System Sharma–Stebe–Tung

The system was an upgrade of the Benz system and it was described in 1996 [5,31]. FET switch was replaced by fast two pole analog switch. One of the switch poles was connected to the signal generator output and the other pole was connected on resistor of 1 MΩ. Voltage source consisted of an arbitrary waveform synthesizer board interfaced to a computer (Fig. 14). A square voltage pulse, which decayed linearly to zero to constitute a negative sloped ramp, and square voltage pulses from 10 μs to 10 s were generated. Four Ag–AgCl electrodes were inserted in the Teflon chamber via agar bridges. Two electrodes served to measure voltage across the bilayer by differential amplifier and the other two to apply voltage across the planar

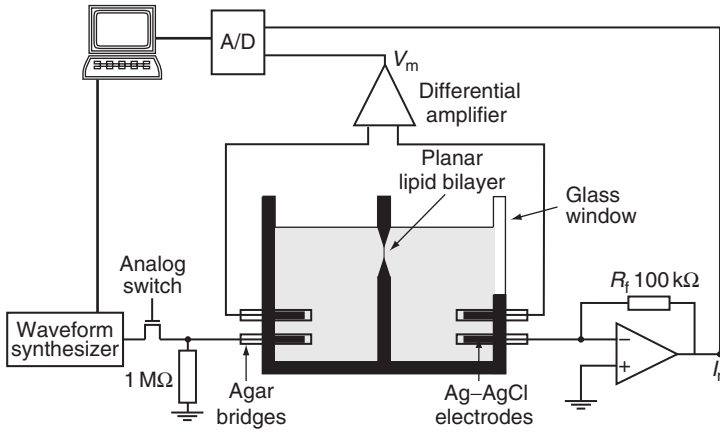

Figure 14 Sistem Sharma–Stebe–Tung [5,31].

lipid bilayer and measure the transmembrane current. The volume of each compartment of the Teflon chamber was about 3 cm^3. The diameter of the aperture in the Teflon foil was 105 μm. The planar lipid bilayers were formed by the folding technique. Current and voltage signals were recorded by a digital oscilloscope.

The system was used for studying the effects of nonionic surfactants (poloxamer 188, $C_{12}E_8$) on capacitance, conductance, and voltage breakdown of planar lipid bilayers [5,31]. Breakdown voltages of planar lipid bilayers were determined by applying a rectangular voltage pulse.

2.1.12. System Gallucci–Micelli

The system was published in 1996 [32]. The dynamic capacitance and resistance of a planar lipid bilayer could be measured simultaneously as is described in Section 3.2. Voltage signal of 1 kHz and adjustable amplitude, which was applied on planar lipid bilayer, was modulated by the signal of amplitude 2 mV and frequency of 1 kHz (Fig. 15). The electrodes were made of platinum. Experiments were performed in a Teflon chamber. The volume of each compartment was 4 ml. The aperture between the two compartments had a diameter of 1.3 mm. Current through the planar lipid bilayer was measured and amplified. The output signal from the amplifier was divided into two parts. In the first part, active two-stage 1 kHz band filter was used. The signal was then amplified, level adjusted, and rectified. Rectified voltage corresponds to the capacitance of planar lipid bilayer. In the second part, two-stage 1 kHz low pass filter was used. The signal was then amplified, level adjusted, and filtered to measure the resistance of the planar lipid bilayer [32–34].

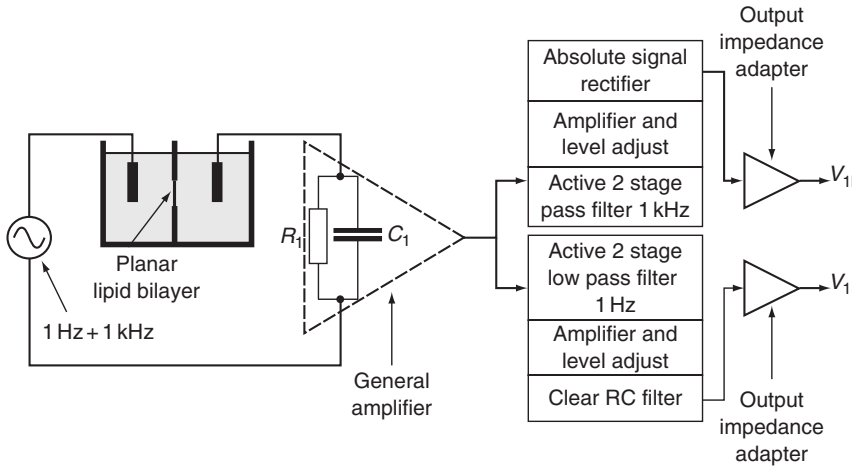

Figure 15 Sistem Gallucci–Micelli [32–34].

The general aim of the authors was investigation of channel insertion into planar lipid bilayer and corresponding electrical properties. The dynamic capacitance and resistance of planar lipid bilayers were measured simultaneously.

2.1.13. System Hanyu–Yamada–Matsumoto

Hanyu and coworkers [11] developed an experimental system that could measure ionic current and fluorescence emission of an artificial planar lipid bilayer, while controlling the membrane potential. Their experimental work was mostly dedicated to structural changes and functioning of ion channels.

The main part of the measuring system was an Axopatch200A (Axon Instruments, Inc. Foster City, USA). The program pClamp was used for voltage generation as well as for measuring the current through the planar lipid bilayer and analyses. Four Ag–AgCl electrodes were inserted in the specially designed chamber via agar bridges. Two electrodes served to measure current across the bilayer while the other two applied the voltage across the planar lipid bilayer. As in the previous system designed by Sharma *et al.* [31], the thin Teflon foil (25 μm thick) was inserted between two symmetrical parts of Teflon chamber. The diameter of the hole in the foil was 120 μm. The planar lipid bilayer was formed by the folding technique.

Schematic diagram of the experimental system developed for measuring the fluorescent emissions from the planar lipid bilayer is shown in Fig. 16. The excitation light was focused on the planar lipid bilayer (80 μm in diameter) with an objective lens so that only an area of the planar lipid bilayer was irradiated. The fluorescent emissions were collected through the

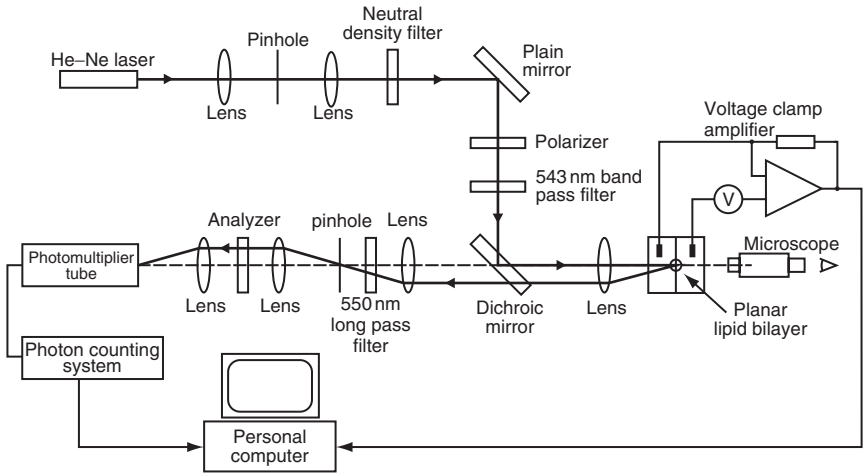

Figure 16 Hanyu–Yamada–Matsumoto [11].

objective lens and sent to the photomultiplier. Any fluorescence from other areas was blocked and scattered light was eliminated. The intensity of fluorescence was measured by photon-counting methods, while a multichannel analyzer was used for the emission spectrum measurements.

2.1.14. System Naumowicz–Petelska–Figaszewski

The system was published in 2003 [35]. It was similar to the system of Wobschall and the Rosen–Sutton system. It allowed applying pressure to planar lipid bilayer. Electrical properties of BLM were examined by impedance spectroscopy (Fig. 17). Four electrodes were immersed in a salt solution: two platinum current electrodes (CE) and two Ag–AgCl measuring electrodes (RE). The volume of one side of the organic glass chamber was modulated by external thread screw. The planar lipid bilayer was formed by the painted technique. Impedance measurement was carried out using an AC impedance system with a personal computer, two-phase lock-in amplifier, and potentiostat/galvanostat. Measuring electrodes were connected with a potentiostat via a high impedance input differential amplifier.

Impedance spectroscopy was used to measure planar lipid bilayer capacitance and resistance [35–37]. The interfacial tension of planar lipid bilayer was measured [38].

