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# A four-electrode potentiostat–galvanostat for studies of bilayer lipid membranes

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**Abstract.** The use of four-electrode systems in the studies of electric phenomena occurring in membranes makes it possible to reduce errors caused by electrode and electrolyte resistance. A four-electrode potentiostat–galvanostat is described in this paper. It allows for electric measurements of membranes in controlled potential and current conditions or measurements of transmembrane potential. The method of selection of the operation mode of the system is described.

## 1. Introduction

Bilayer lipid membranes are the object of study of investigators from several disciplines of science. They are used as biological membrane models [1, 2], allow for studying properties and functions of biological membrane components, e.g. of lipids or proteins and they make it possible to study the effects of biologically active substances on the membranes. Lipid bilayers are also used in constructing biosensors [3, 4].

The invention of a simple method of planar lipid membrane formation [5] made the solutions on its two sides accessible by the insertion of electrodes into the solutions. It became possible to measure the transmembrane potential and to impose on external potential. A number of classical analytical techniques are used in the studies of electric phenomena, e.g. potential measurements, chronoamperometry, voltammetry and pulse techniques.

The vessels in which the membranes are formed consist of two chambers separated by a hydrophobic septum with a hole 0.1–3 mm in diameter [1]; the lipid membrane is formed in this hole. Usually, the volumes of electrolytes contacting the membrane are a few millilitres. In such situations, it is necessary to use small electrodes. The electrodes used in these studies should not undergo polarization, i.e. they should not change their potential with respect to the electrolyte when a current is flowing through the electrode. Silver–silver chloride electrodes are commonly used. A silver wire covered with silver chloride and directly immersed in a KCl or NaCl solution in the measuring vessel has some advantages. The electrode is of small size, low resistance and low noise but its use is limited. It cannot be used if substances which react with electrode materials are present in the solution, e.g. $Br^-$, or $I^-$ ions. The $Ag^+$ ions arising from AgCl dissociation may sometimes react with peptides used in the study. Such limitations are removed if salt bridges are used but can cause an increase in electrode size and resistance.

Usually, two-electrode measuring systems are used in studies of electrical phenomena in membranes. If the membrane resistance is of the same order of magnitude as the resistance of the electrode and the electrolyte, then the membrane potential is significantly lower than the voltage applied to the electrodes. Thus, the measurements are subject to an error. With 1 MΩ membrane resistance and with total electrode and electrolyte solution resistance equal to 100 kΩ for each membrane side, the membrane potential amounts only 83% of the voltage applied to the electrodes—the remaining voltage is lost with the electrode and electrolyte resistances.

It is possible to use a classical three-electrode potentiostat in the studies of electrical phenomena in bilayer lipid membranes but such systems are imperfect for membrane studies: they compensate the potential drop on the electrode and electrolyte resistance on one side of the membrane only. The error in the membrane potential is half that of the two-electrode system. In the above cited example, the real potential applied to the membrane is then 91% of the potential applied to the electrodes.

It is advantageous to carry out measurements using a four-electrode system (figure 1) if high-impedance electrodes are used. Two current electrodes, CE1 and CE2, and two control electrodes, RE1 and RE2, are used in the system. A current flow is forced between

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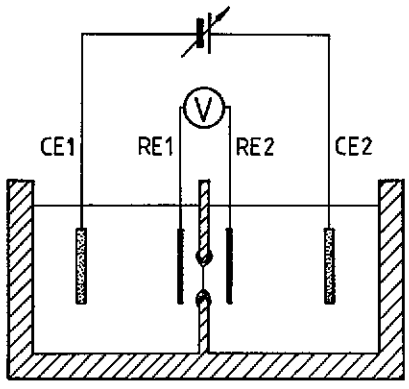


**Figure 1.** The vessel and electrodes for studies of the electrical properties of lipid membranes in the four-electrode system: CE1, CE2, current electrodes, RE1, RE2, control electrodes.

the current electrodes. The control electrodes are used for measuring the potential difference between the electrolytes. If a measuring system of sufficiently high internal resistance is used then the potential drops caused by electrode resistance are negligible. The difference between the control electrode potentials is equal to the potential difference between the corresponding points of the solution. If the electrodes are situated close to the membrane and the solution conductivity is high, then the potential between the control electrodes is equal to the transmembrane potential.