2.1.15. System Kramar–Miklavcic–Macek Lebar

The system was published in 2007 [8,39]. It was based on the Sharma–Stebe–Tung system. It included a signal generator, Teflon chamber, voltage and current measurement circuit, and digital storage oscilloscope (Fig. 18). Signal generator was a generator of an arbitrary type. It provided voltage amplitudes

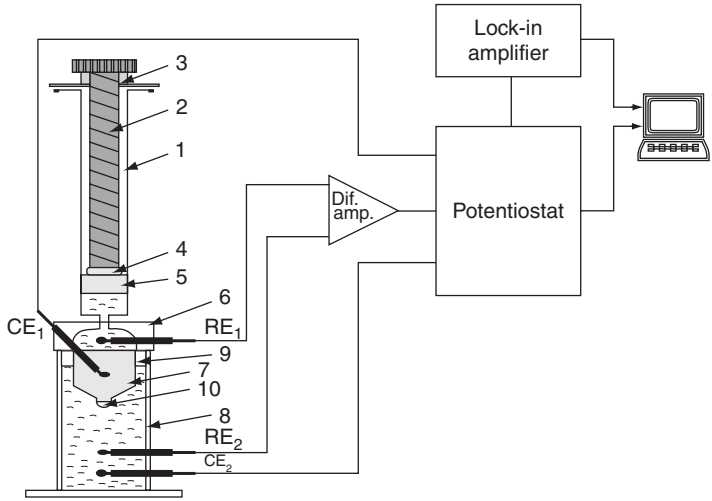

Figure 17 System Naumowicz–Petelska–Figaszewski [37]. 1. Syringe, 2. External thread screw, 3. Handwheel, 4. Steel tube, 5. Tight Teflon Piston, 6. Connector made of organic glass, 7. Platinum current electrode, 8. The chamber made of organic glass, 9. A tight Teflon attachement, 10. A forming sphere for planar lipid bilayer.

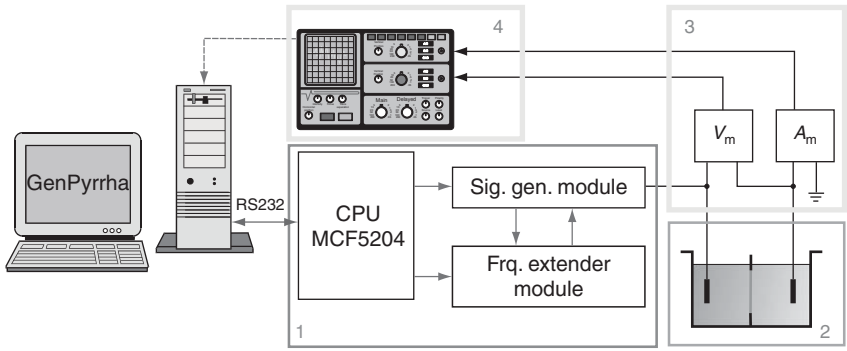

Figure 18 Experimental system. 1. The microprocessor board with MCF5024 processor and two modules. Signal generator module generates arbitrary signals. Frequency extender module is realized in programmable integrated circuit (FPGA) and is used for frequency extension. 2. Chamber for forming planar lipid bilayer and two Ag–AgCl electrodes. 3. Modules for current and voltage amplification. 4. Oscilloscope for data collection and storage.

from −5 to +5 V. It was controlled by custom designed software (Genpyrrha), which allowed drawing of arbitrary voltage signals. On the output of the signal generator was a switch that disconnected the output of the signal generator and connected the electrodes to the 1 MΩ resistor. The switch was able to turn off

the signal generator in 2 ns. This way planar lipid bilayer capacitance was measured. Two Ag–AgCl electrodes, one on each side of the planar lipid bilayer, were inserted into the salt solution. The Teflon chamber consisted of two parts—each part was a cubed reservoir of 5.3 cm^3 in volume. Between the reservoirs a 25 μm thin Teflon sheet was inserted. A diameter of the aperture was 105 μm. The planar lipid bilayer was formed by the folding method. Transmembrane voltage was measured by LeCroy differential amplifier 1822. The same electrodes were also used to measure the transmembrane current. Both signals were stored by the oscilloscope LeCroy Waverunner-2 354M in Matlab format. All sampled signals could be analyzed in Matlab^{TM} software after the experiments.

The authors measured U_{br} by means of a linearly rising signal and the capacitance of the planar lipid bilayers of various compositions [8].

2.2. Current Clamp

2.2.1. System Carius

The system was published in 1976 [40]. Symmetric AC Bridge was the main part of the measuring system (Fig. 19). The variable resistor R_K and the capacitor decade C_K, used for compensation, were in series. Without a planar lipid bilayer in the chamber, the electrode and electrolyte resistance were compensated with $R_K = R_0$ and $C_K = C_0$, when the capacitor C_0 was in series with the cell by means of switch S. With the planar lipid bilayer in the chamber, the increase in the compensation resistance needed ($R_K - R_0$) corresponded to the loss of the planar lipid bilayer capacitor, represented by the equivalent series planar lipid bilayer resistance. A DC bias voltage up to ± 200 mV was provided at the planar lipid bilayer by a voltage controlled current source. The bridge balance was controlled by a phase-sensitive detector (lock-in amplifier) tuned to the frequency of the oscillator. When the out-of-phase signal vanished at proper settings R_K, the in-phase signal output of the lock-in amplifier was proportional to ($C_{BLM} - C_K$), provided that the difference was small compared to C_K. The AC voltage on the planar lipid bilayer was 3 or 6 mV_{RMS}, when only the capacitance was recorded. Another lock-in amplifier was used for the detection of the second harmonic. For these measurements, the AC voltage on the planar lipid bilayer was 20–60 mV_{RMS} and the DC voltage varied between 0 and ± 160 mV. When the second and third harmonics were measured simultaneously by the two lock-in amplifiers, bridge balance was controlled by an AC voltmeter with a band pass filter at the input. The electrodes were Ag–AgCl–platinum black electrodes. The chamber was made of Teflon. In most cases, the diameter of the aperture was about 0.9 mm. The planar lipid bilayer was formed by the painted technique. The output signals were recorded.

The system was applied to measurements of the transmembrane voltage dependence on the BLM capacitance.

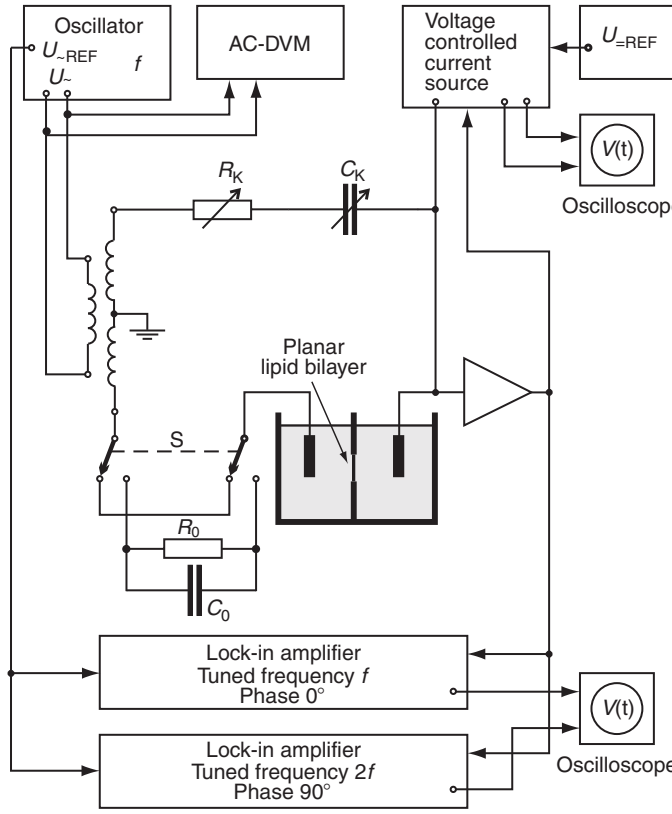

Figure 19 System Carius [40].

2.2.2. System Robello–Gliozzi

The system was published in 1989 [41]. The group performed experiments on current–voltages relationship of planar lipid bilayer under voltage-clamp condition in previous years [42]. Later on, they changed their measuring system to a current-clamp mode. The planar lipid bilayer was in a feedback network of the operational amplifier which acted as a current–voltage converter (Fig. 20). The current value was selected with resistor on the amplifier input. The current–voltage characteristics were obtained by exposing the planar lipid bilayer to a triangular signal with 8–10 min period [41]. By constant current of 10–20 pA, the fluctuations in planar lipid bilayers were studied [43–45]. Two Ag–AgCl electrodes were immersed in salt solution. The volume of each compartment of the Teflon chamber was about 2 ml. The Teflon foil between two compartments was 12 μm thick. The diameter of the apertures was from 100 to 200 μm. The planar lipid bilayer was formed by the folding method. The output signal was low-pass filtered at 250 Hz (24 db/octave) and recorded to the computer with a sampling frequency of 1 kHz.

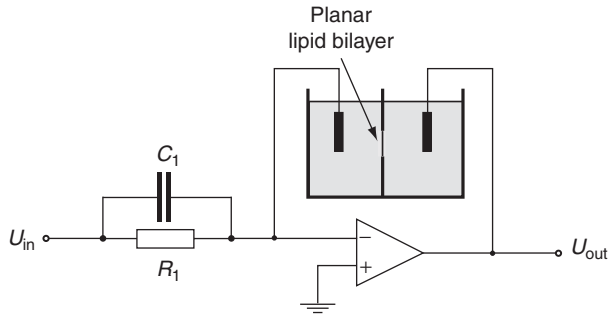

Figure 20 System Robello–Gliozzi [41–47].

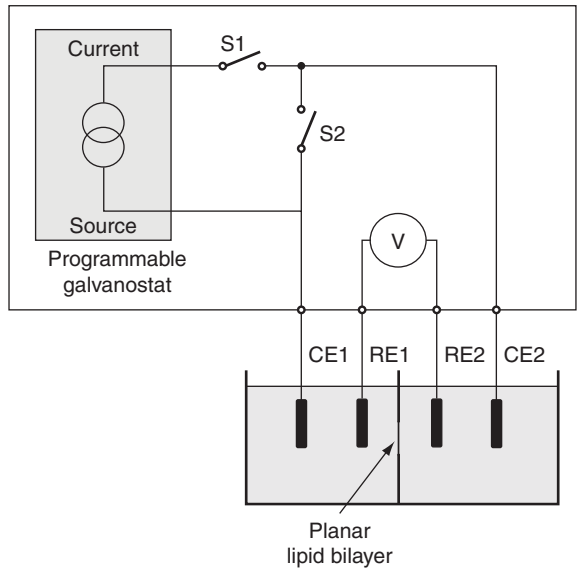

Figure 21 System Kalinowski–Figaszewski [48–51].

The authors observed voltage breakdown, current–voltage characteristic, and fluctuations of planar lipid bilayer. The system was later on upgraded to extremely low current value source (10 pA) [47].

2.2.3. System Kalinowski–Figaszewski

The system was published in 1992 [48]. The system included two modules (Fig. 21). The first module was capacity to period converter, used for measuring the BLM capacitance [48,49] (see Section 3.1). The second module was a potentiostat–galvanostat for planar lipid bilayer studies under current clamp [50]. Both modules were controlled with a personal computer.

The output signal was programmable [51]. The potentiostat had a negative feedback for equalization of operational amplifier input voltage. The chamber was made of one piece Teflon with two compartments, each 10 cm^3 of volume. Between the compartments was the aperture of 1 mm in diameter. Four Ag–AgCl electrodes were immersed in a salt solution; two of them were CE and two other reference electrodes (RE). The switch S1 disconnected the current flowing through the electrodes. The switch S2 caused short circuit of the CE and forced planar lipid bilayer potential to zero.

The system was applied for recording the transmembrane voltage, especially for the electroporation studies the trace of building voltage on planar lipid bilayer was observed due to constant current clamp [9,52].

3. Methods for Determination of Planar Lipid Bilayer Properties

3.1. Capacitance (*C*)

The capacitance (*C*) is the parameter considered the best tool for probing the stability and integrity of planar lipid bilayers and for this reason it is measured for every bilayer, even when other properties are the main focus of the measurements. There are three main methods for determination of planar lipid bilayer capacitance: a discharge method, a capacitance to period conversion method, and a capacitance to voltage conversion method. For comparison between different studies, the measured value of the capacitance must be normalized to the size of the planar lipid bilayer surface and the specific capacitance of the planar lipid bilayer, that is, the capacitance per unit area, is usually given.

3.1.1. Discharge method

The most common and simplest method for measuring planar lipid bilayer capacitance is measurement of the voltage discharge time constant [5,6,8,26,27,30,31,39,53]. Only a voltage generator, a fast switch, and an oscilloscope are needed for its implementation. To make the measurement, a planar lipid bilayer is first charged by a voltage pulse. At the end of the pulse, the charged lipid bilayer is discharged through a resistor of known resistance and the discharging process monitored with an oscilloscope (Fig. 22).

The voltage $u(t)$ on the planar lipid bilayer decreases exponentially:

$$u(t) = U_0 e^{-t/\tau}. \tag{1}$$

Here, U_0 is the amplitude of the voltage pulse and τ is a time constant. The time constant depends on the capacitance (C) and resistance (R) which come from the planar lipid bilayer and the electronic system,

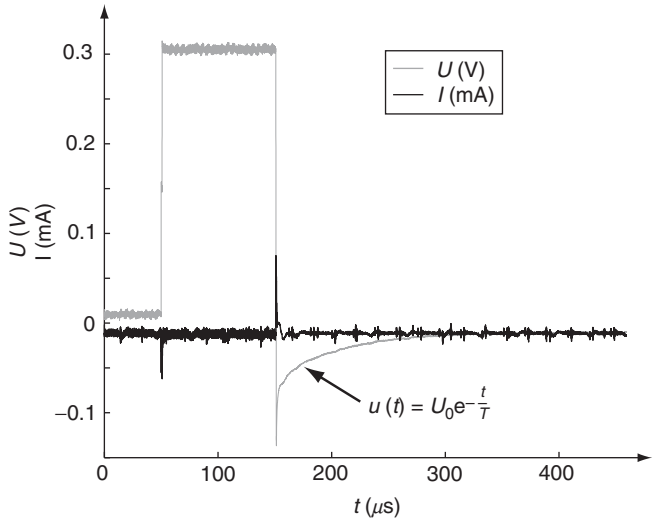

Figure 22 Planar lipid bilayer capacitance measurement by discharge method.

$$\tau = RC. \tag{2}$$

The resistance of the electronic system is usually known and is much lower than the resistance of planar lipid bilayer ($\sim 10^8$ **Ω**); therefore, the capacitance of planar lipid bilayer can be determined in two steps. First, the capacitance of the electronic system is measured without the planar lipid bilayer, C_{SYS}. Then, the capacitance of the electronic system with the planar lipid bilayer and salt solution C_{SBLM} is determined. The capacitance of planar lipid bilayer C_{BLM} is then obtained as a difference between C_{SYS} and C_{SBLM}:

$$C_{BLM} = C_{SYS} - C_{SBLM}. \tag{3}$$

In early experiments, the planar lipid bilayer charging process was also used for planar lipid bilayer capacitance determination. Montal and Mueller [3] calculated the capacitance of planar lipid bilayers from the current records in response to a voltage step signal:

$$C = \frac{I}{\Delta U} \int_0^\infty I \, \mathrm{dt}, \tag{4}$$

where I is the current and ΔU the amplitude of the voltage. In the constant voltage mode, the time constant and gain of the current record depends on the value of the feedback resistor in the current measuring amplifier. Because the capacity currents are small, the feedback resistance was kept about 100 k**Ω**.

3.1.2. A capacitance to period conversion method

The electrical parameters of the planar lipid bilayer can also be measured by means of an alternating voltage signal, which offers the advantage of eliminating the effect of possible electrode polarization.

Kalinowski and Figaszewski [48] constructed an instrument (Fig. 23), which converts planar lipid bilayer capacitance to a train of rectangular pulses. During the measurement, the planar lipid bilayer is charged and discharged with a constant current. The charge–discharge cycle duration is proportional to the membrane capacitance.

In the circuit in Fig. 23, the capacitance of planar lipid bilayer is represented by the capacitor C_{BLM}. The voltage at the point D is amplified by a noninverting amplifier with the gain k:

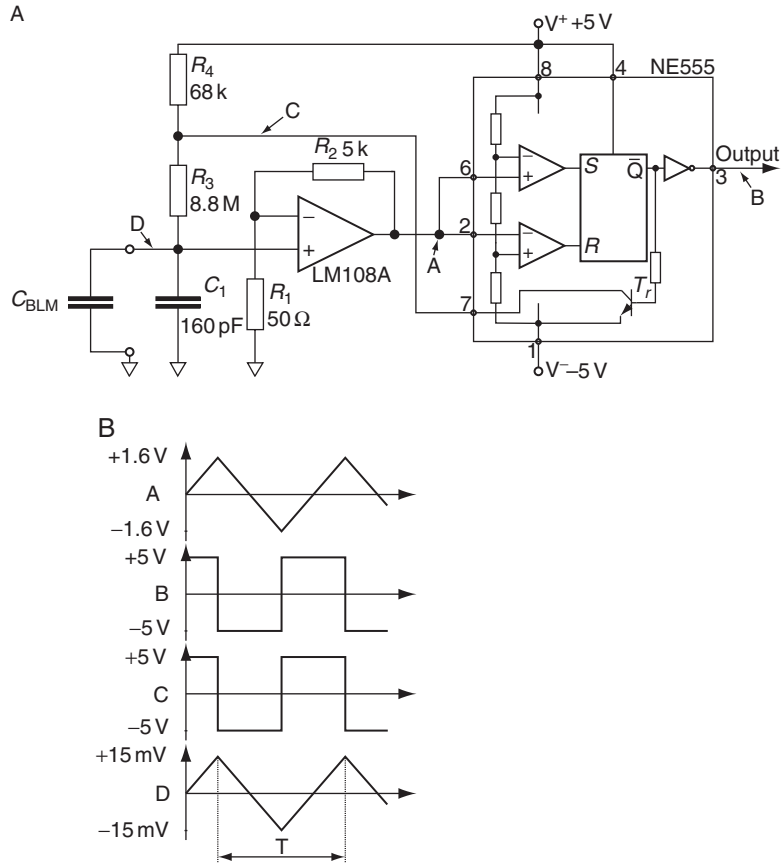

Figure 23 Measurement of planar lipid bilayer capacitance by capacitance to period conversion method. (A) Schematic diagram of the capacity-to-period converter [48]. (B) Voltage wave of the capacity-to-period converter [48].