Four-electrode systems have been known for a long time. They have been used, for example, in electrophoresis [6], in coulometric titration [7] and in studies of interfaces between immiscible electrolyte solutions [8–10]. Four-electrode measuring systems were also used in the studies of lipid membranes [1, 11]. The development of microelectronics has made it possible to construct simple four-electrode potentiostats [8, 9].

## 2. Structure and characteristics of the system

### 2.1. Description of the potentiostat–galvanostat

Our four-electrode potentiostat–galvanostat is based on six operational amplifiers. The operation mode depends on the way the negative feedback is realized. Either the determined voltage is maintained between the RE1 and RE2 electrodes (the potentiostat) or a determined current is forced through the CE1 and CE2 electrodes (the galvanostat).

**2.1.1. Potentiostat.** The connections of the system making the potentiostat are presented in figure 2(*a*). The OA1 operational amplifier and the resistor $R_I$ make a current source which forces the current through the CE1 and CE2 electrodes. It is controlled by the OA5 amplifier, the main amplifier of the potentiostat. The control signal $U_{inp}$ is fed to the non-inverting input and the signal from the difference amplifier based on OA2-OA4 operational amplifiers is fed to the inverting input.

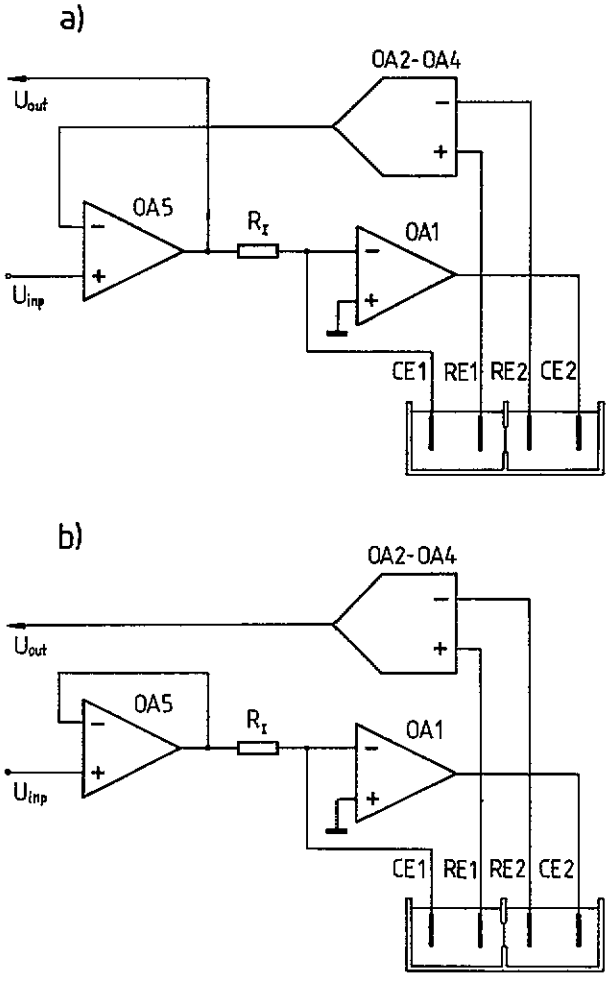


**Figure 2.** Connections of the elements in the working modes: (*a*) the potentiostat and (*b*) the galvanostat.

In this way, a negative feedback is created making the tendency of the system to equalization of the operational amplifier OA5 input voltages. For this reason, the potential difference of the electrodes RE1 and RE2 is equal to the input voltage, $U_{inp}$. The current flowing through the current electrodes is equal to that of the resistor $R_I$. The potential of the resistor lead connected with the inverting input of the OA1 operational amplifier is equal to the ground. The output voltage $U_{out}$ depends on the resistance $R_I$ and on the intensity of the current flowing through the resistor and the current electrodes:

$$U_{out} = i \cdot R_I. \tag{1}$$

**2.1.2. Galvanostat.** The configuration in which the system works as a galvanostat is simpler than the potentiostat (figure 2(*b*)). The current source is based on the OA1 operational amplifier; it forces the current flow through the CE1 and CE2 electrodes:

$$i = \frac{U_{inp}}{R_I}. \tag{2}$$

The difference amplifier yields a voltage equal to the RE1 and RE2 electrode potential difference in the output ($U_{out}$).