$$k = \frac{R_1 + R_2}{R_2}. \tag{5}$$

The amplified signal is an input of the integrated circuit NE555. Two voltage comparators are contained in the integrated circuit, both with one of their inputs connected to the voltage from the amplifier output (A) while the other input is one of the voltages from the voltage divider, which is realized by three resistors. Depending on the voltage levels at the inputs R and S, the output of the cell (B) is switched to a low or high state. The result is a square wave signal, which has a well-defined period. The voltage across the planar lipid bilayer can be calculated as

$$U_{\mathrm{BLM}} = \frac{2}{3} V \frac{1}{k} = \frac{2VR_1}{3(R_1 + R_2)}. \tag{6}$$

The measurement is divided into two steps. First, the capacitor is charged with the current passing across resistors R_4 in R_3 and transistor T_{r} is off. Then, the capacitor is discharged due to the current across the transistor, which is a consequence of a changed state of the cell. The current flows across resistor R_3 and transistor T_{r}. The product of the voltage and time in one period is:

$$TV = U_{\mathrm{BLM}}(R_3 + R_4)C_{\mathrm{BLM}} + U_{\mathrm{BLM}}R_3C_{\mathrm{BLM}}. \tag{7}$$

If $R_4 \ll R_3$ and $V = V^+ = |V^-|$, then the period is:

$$T = \frac{2U_{\mathrm{BLM}}R_3C_{\mathrm{BLM}}}{V}. \tag{8}$$

3.1.3. A capacitance to voltage conversion method

When a sinusoidally varying signal is applied to the planar lipid bilayer, its impedance is important. Since capacitors "conduct" current in proportion to the rate of voltage change, they pass more current for faster changing voltages, and less current for slower changing voltages. Therefore, the capacitive part of the impedance–capacitive reactance in ohms for any capacitor is inversely proportional to the frequency of the alternating current. According to this theory, the capacitances of planar lipid bilayers were often measured using AC Wheatstone bridge [15–17], which contain a variable resistor in parallel with a variable capacitor in the known arm. When the bridge is balanced at a given frequency, the settings of the known arm give the parallel equivalent capacitance and resistance of the circuit connected to its unknown terminals. Since the planar lipid bilayer is immersed in electrolyte, the bridge measures the parallel equivalent impedance of the membrane–electrolyte system.

The parallel equivalent capacitance can be represented by the membrane capacitance and the stray capacitance associated with electrodes in series with electrolyte resistance; therefore, appropriate equivalent circuit and transform equations should be used to relate membrane capacitance to the elements of the bridge. A convenient technique for displaying AC impedance data is the Cole–Cole diagram.

Micelli *et al*. [33] measured the capacitance of planar lipid bilayers by applying sinusoidally varying voltage with amplitude of 2 mV and the frequency of 1 kHz. At this high frequency, almost all of the current crosses the reactive part of the planar lipid bilayer and its resistance is negligible. The rectified voltage is proportional to the planar lipid bilayer capacitance. By using a set of test values for the capacitance, which were one by one included in measuring system, they parameterized the relation between measured voltage and capacitance. The hyperbolic relation with two known parameters a and b was obtained:

$$C_{\mathrm{BLM}} = a\frac{V_{1h}}{b - V_{1h}}. \tag{9}$$

3.2. Resistance (*R*)

Planar lipid bilayer membranes exhibit resistance in the range of few gigaohms. The resistance is usually calculated in accordance with Ohm's law as a ratio of voltage applied to (or measured on) the planar lipid bilayer and current which flows through it. As mentioned earlier, the electrical parameters of planar lipid bilayer can also be measured by means of an alternating current. The continuous monitoring of capacitance is useful in tracking membrane thickness, while the continuous monitoring of the resistance allows studies of protein–lipid interactions and planar lipid bilayer fluctuations.

Gallucci and coworkers [32] presented an electrical circuit appropriate for continuous monitoring of planar lipid bilayer capacitance and resistance simultaneously. An input voltage was composed of two sinusoidally varying signals: one with variable amplitude (0.1–1.5 V) and frequency of 1 Hz and another with amplitude of 2 mV and frequency of 1 kHz. The planar lipid bilayer and the measuring device are shown with equivalent circuits on the left side in Fig. 24. According to the associated vector graph on the right side in Fig. 24, the following relations can be written:

$$V_1\cos(\varphi - \varphi_1) + V_{\mathrm{BLM}}\cos(\varphi_{\mathrm{BLM}} - \varphi) = V_{\mathrm{s}} \tag{10}$$

$$V_1\sin(\varphi - \varphi_1) = V_{\mathrm{BLM}}\sin(\varphi_{\mathrm{BLM}} - \varphi) \tag{11}$$

$$\omega C_{\mathrm{BLM}} V_{\mathrm{BLM}} = I\sin\varphi_{\mathrm{BLM}}. \tag{12}$$

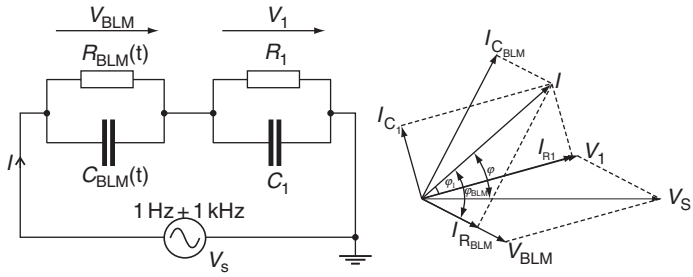

Figure 24 Left: Equivalent circuit of the planar lipid bilayer (BLM) and of the measurement device. V_s is an input voltage. V_{BLM} and V_1 are the planar lipid bilayer and output voltages, respectively. R_1 is electrical resistance and C_1 the capacitance of the measuring circuit; R_{BLM} and C_{BLM} are the resistance of planar lipid bilayer and capacitance, respectively. Right: Vector scheme of the voltages and currents in the circuit no the left [32–34].

The current I is the vector sum of the currents crossing the resistance R_1 and capacitance C_1 of the measuring device:

$$I = V_1 \sqrt{(\omega C_1)^2 + \left(\frac{1}{R_1}\right)}. \tag{13}$$

If the capacitance of the planar lipid bilayer has already been measured (see Section 3.1), the phase angles φ_{BLM} and φ as well as the voltage V_{BLM} can be determined. The resistance of the planar lipid bilayer is then obtained from the relation:

$$R_{BLM} = \frac{V_{BLM}}{I \cos\varphi}. \tag{14}$$

3.3. Breakdown Voltage (U_{br})

The electrical modulation of biological membrane physical properties caused by electrical oscillations and excitations are natural processes in living organisms. Applications of external electric fields, especially those based on the phenomenon of electroporation, have gained increasing importance for manipulations in biological cells and tissues [54]. The structural changes in biological membranes induced by an external electric field involve rearrangement of the phospholipid bilayer and lead to the formation of aqueous pores. If the electric field does not exceed some critical adequate strength and duration, the membrane returns to its normal state after the end of the exposure to the electric field; the electroporation is reversible. However, if the exposure to the electric field is too long or the strength of the electric field is too high, the membrane does not reseal after the end of the exposure,

and the electroporation is irreversible. The underlying mechanisms for these properties are dependent on the lipid component of biological membrane, and can be studied on planar lipid bilayers.

Application of a steady voltage in the order of few hundred millivolts across a planar lipid bilayer causes the membrane to break. Most often the breakdown voltage (U_{br}) of the planar lipid bilayer is determined by applying a rectangular voltage pulse (10 μs–10 s) (Fig. 25). The amplitude of the voltage pulse is increased in small steps until the breakdown of the bilayer is obtained [5]. First, the voltage pulse charges up the planar lipid bilayer. Above a critical voltage (U_{br}) defects are created in the planar lipid bilayer allowing an increase of the current through the bilayer [4]. Usually, planar lipid bilayer collapses when the breakdown voltage is exceeded.

Using the rectangular voltage pulse measuring protocol, the number of applied voltage pulses is not known in advance and each planar lipid bilayer is exposed to a voltage stress many times. Such a pretreatment of the planar lipid bilayer affects its stability and consequently the determined breakdown voltage of the planar lipid bilayer [4]. Another approach for the breakdown voltage determination was suggested by Kramar *et al.* [8]. Using a linear rising signal, the breakdown voltage of a planar lipid bilayer is determined by only a single voltage exposure (Fig. 26).

The slope of the linear rising signal and the peak voltage of the signal must be selected in advance. The breakdown voltage (U_{br}) is defined as the voltage at the moment t_{br} when a sudden increase of the current through the planar lipid bilayer is observed. Time (t_{br}) is defined as the lifetime of the planar lipid bilayer at a chosen slope of the linear rising signal (Fig. 26). Because the planar lipid bilayer lifetime depends on the applied voltage [5,55] and the planar lipid bilayer pretreatment [4], U_{br} and t_{br} are measured at a variety of slopes. Using nonlinear regression (Fig. 27), a two parameter curve can be fitted to the data

$$U = \frac{a}{1 - e^{-t/b}}, \tag{15}$$

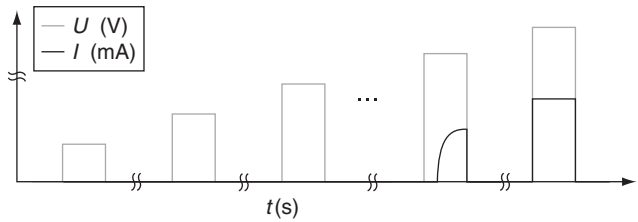

Figure 25 Measurement of planar lipid bilayer breakdown voltage (U_{br}) by successional rectangular pulses. The amplitude of the voltage pulse (gray) is incremented in small steps until the breakdown of the bilayer is observed as sudden increase of current (black) [5].

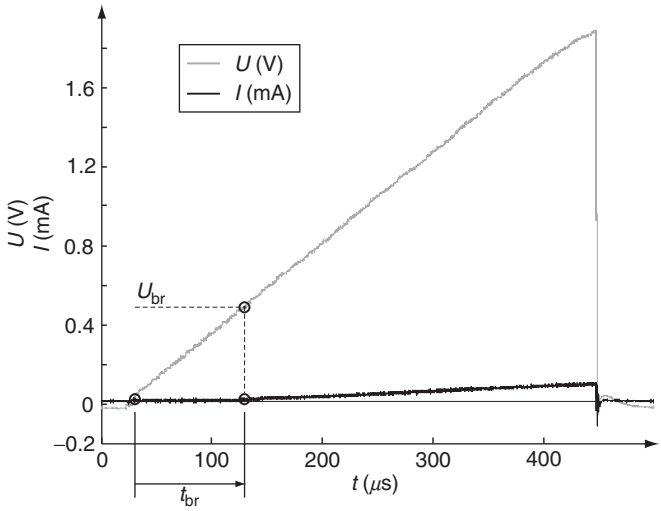

Figure 26 Measurement of planar lipid bilayer breakdown voltage (U_{br}) by linear rising signal. Breakdown voltage is defined as the voltage (gray) at the moment t_{br} when sudden increase of the current (black) through the planar lipid bilayer is observed [8].

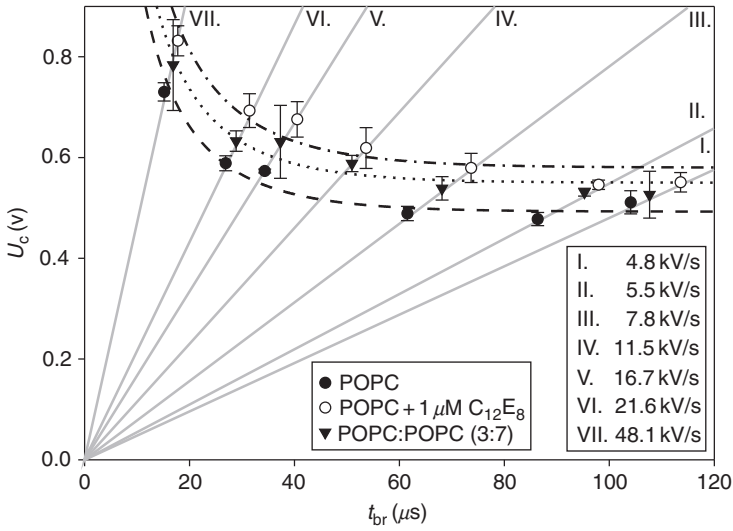

Figure 27 The breakdown voltage (U_{br}) (dots and triangles) of planar lipid bilayers with different chemical composition as a function of lifetime t_{br}. The gray lines show seven different slopes of applied liner rising voltage signal. Dash, dotted, and dash-dotted curves represent two parameters curve [15] fitted to data.

where U is U_{br} measured at different slopes; t is corresponding t_{br}; and a and b are parameters. Parameter a is an asymptote of the curve which corresponds to minimal breakdown voltage U_{brMIN} for a specific planar lipid bilayer chemical composition. Parameter b governs the inclination of the curve.

3.4. Fluctuations (ψ)

Fluidity of the lipid membrane must produce local fluctuations of the membrane microscopic parameters. Appearance of transient defects and pores in the membrane structure affects its conductance, producing fluctuations. Transient changes of the membrane electrical properties also accompany protein insertion into the membrane. Voltage-clamp studies with low-value fields are typically applied for recording capacitance and conductance changes, following insertion of channel-forming proteins into the planar lipid bilayer (e.g., Ref. [25]).

The fluctuations are even more pronounced under a strong electric field that is sufficient to electroporate the membrane. The pore appearance is preceded by lipid reorganization resulting in the events of transient membrane permeability to ions. Related to these phenomena, fluctuations of the current were observed prior to an irreversible breakdown of a planar lipid bilayer [4]; the fluctuation amplitude was about 10^{-11} A. After electroporation, it is very unlikely for an electropore to maintain its rim fixed, hence pore fluctuations are theoretically expected. Since the electroporation under voltage-clamp conditions results in very fast pore expansion leading to rapid membrane breakdown, an experimental study on the pore dynamics, in the voltage-clamp mode, required application of very short pulses that could protect the membrane from destruction [56,57]. The experiment reported in Ref. [57] approximated a typical lifetime of an electropore created under voltage-clamp conditions (250 mV) as 3 ms. Conductance fluctuations recorded in these experiments were attributed to a pore dynamics. In such a study, however, the voltage was clamped above the breaking potential and, because of the high value of the potential, the appearance of multiple pores is almost certain. The combined dynamics of several pores may have accounted for the observed fluctuations and single pore dynamics was blurred. At higher voltages, an irreversible membrane breakdown was studied by voltage-clamp techniques [27,31].

Exposure of the planar lipid bilayer to a constant current (0.1–2.0 nA) does not rupture the bilayer rapidly. The membrane slowly accumulates the charge and when the first pore appears, the transmembrane potential decreases, preventing subsequent electropore appearance, which permits the hypothesis of a single pore formation. Fluctuations observed in these current-clamp experiments are caused by opening and closing of a single pore [9,49–52,58]. The natural electropore fluctuations are enhanced by a negative feedback inherent to the current-clamp electroporation method.

The feedback results from interplay between the pore surface and the transmembrane voltage. As a consequence of the electropore expansion, the membrane resistance decreases and the voltage across planar lipid bilayer is reduced. This prevents further increase of the electropore, which usually starts shrinking instead—increasing the transmembrane voltage. This chain of events accounts for the pore stabilization and its fluctuations. The pore can live for several hours. The fluctuations show regular stochastic properties, which are partly due to the feedback and partly due to the pore dynamics and membrane elastic properties [58,59]. Elimination of the feedback in the voltage-clamp experiment, before which the pore that is formed and stabilized under current-clamp conditions, shows very interesting non-Gaussian properties of the electropore dynamics. The parameters of the long-tailed Levy-stable probability density function, which characterizes electropore dynamics, are related to the lipid composition of the membrane, salt properties, and the pore diameter (Fig. 28) [52].

3.5. Other Physical Properties

3.5.1. Thickness (*d*)

The thickness of planar lipid bilayer is usually determined from its capacitance according to the relation valid for parallel plate capacitor:

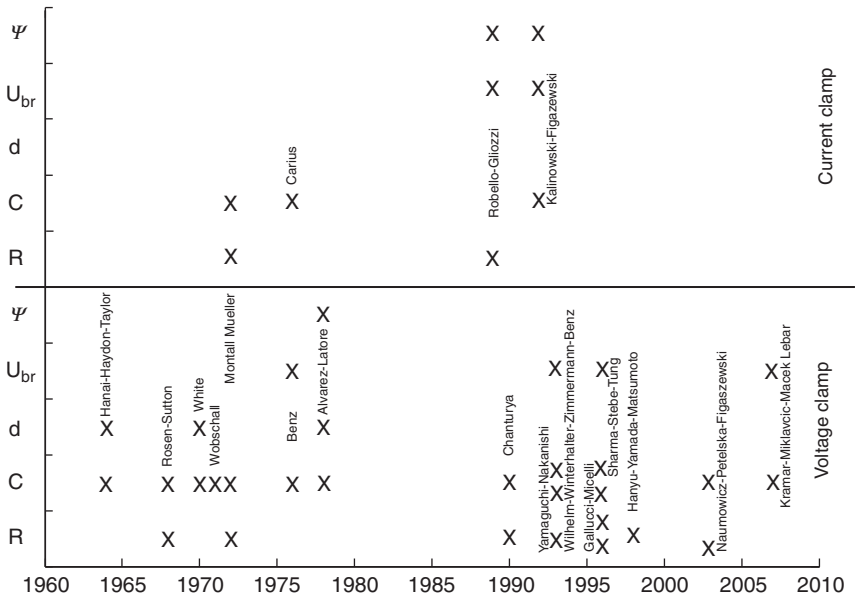

Figure 28 Time perspective of systems for planar lipid bilayer properties determination.

$$d = \varepsilon\varepsilon_0 \frac{A}{C_{\mathrm{BLM}}}. \tag{16}$$

In this equation, A stands for the area of planar lipid bilayer and C_{BLM}, ε_0, and ε for its capacitance, the permittivity of the free space, and the relative static permittivity, respectively. It is usually assumed that the relative static permittivity inside lipid bilayer equals to 2.1 [24].