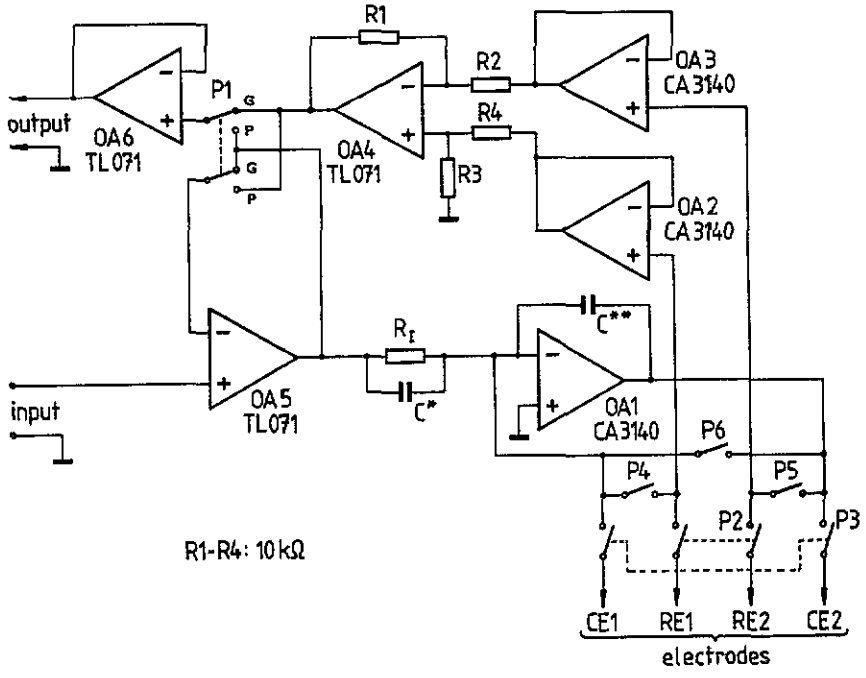


**Figure 3.** Scheme of the four-electrode potentiostat–galvanostat.

**2.1.3. Potentiostat–galvanostat.** The complete diagram of the instrument is shown in figure 3. The operation mode of the instrument can be selected with switches P1–P6:

P1 (P, the potentiostat, G, the galvanostat). It changes the kind of feedback of the OA5 amplifier and switches the site from which the signal is fed to the instrument output.

P2 connects the control electrodes RE1 and RE2.

P3 connects the current electrodes CE1 and CE2.

P4 connects the inputs CE1 and RE1, the two- or three- electrode operation mode.

P5 connects the inputs CE2 and RE2, the two-electrode operation mode.

P6 connects the current source outputs. It is used in the galvanostat operation mode. If the electrodes are disconnected then the current flows through the switch. The feedback circuit is not disconnected and overload is avoided. The initial potential of the membrane is zero when the electrodes are connected to switch P6.

## 2.2. Working mode selection

Bilayer lipid membranes are very voltage sensitive; their breakdown voltage may range from 80 to 500 mV or more, depending on the membrane and electrolyte composition [1]. Connecting the electrodes and switching the operation mode must not result in even short-duration voltage pulses which can destroy the membrane. Accidental pulses across the membrane are avoided by selecting switches P1–P6 in the correct way and in the correct sequence.

(a) The potentiostat–galvanostat working mode selection (P6) should be made with disconnected electrodes (P1, P2—OFF),

(b) Potentiostat. The two-electrode working mode (P3, P4—ON) should be set before connecting the electrodes; thereafter, the desired three- or four-electrode system can be set. Thus, the situation is avoided where the negative feedback loop is inoperative and the amplifiers are overloaded.

(c) Galvanostat. The current source (OA1) should be shorted (P5—ON) and the P5 switch can be opened only after the electrodes are connected. In this way, a pulse due to equilibration of the OA1 amplifier is avoided.

If the potentiostat-galvanostat works with less than four electrodes then the inputs have the following functions:

RE1, working electrode (two- or three-electrode system);

RE2, reference electrode (two- or three-electrode system);

CE2, auxiliary electrode (three-electrode system).

The current ranges are varied by selecting the resistance $R_I$. Changes in $R_I$ are accompanied by changes in capacitances $C^*$ and $C^{**}$ which prevent oscillations of the system. The current ranges and the corresponding $R_I$ values are presented in table 1.

## 2.3. Selection of the electronic parts

The CA3140 operational amplifiers (Harris) made with BiMOS technology and low-noise TL071 amplifiers (SGS-Thomson) made with BiFET technology were used in the system. The amplifiers have a high limiting frequency which is necessary due to the potentiostat working mode (four amplifiers, OA1, OA2/3, OA4, OA5 within a feedback loop). The CA3140 amplifiers were applied to the measuring of electrode potential (OA2, OA3) because of low input bias and offset currents.