3.5.2. Elasticity (E) and surface tension (σ)

A macroscopic approach using the theory of elasticity of solid bodies and liquid crystals can be applied to describe mechanical properties of lipid bilayers. In 1973, Helfrich proposed a theory and possible experiments of elastic properties measurements on planar lipid bilayers [60]. As the anisotropy of lipid bilayers is clearly expressed, several elasticity modules are required to describe its viscoelastic properties. Depending on the directions of the membrane deformation, we distinguish volume compressibility, area compressibility, unilateral extension along membrane plane, and transversal compression.

Experimentally, lipid bilayer mechanical properties were commonly measured on giant unilamellar vesicles [61–64]. Pressure was applied on a membrane with micropipette aspiration method; the properties were measured by means of video microscopy [65]. From experiments on planar lipid bilayer, Winterhalter and coworkers [66] reported that dynamics light scattering allowed quantifying viscoelastic properties in nonperturbative way, while Wobschall calculated membrane elasticity and breaking strength from measurement of capacitance of the planar lipid bilayer as it was bowed under a known pressure. Transversal elasticity modulus cannot be measured directly due to small thickness of the membrane and extremely small changes of the thickness upon deformation. It can be estimated through capacitance measurement with a special electrostriction method which is based on measurements of the amplitude of higher current harmonics [67].

Sabotin with coworkers [68] presented an estimation of the planar lipid bilayer transversal elasticity (E) and surface tension (σ) by means of viscoelastic predictive model of Dimitrov [69] and measured planar lipid bilayer capacitance and break down voltage. The model considers the lipid bilayer as a viscoelastic, isotropic material that can be represented as a standard solid model, composed of a Kelvin body in series with a linear spring. Originally, this model predicts the critical voltage and critical time needed to collapse a membrane at applied voltage. Critical voltage corresponds to breakdown voltage (U_{brMIN}) and critical time to life time (t_{br}) of planar lipid bilayer. The parameters of model are Young's transversal elasticity modulus (E), surface tension (σ), viscosity (μ), thickness of the membrane (h), and

permittivity of membrane (ε_m). If U_{br} is measured by linear rising signal (Fig. 26), the corresponding planar lipid bilayer lifetime (t_{br}) is always finite [8]. Generic model equation that still contains t_{br} [69] gives the relation:

$$U_{br} = \sqrt[4]{n + \frac{k}{t_{br}}}\,. \tag{17}$$

The relation can be fitted to the data obtained experimentally by the breakdown voltage determination using linear rising signal [8]. Parameters n and k, which are obtained through fitting, served to calculate Young's elasticity modulus (E) and surface tension (σ) of planar lipid bilayer. Specific capacitance (c_{BLM}) has to be measured, while other parameters such as thickness ($h = 3.5$ nm) and viscosity ($\mu = 6$ Ns/m^2) can be taken from Ref. [69].

4. Conclusions

In this chapter, we have reviewed setups and experimental methods applied to study of the properties of planar lipid bilayers. The planar lipid bilayer presents a good model of the plasma membrane where the behavior of the lipid part is not obscured by other components of the real cell membrane. In particular, the influence of the conductive protein ion channels could be eliminated. The development of measuring systems was enabled by discovery of the first technique for forming stable planar lipid bilayers in 1962. This discovery permitted design of a range of instruments useful for measuring planar lipid bilayer characteristics by different methods, allowing a more complete picture of planar lipid bilayer physical properties and membrane-related phenomena like electroporation. The measurement methods vary from simple electrical setups, which allowed for the first experiments and demonstrated basic lipid bilayer characteristics, to more recent advanced systems, frequently combining electrical and nonelectrical methods, such as optical or mechanical.

In the field of electrical measurements on biological objects with non-constant resistance, there are two major approaches to the topic. One method is based on the voltage-controlled measurements, in which different shapes of an alternating voltage are applied on the planar lipid bilayer. In the other method, the current shape and value are controlled. The planar lipid bilayer characteristics that are observed during the experiment dictate the choice of the measuring principle. The experiments carried out by the presented systems showed viscoelastic properties of planar lipid bilayers, temporal changes in the electroporated membrane, and fluctuation characteristics of an electropore.

Appendix A. Review of Measuring Systems

Table 1 Description of measuring systems

System	Stimulating signal	Lipid	Salt solution	Lipid preparation	Volume of the chamber	Electrodes	Aperture diameter	Temperature (°C)
Rosen–Sutton [16]	AC: 5 mV/500 Hz, DC: ±200 mV	Lecitin	1 mM, 10 mM, 100 mM, 1 M KCl or NaCl	1% (w/v)	–	2 Calomel + 2 Pt	1 mm	22
White [17,18]	AC: 20 mV/100 Hz	Oxidized cholesterol, lecithin	KCl, pH 7.1	35 mg/ml	–	4 platinized Ag–AgCl	1.6 mm	20
Wobshall [19,20]	AC: 5–15 mV_{RMS}/4kHz	Cholesterol, HDTAC	40 mM KCl, pH 6.7	12 mg/ml	–	4 Ag–AgCl	1.68 mm	30
Montal–Mueller [3]	Voltage pulse 10 ms, 37.5 mV; Constant current 20 pA	Egg lecithin, cholesterol, glyceroldioleate, bovin cardiolipin, gramicidin	0.01 M NaCl, pH 5.5	–	(18 × 12) mm^2/compartment	2 Calomel	0.25 mm	Room temperature
Benz [6,7]	10 mV–2 V/500 μs–500 ms	PC, DPhPC, DOPC, POPC, PE, Ox Ch	1 M KCl, 0.1 M NaCl, pH 6	2% (w/v)	3 cm^3/compartment	2 Ag–AgCl	2 mm^2 (area); 0.2–0.3 mm	25
Abidor-Chernomordik-Chizmadzhev-Pastushenko [4]	100 mV/1 μs, 400 mV/1 μs	Egg lecithin; Ch: lecitin 2:1	0.1 M NaCl + 10 M Tris–HCl, pH 7.4	40 mg/cm^3	–	–	1 mm	27

(*continued*)

Table 1 (*continued*)

System	Stimulating signal	Lipid	Salt solution	Lipid preparation	Volume of the chamber	Electrodes	Aperture diameter	Temperature (°C)
Alvares-Latore [24]	10 mV/5 kHz	–	–	–	4 cm^2/ compartment	2 Ag–AgCl	–	–
Robello–Gliozzi [41,43–45]	Square waves 40 mV, current ramp (0.17 pA/s), triangular signal 2.5×10^{-3} Hz	Egg lecithin:Ch 1:1, PC:Ch (4:1), PC, Ch, DPhPC, PS	10 mM Tris–Cl, pH=7.5, 0.1 M, 1 M KCl	10 mg/ml	2 ml / compartment	2 Ag–AgCl	100–150, 160, 200 μm	25
Chanturya [25,70]	10 mV–1 V/10–100 μs	PC:Ch 2:1	10 mM Tris, pH=7.4	20 mg/ml	9 ml	Ag–AgCl	0.6 mm	26–29
Kalinovski-Figazewski [48–50]	Constant current: 0.005, 0.2, 0.3, 2 nA; step 0.2 nA/10 s; rectangular signal: 8 × 0.2 nA/10 s	PC:Ch, lecithin, PS	0.1 M KCl, pH 7.0	20 mg/ml	10 cm^3/ compartment	4 Ag–AgCl (0.5 × 80) mm	1 mm	23–25
Wilhelm–Winterhalter–Zimmermann–Benz [26–30]	10 mV–2 V/500 μs–500 ms	Ox Ch (+ DOPC or + PE), azolectin, DPHPC, PS, DOPC, DOPE	10 mM, 100 mM, 1 M, 2 M, 3 M KCl. pH=6,7	1 μl, 5 mg/ml, 10 mg/ml, 40 mg/ml	5 ml/ compartment, 15 ml/ compartment	2 Ag–AgCl	100–200 μm, 0.3–3 mm, 1 mm, 2 mm^2 (area)	20, 22, 25, 30
Yamaguchi–Nakanishi [12]	7 $mV_{R.MS}$/1 kHz	GMO	0.1 M KCl	62 mg/ml	–	2 Pt	0.7 mm	25
Sharma–Stebe–Tung [5,31]	100 mV/10 μs, 510 μs ⎍	POPC, azolectin	100 mM KCl, pH=7.4	10 mg/ml 20 mg/ml	3 ml/compartment	4 Ag–AgCl	75–100 μm, 105 μm	22–24

Gallucci–Micelli [32–34]	Variable amplitude V_{pp}/1 Hz + 2 mV/1 kHz	PI, Ox Ch	0.1 M, 0.5 M, 1 M KCl, pH 7.0	1% (w/v)	4 ml/compartment	2 Pt	1.3 mm	22–24
Hanyu–Yamada–Matsumoto [11]	0.1–1 V	Azolectin	–	5 mg/ml	–	4 Ag–AgCl	120 μm	–
Naumowicz–Petelska–Figaszewski [35–37]	4 mV/0.01–10 kHz	Lecithin, Ch, lecithin:Ch 1:1	0.1 M KCl, pH 7.4	20 mg/cm^3	–	2 Ag–AgCl, 1 Pt	–	–
Kramar [8,68]	Linar rising voltage (4.8–48.1 kV/s)	POPC, POPS	0.1 M KCl	10 mg/ml	5.3 cm^3/ compartment	2 Ag–AgCl	105 μm	Room temperature

For each system the stimulating signal, number and material of electrodes, volume of the chamber, and a diameter of the aperture are given. Lipids, which were used in described experiments, corresponding salt solutions and the temperature, at which the experiments were conducted, are also listed. Ch, cholesterol.

Appendix B. Review of Measured Properties on Planar Lipid Bilayer

Table 2 Properties of planar lipid bilayers of various lipid compositions and parameters at which they were measured

Lipid	Salt solution	T (μs)	Shape of the signal	U_{br} (mV)	C (μF/cm^2)	R (MΩcm^2)	d (nm)	References
Azolecitin	0.1 M KCl	10		423.4±29.4	0.59±0.21	–	–	[31]
		510		441.6±23.2				
DOPC	0.1 M NaCl	–		–	0.37±0.01	0.40	4.97±0.17	[6]
DOPE	0.1 M NaCl	–		–	0.37±0.01	0.40	5.00±0.16	[6]
DPhPC	0.1 M KCl	–		390±20	0.6–0.75	7.85–17.76	–	[41,43,45,46]
	1 M KCl	–		–	0.74–1.13	24.3–54.9	–	[43,44]
	0.1 M KCl	10		546±15	–	–	–	[27,30,44]
	0.1 M NaCl	–		–	0.36±0.02	0.40	5.08±0.21	[6]
	0.1 M KCl	–		–	0.9–1	–	–	[57]
DPhPS	0.1 M KCl	10		530±15	–	–	–	[27,30]
Lecithin	1e-3–1 M NaCl or KCl	–		–	0.32–0.64	1–10	–	[16]
Ox Ch	1 M KCl	–		–	0.40	0.25	–	[34]
	0.1 M KCl	–		–	0.45±0.01	0.21±0.01	–	[32]
	0.5 M KCl				0.47±0.04	0.23±0.01		
	1 M KCl				0.40±0.01	0.20±0.03		
	1 M KCl	10		–	0.56	–	3.3	[7]
PC	0.1 M KCl	–		280±30	0.75	–	–	[41,46]
	0.1 M NaCl	–		–	0.34±0.01	0.40	5.48±0.17	[6]
PE	0.1 M NaCl	–		–	0.33±0.01	0.40	5.67±0.22	[6]

PI	1 M KCl	–	∿	–	0.25	0.4	–	[34]
	0.1 M KCl	–	∿	–	0.30±0.01	0.37±0.01	–	[32]
	0.5 M KCl				0.27±0.01	0.34±0.01		
	1 M KCl				0.25±0.01	0.38±0.06		
POPC	0.1 M KCl	10	⊓	400±6	–	–	–	[27,30]
	0.1 M KCl	10	⊓	450±24	0.59±0.15	–	–	[5]
		100		398±19				
		10^3		331±20				
		10^4		282±26				
		10^5		258±9				
		10^6		213±18				
		10^7		167±6				
POPS	0.1 M KCl	10	⊓	410±20	–	–	–	[27,30]
PS	0.1 M KCl	–	⊓	500 ± 50	–	–	–	[46]
PC + Ch	0.1 M KCl	–	⊓	270 ± 20	–	–	–	[46]
PC + PE	0.1 NaCl	–	∿	–	0.1	–	–	[25]
Lecithin	0.1 M KCl	–	∿	–	0.38–0.61	0.014–2.12	–	[20]
+ Ch 1:1	10 mM Tris–Cl	–	⊓	–	–	–	–	[21]

For each lipid composition break down voltage ($U_{\mathbf{br}}$), capacitance (C), resistance (R), and thickens (d) are given. Salt solution that was used in experiments, as well as shape and duration of the stimulating signal are also presented.

REFERENCES

[1] R.B. Gennis, Biomembranes, Molecular Structure and Function, Springer, New York, 1989.
[2] P. Mueller, D.O. Rudin, H.T. Tien, W.C. Wescott, Methods for the formation of single bimolecular lipid membranes in aqueous solution, J. Phys. Chem. 67 (1963) 534–535.
[3] M. Montal, P. Mueller, Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties, PNAS 69 (1972) 3561–3566.
[4] I.G. Abidor, V.B. Arakelyan, L.V. Chernomordik, Y.A. Chizmadzhev, V.F. Pastushenko, M.R. Tarasevich, Electric breakdown of bilayer lipid membranes I. The main experimental facts and their qualitative discussion, Bioelectrochem. Bioenerg. 6 (1979) 37–52.
[5] G.C. Troiano, L. Tung, V. Sharma, K.J. Stebe, The reduction in electroporation voltages by the addition of surfactant to planar lipid bilayer, Biophys. J. 75 (1998) 880–888.
[6] R. Benz, K. Janko, Voltage-induced capacitance relaxation of lipid bilayer membranes; effects on membrane composition, Biochim. Biophys. Acta 455 (1976) 721–738.
[7] R. Benz, F. Beckers, U. Zimmermann, Reversible electrical breakdown of lipid bilayer membranes: A charge-pulse relaxation study, J. Membr. Biol. 48 (1979) 181–204.
[8] P. Kramar, D. Miklavčič, A. Maček Lebar, Determination of the lipid bilayer breakdown voltage by means of a linear rising signal, Bioelectrochemistry 70 (2007) 23–27.
[9] S. Kalinowski, G. Ibron, K. Bryl, Z. Figaszewski, Chronopotentiometric studies of electroporation of bilayer lipid membranes, Biochim. Biophys. Acta 1396 (1998) 204–212.
[10] A. Blume, Lipids, in: D. Waltz, J. Teissie, G. Milazzo (Eds.), Bioelectrochemistry of Membranes Birkhauser, Basel-Boston-Berlin, 2004, pp. 24–61.
[11] Y. Hanyu, T. Yamada, G. Matsumoto, Simultaneous measurement of spectroscopic and physiological signals from a planar bilayer system: Detecting voltage-dependent movement of a membrane-incorporated peptide, Biochemistry 1998 (1998) 15376–15382.
[12] H. Yamaguchi, H. Nakanishi, Characterization of the preparation process and the photochemical control of electrical properties of bilayer lipid membranes containing azobenzene chromopores, Biochim. Biophys. Acta 1148 (1993) 179–184.
[13] T.F. Eibert, M. Alaydrus, F. Wilczewski, V.W. Hansen, Electromagnetic and thermal analysis for lipid bilayer membranes exposed to RF fields, IEEE Trans. Biomed. Eng. 46 (1999) 1013–1021.
[14] P. Mueller, D.O. Rudin, H.T. Tien, W.C. Wescott, Reconstitution of a cell membrane structure in vitro and its transformation into an excitable system, Nature 194 (1962) 979–980.
[15] T. Hanai, D.A. Haydon, J. Taylor, An investigation by electrical methods of lecitin-in-hydrocarbon films in aqueous solutions, Proc. R. Soc. Lond. Ser. A Math. Phys. Sci. 281 (1964) 377–391.
[16] D. Rosen, A.M. Sutton, The effects of a direct current potential bias on the electrical properties of bimolecular lipid membranes, Biochim. Biophys. Acta 163 (1968) 226–233.
[17] S.H. White, A study of lipid bilayer membrane stability using precise measurements of specific capacitance, Biophys. J. 10 (1970) 1127–1147.
[18] S.H. White, T.E. Thompson, Capacitance, area, and thickness variations in thin lipid films, Biochim. Biophys. Acta 323 (1973) 7–22.
[19] D. Wobschall, Bilayer membrane elasticity and dynamic response, J. Colloid Interface Sci. 36 (1971) 385–423.