**Table 1.** Resistance $R_f$ and approximate $C^*$ and $C^{**}$ capacitance values for respective current ranges.

| Current range | Resistance $R_f$ | Capacitance $C^*$ | Capacitance $C^{**}$ |
|---|---|---|---|
| 10 nA | 100 MΩ | — | 10 pF |
| 100 nA | 10 MΩ | — | 18 pF |
| 1 μA | 1 MΩ | — | 33 pF |
| 10 μA | 100 kΩ | 33 pF | 100 pF |
| 100 μA | 10 kΩ | 47 pF | 470 pF |
| 1 mA | 1 kΩ | 560 pF | 10 nF |

The input currents of the OA1 amplifier constituting the current source should also be as low as possible. The low-noise TL071 amplifiers were used wherever the input current values were unimportant. Other amplifiers were also tested in the system: OA1-ICL7650 (Intersil), 3527 (Burr Brown), OA2, OA3-CA3130 (Harris). The OA6 operational amplifier can be of any type. The potentiostat–galvanostat was supplied with a ±5 V voltage. The input currents of the operational amplifiers were lower at low feed voltage.

### 2.4. Frequency compensation

The $C^*$ and $C^{**}$ capacitors which prevent system oscillations should be adjusted to each current range of the described galvanostat–potentiostat (Table 1). The capacitance values of the capacitors are approximate as they are individually selected for each instrument. The electrodes should be connected to the instrument with shielded wires of as low as possible inner capacitance.

### 2.5. Digital control

The potentiostat–galvanostat described here was used as part of a measuring system for investigation of bilayer lipid membranes. The system works under the control of PC/AT/386/486 microcomputers. The digital control enables simple switching of the potentiostat–galvanostat functions.

A block diagram of the measuring module comprising the described potentiostat–galvanostat is presented in figure 4. The current range and working modes are controlled by digital systems. Miniature V23042-A relays (Siemens) supplied with 12 V voltage are used as the switches P1–P6 and range switches. P1–P6 are operated directly by the SN7416 (Texas Instruments) gate outputs and the current range relays by SN74145 (Texas Instruments) demultiplexer outputs.

The module has a galvanostat–potentiostat overload control relying on two window comparators which control the output voltages of the amplifiers OA4 and OA5. Voltages exceeding those admissible are signalled optically (LED) and they are controlled by a computer.

The potentiostat–galvanostat, together with the relays and the digital control part, is accomodated on a printed board of Eurocard format (10 cm × 16 cm).

A block diagram of the measuring system is shown in figure 5. The measuring modules, the voltage supply and

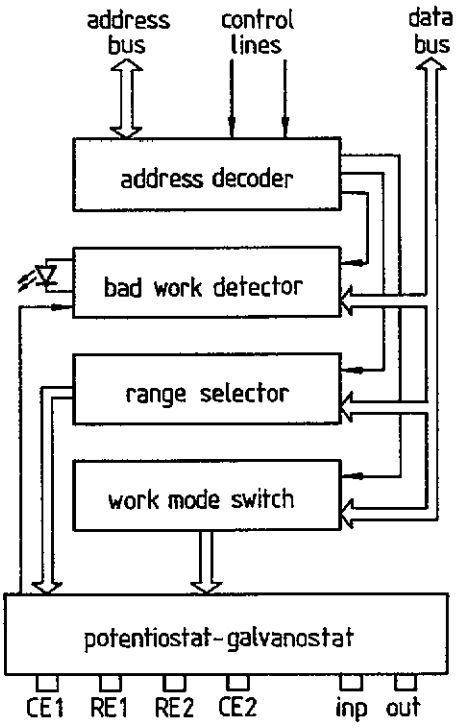


**Figure 4.** Block diagram of the measuring module containing a digitally controlled potentiostat–galvanostat.

the interface are located in the cassette. The system bus of the cassette is a continuation of the PC/AT/386/486 microcomputer bus. The 16-bit data bus, 8-bit address bus, and selected control lines are led to the cassette. The potentiostat–galvanostat module cooperates with the digital-to-analogue and analogue-to-digital converters. The digital-to-analogue converter is the source of the signal fed to the input ($U_{inp}$) of the potentiostat–galvanostat. The current or the potential is measured with the analogue-to-digital converter connected to the potentiostat–galvanostat output.