[20] D. Wobschall, Voltage dependance of bilayer membrane capacitance, J. Colloid Interface Sci. 40 (1972) 417–423.
[21] K.U. Maheswari, T. Ramachandran, D. Rajaji, Interaction of cisplatin with planar model membranes—dose dependent change in electrical characteristics, Biochim. Biophys. Acta 1463 (2000) 230–240.
[22] L.V. Chernomordik, S.I. Sukharev, I.G. Abidor, Y.A. Chizmadzhev, Breakdown of lipid bilayer membranes in an electric field, Biochim. Biophys. Acta 736 (1983) 203–213.
[23] L.V. Chernomordik, S.I. Sukharev, S.V. Popov, V.F. Pastushenko, A.V. Sokirko, I.G. Abidor, et al. The electrical breakdown of cell and lipid membranes: the similarity of phenomenologies, Biochim. Biophys. Acta 902 (1987) 360–373.
[24] O. Alvarez, R. Latorre, Voltage-dependent capacitance in lipid bilayers made from monolayers, Biophys. J. 21 (1978) 1–17.
[25] A.N. Chanturiya, Detection of transient capacitance increase associated with channel formation in lipid bilayers, Biochim. Biophys. Acta 1026 (1990) 248–250.
[26] C. Wilhelm, M. Winterhalter, U. Zimmermann, R. Benz, Kinetics o pore size during irreversible electrical breakdown of lipid bilayer membranes, Biophys. J. 64 (1993) 121–128.
[27] A. Diederich, G. Bahr, M. Winterhalter, Influence of surface charges on the rupture of black lipid membranes, Phys. Rev. E 58 (1998) 4883–4889.
[28] A. Diederich, G. Bahr, M. Winterhalter, Influence of polylysine on the rupture of negatively charged membranes, Langmuir 14 (1998) 4597–4605.
[29] A. Diederich, M. Strobel, W. Meier, M. Winterhalter, Viscosity- and inertia-limited rupture of dextran-supported black lipid membranes, J. phys. Chem. B 103 (1999) 1402–1407.
[30] W. Meier, A. Graff, A. Diederich, M. Winterhalter, Stabilization of planar lipid membranes: a stratified layer approach, Biochim. Biophys. Acta 2000 (2000) 4559–4562.
[31] V. Sharma, K. Uma Maheswari, J.C. Murphy, L. Tung, Poloxamer 188 decreases susceptibility of artificial lipid membranes to electroporation, Biophys. J. 71 (1996) 3229–3241.
[32] E. Gallucci, S. Micelli, G. Monticelli, Pore formation in lipid bilayer membranes made of phosphatidylinositol and oxidized cholesterol followed by means of alternating current, Biophys. J. 71 (1996) 824–831.
[33] S. Micelli, E. Gallucci, V. Picciarelli, Studies of mitochondrial porin incorporation parameters and voltage-gated mechanism with different black lipid membranes, Bioelectrochemistry 52 (2000) 63–75.
[34] S. Micelli, E. Gallucci, D. Meleleo, V. Stipani, V. Picciarelli, Mitochondrial porin incorporation into black lipid membranes: ionic and gating contribution to the total current, Bioelectrochemistry 75 (2002) 97–106.
[35] M. Naumowicz, A.D. Petelska, Z.A. Figaszewski, Capacitance and resistance of the bilayer lipid membrane formed of phosphatidylcholine and cholesterol, Cell. Mol. Biol. Lett. 8 (2003) 5–18.
[36] M. Naumowicz, Z.A. Figaszewski, Impedance spectroscopic investigation of the bilayer lipid membranes formed from the phosphatidylserine–ceramide mixture, J. Membr. Biol. 227 (2009) 67–75.
[37] M. Naumowicz, A.D. Petelska, Z.A. Figaszewski, Impedance analysis of phosphatidylcholine–cholesterol system in bilayer lipid membranes, Electrochim. Acta 50 (2005) 2155–2161.
[38] A.D. Petelska, M. Naumowicz, Z.A. Figaszewski, Complex formation equilibria in two-component bilayer lipid membrane: interfacial tension method, J. Membr. Biol. 228 (2009) 71–77.

[39] P. Kramar, D. Miklavčič, A. Maček-Lebar, A system for the determination of planar lipid membrane voltage and its applications, IEEE Trans. Nanobiosci. 8 (2009) 132–138.
[40] W. Carius, Voltage dependence of bilayer membrane capacitance. Harmonic response to ac excitation with dc bias, J. Colloid Interface Sci. 57 (1976) 301–307.
[41] M. Robello, A. Gliozzi, Conductance transition induced by an electric field in lipid bilayers, Biochim. Biophys. Acta 982 (1989) 173–176.
[42] M. Robello, M. Fresia, L. Maga, A. Grasso, S. Ciani, Permeation of divalent cations through α-latrotoxin channels in lipid bilayers: steady-state current-voltage relationships, J. Membr. Biol. 95 (1987) 55–62.
[43] A. Ridi, E. Scalas, M. Robello, A. Gliozzi, Linear response of a fluctuating lipid bilayer, Thin Solid Films 327–329 (1998) 796–799.
[44] E. Scalas, A. Ridi, M. Robello, A. Gliozzi, Flicker noise in bilayer lipid membranes, Europhys. Lett. 43 (1998) 101–105.
[45] A. Ridi, E. Scalas, A. Gliozzi, Noise measurements in bilayer lipid membranes during electroporation, The Eur. Phys. J. E 2 (2000) 161–168.
[46] I. Genco, A. Gliozzi, A. Relini, M. Robello, E. Scalas, Electroporation in symmetric and asymmetric membranes, Biochim. Biophys. Acta 1149 (1993) 10–18.
[47] E. Pescio, A. Ridi, A. Gliozzi, A picoampere current generator for membrane electroporation, Rev. Sci. Instrum. 71 (2000) 1740–1744.
[48] S. Kalinowski, Z. Figaszewski, A new system for bilayer lipid membrane capacitance measurements: method, apparatus and applications, Biochim. Biophys. Acta 1112 (1992) 57–66.
[49] S. Kalinowski, Z. Figaszewski, A four-electrode system for measurement of bilayer lipid membrane capacitance, Meas. Sci. Technol. 6 (1995) 1034–1049.
[50] S. Kalinowski, Z. Figaszewski, A four-electrode potentiostat–galvanostat for studies of bilayer lipid membranes, Meas. Sci. Technol. 6 (1995) 1050–1055.
[51] S. Koronkiewicz, S. Kalinowski, K. Bryl, Programmable chronopotentiometry as a tool for the study of electroporation and resealing of pores in bilayer lipid membranes, Biochim. Biophys. Acta 1561 (2002) 222–229.
[52] M. Kotulska, Natural fluctuations of an electropore show fractional Levy stable motion, Biophys. J. 92 (2007) 2412–2421.
[53] J. Vargas, J.M. Alarcon, E. Rojas, Displacement currents associated with the insertion of Alzheimer Disease amyloid (beta)-peptide into planar bilayer membranes, Biophys. J. 79 (2000) 934–944.
[54] D. Miklavčič, M. Puc, Electroporation, Wiley Encyclopedia of Biomedical Engineering, Wiley, New York, 2006.
[55] A. Maček-Lebar, G.C. Troiano, L. Tung, D. Miklavčič, Inter-pulse interval between rectangular voltage pulses affects electroporation threshold of artificial lipid bilayers, IEEE Trans. Nanobiosci. 1 (2002) 116–120.
[56] V.F. Antonov, V.V. Petrov, A.A. Molnar, D.A. Predvoditelev, A.S. Ivanov, The appearance of single ion channels in unmodified lipid bilayer membrane at the phase transition temperature, Nature 283 (1980) 585–588.
[57] K.C. Melikov, V.A. Frolov, A. Shcherbakov, A.V. Samsonov, Y.A. Chizmadzhev, L.V. Chernomordik, Voltage-induced nonconductive pre-pores and metastable single pores in unmodified planar lipid bilayer, Biophys. J. 80 (2001) 1829–1836.
[58] M. Kotulska, S. Koronkiewicz, S. Kalinowski, Self-similar processes and flicker noise from a fluctuating nanopore in a lipid membrane, Phys. Rev. E 69 (2004) 031920–031930.
[59] S. Koronkiewicz, K. Bryl, Cholesterol-induced variations in fluctuations of the pores in bilayer lipid membrane, Cell. Mol. Biol. Lett. 4 (1999) 567–582.

[60] W. Helfrich, Elastic properties of lipid bilayers—theory and possible experiments, Z. Naturforsch. C J. Biosci. C 28 (1973) 693–703.
[61] R. Kwok, E.A. Evans, Thermoelasticity of large lecithin bilayer vesicles, Biophys. J. 35 (1981) 637–652.
[62] E. Evans, D. Needham, Physical properties of surfactant bilayer membranes: thermal transitions, elasticity, rigidity, cohesion, and colloidal interactions, J. Phys. Chem. 91 (1987) 4219–4228.
[63] B.L.-S. Mui, P.R. Cullis, E.A. Evans, T.D. Madden, Osmotic properties of large unilamellar vesicles prepared by extrusion, Biophys. J. 64 (1993) 443–453.
[64] E. Evans, W. Rawicz, Elasticity of ''fuzzy'' biomembranes, Phys. Rev. Lett. 79 (1997) 2379–2382.
[65] J. Genova, A. Zheliaskova, M.D. Mitov, The influence of sucrose on the elasticity of SOPC lipid membrane studied by the analysis of thermally induced shape fluctuations, Colloids Surf. A—Physicochem. Eng. Asp. 282 (2006) 420–422.
[66] M. Winterhalter, Black lipid membranes, Curr. Opin. Colloid Interface Sci. 5 (2000) 250–255.
[67] T. Hianik, Structure and physical properties of biomembranes and model membranes, Acta Phys. Slovaca 56 (2006) 687–806.
[68] I. Sabotin, A. Maček Lebar, D. Miklavčič, P. Kramar, Measurement protocol for planar lipid bilayer viscoelastic properties, IEEE Trans. Dielectr. Electr. Insul. 16 (2009) 1236–1242.
[69] D.S. Dimitrov, Electric field-induced breakdown of lipid bilayers and cell-membranes—a thin viscoelastic film model, J. Membr. Biol. 78 (1984) 53–60.
[70] A.N. Chanturiya, G. Basanez, U. Schubert, P. Henklein, J.W. Yewdell, J. Zimmerberg, PB1-F2, an influenza A virus-encoded proapoptotic mitochondrial protein, creates variably sized pores in planar lipid membranes, J. Virol. 78 (2004) 6304–6312.