## 3. Application of the potentiostat–galvanostat

### 3.1. Effect of gramicidin on the conductance of lipid membranes

Gramicidin is a peptide widely used in the studies of transport through bilayer lipid membranes [2, 12]. Gramicidin incorporates itself in the membrane; it appears in the form of dimers connected by N-ends. Its chain is coiled and the coil interior forms a channel which selectively transports monovalent cations.

The chronoamperometric curve of a membrane in the presence of gramicidin is presented in figure 6. The membrane had been formed in the absence of gramicidin and after membrane formation (its capacitance having been recorded), gramicidin solution was added to both cells. The solutions in the cells had been stirred for a short time and then the membrane current was recorded. An increase in current was due to gramicidin incorporation into the membrane and ion channel formation. Stabilization of the current after a

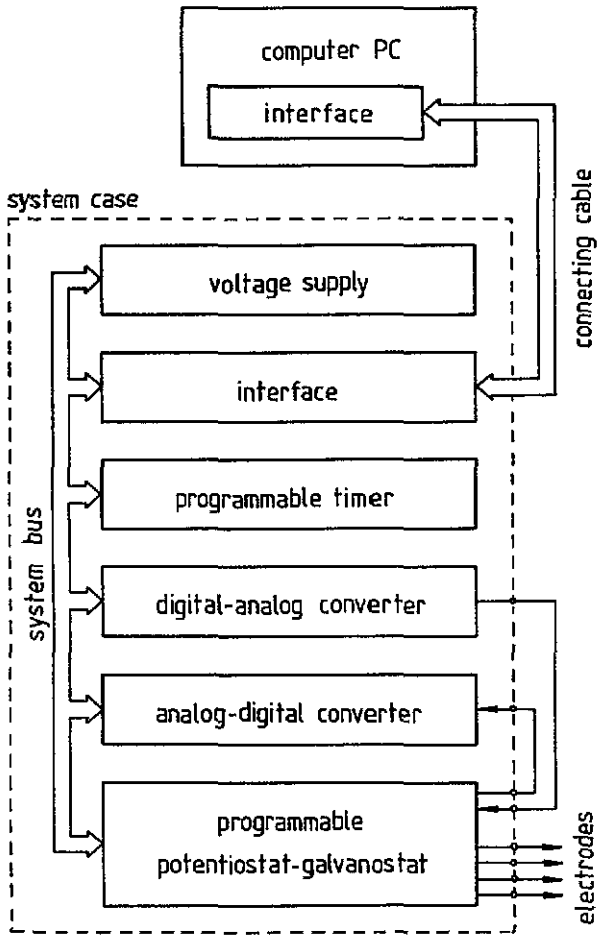


**Figure 5.** Block diagram of the PC microcomputer-controlled measuring system.

period of time is due to equilibrium of the gramicidin distribution between the electrolyte solution and the membrane.

### 3.2. Chronopotentiometric studies of pore formation in bilayer lipid membranes

A strong electric field causes pore formation in cell membranes and in artificial lipid membranes [13, 14]. The phenomenon has been practically applied in genetic engineering to introduce macromolecules into cells and it has also been used to cause cell fusions. A high voltage should be applied to the electrodes inserted in the solution containing the cells. Short-duration pulses are usually used to cause a reversible membrane breakdown; the pores thus formed close when the voltage is removed.

Artificial planar lipid membranes undergo reversible perforation at 150–500 mV voltage applied when the voltage pulse duration is of the order of microseconds to milliseconds [13].

The phenomenon of opening and closing pores in bilayer lipid membranes can be observed in voltammetric curves (figure 7). Short-duration current jumps appear at voltages close to breakdown. In constant current conditions, it is possible to attain a state where the formed pores do not close and the membrane is not destroyed (figure 8). The current flowing in the initial stage causes a voltage increase in the membrane. The

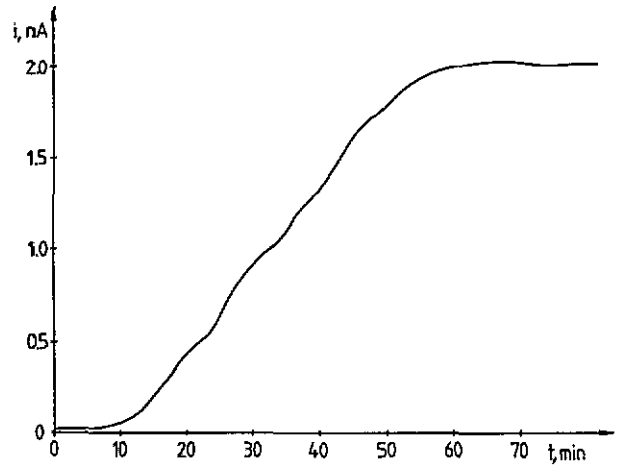


**Figure 6.** Current flowing through the bilayer lipid membrane against time after gramicidin addition. Potential, 50 mV; electrolyte, 0.1 M KCl, 32 nM gramicidin; forming solution, lecithin in decane, 20 mg $ml^{-1}$. The membrane was formed by the Mueller–Rudin method in a hole 1.1 mm in diameter.

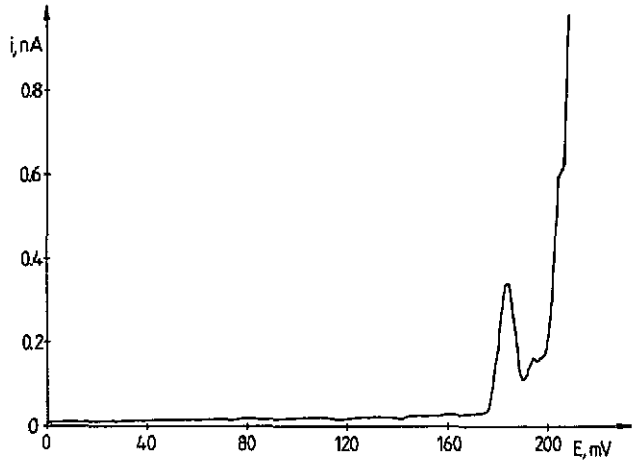


**Figure 7.** Voltammetric curve of a bilayer lipid membrane. Potential sweep speed, 10 mV $s^{-1}$; electrolyte, 0.1 M KCl; forming solution, lecithin in decane, 20 mg $ml^{-1}$. The membrane was formed by the Mueller–Rudin method in a hole 1.1 mm in diameter.

voltage increase rate depends on the current intensity, the membrane capacitance and the membrane resistance. The membrane voltage increases to a value at which a pore is formed. The membrane conductance rapidly increases, causing a drop of the membrane voltage. The force which has caused pore formation is decreased and provokes, in turn, pore opening. Hence voltage oscillations are observed in the chronopotentiometric curve after membrane perforation.

## 4. Summary

The potentiostat–galvanostat described has been in use for about two years for studies of lipid membranes and in cooperation with the membrane capacitance measurement module [15]. The apparatus is controlled by software working in the Windows 3.1 (Microsoft)

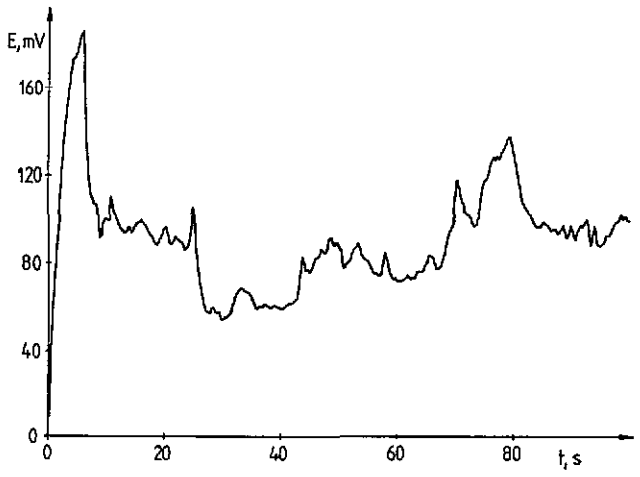


**Figure 8.** The chronopotentiometric curve of a bilayer lipid membrane. Current, 0.25 nA; electrolyte, 0.1 M KCl; forming solution, lecithin in decane, 20 mg $ml^{-1}$. The membrane was formed by the Mueller–Rudin method in a hole 1.1 mm in diameter. Membrane capacitance was recorded during formation of the membrane. After the lipid bilayer formation, current was applied to the membrane.

environment making it a convenient tool for the study of membrane phenomena.

Application of chronopotentiometry to the studies of membrane phenomena can yield much useful information, e.g. of breakdown voltage, membrane capacitance at various potentials, pore conductance, and oscillation frequency. Some of these parameters are affected by many biologically active substances. It is sometimes necessary to apply chronopotentiometry in studies related to chemical sensor construction [16].

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