Supported Phospholipid Membranes Formation at a Gel Electrode and Transport of Divalent Cations across them

Tomáš Navrátil[*]*, Ivana Šestáková, Vladimír Mareček*

J. Heyrovský Institute of Physical Chemistry of AS CR, v.v.i., Dolejškova 3, 182 23 Prague, Czech Republic
[*]E-mail: navratil@jh-inst.cas.cz
Dedicated to the memory of Prof. RNDr. PhMr. Robert Kalvoda, DrSc.

This contribution deals with study and characterization of transporting processes of lead and cadmium ions across supported artificial phospholipid bilayers (SPLBs), which were formed on the hydrophilic surface of gel-type (agar or agarose) electrode. For the formation of these SPLBs two types of phospholipids (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and the mixture of phospholipids obtained from soybeans (under commercial name Asolectin)) were used. Discussed SPLBs and the transporting processes of selected ions have been characterized using two electrochemical methods: electrochemical impedance spectrometry (EIS) and voltammetry. The impact of some parameters on these systems and processes was investigated.

1. INTRODUCTION

A number of metals, e.g., zinc, iron, copper, are essential micronutrients required for variety of physiological processes, but they can be easily toxic when present in excess as well as their insufficiency can be very dangerous. Therefore, in all organisms, a metal homeostasis network is functioning to adjust fluctuations in micronutrient availability. However, some metals – such as cadmium and lead - have no apparent natural role in organisms and are toxic almost at all concentrations. These metals are prevalent environmental pollutants in industrial countries and apart from acute exposure in the working environment (inhalation of dusts and fumes, occasionally oral intake), the main entry pathway into human as well as animal food chain is plants uptake [1].

To start their role in plants or in human body, the elements, the compounds and the other species which are present in polluted environment, must be transported into these organisms, more

precisely, into their cells. In other words, each particle, which takes part further in metabolic processes, must be transported across the cell membranes [2, 3]. Similar processes are realized in the opposite way – out of the cells as well as into and out of any sub cellular structure. Detail elucidation of membrane transport mechanisms plays a key role and is prerequisite for understanding the distribution of pollutants in real cells of more complex organisms (leaves, roots or the whole plants, animals or men) and for their possible control in the future [4-6]. The biological membrane exists as a surface, at which the hydrophobic parts of phospholipids (PLs) are protected from water, while the hydrophilic ones are in contact with the aqueous medium. Only the ends or edges of the bilayer surface are exposed to unfavorable conditions, however, even these exposed regions can be eliminated by bending them underneath the surface whereby a closed edgeless structure is formed. The closed bilayer is impermeable for most of water soluble molecules, as they would be insoluble in the hydrophobic bilayer core [7].

There are many different principles, on which the molecules, ions, or particles are transported across the PL bilayers (PLB) [8-10]. Gases like oxygen, CO_2 and nitrogen – small molecules hardly interacting with solvents – diffuse easily across the hydrophobic part of the membrane. Lipid molecules, e.g., steroidal hormones, permeate the bilayer easily [10]. Compounds insoluble in fats are transported across amphipathic proteins and can be dipped into equally oriented lipid bilayer. The proteins form channels for ions and small molecules and serve for transport of bigger molecules [11], which would not be otherwise able to pass across the bilayer [2]. The PL membrane acts as a solvent for membrane proteins. They create a suitable medium, in which the proteins can be active [10]. Most of the membrane proteins are integral membrane components (they interact with PLs [10]) and can pass through an entire bilayer of 5-10 nm thickness. Transport can be passive or supplied by some external energy [10]. There are some other principles of transport, we can mention endocytosis and exocytosis (e.g., in cases of larger objects and particles, such as bacteria, viruses), electroporation (DNA) etc. [2, 3, 10].

By means of appropriate techniques, it is possible to prepare an artificial membrane system, which can serve as a model of the real cell (e.g. [2, 3, 5, 8, 12-15]). There are a few various techniques, which have been used for these purposes. It is possible to mention the multilamellar (0.2-50 μm) or small unilamellar (20-50 nm) vesicles. Beside the unsupported BLM [16], the supported PLB on various supporting materials are frequently investigated. Metallic mercury is less suitable for deposition of SPLBs due to is poor mechanical stability and its hydrophobicity. These disadvantages were eliminated by introduction of amalgam electrodes. The problem of hydrophobicity was solved by anchoring SPLB via a sulfur atom, and therefore it is possible to conclude that solid amalgam electrodes covered by a mercury meniscus or by a mercury film (m-AgSAE, MF-AgSAE, m-CuSAE, m-BiAgSAE and m-CdSAE) are convenient for obtaining thiolipid films [17]. Applied thiolipids consist of a hydrophilic chain anchored on the electrode surface at the one end via a sulfhydryl or a disulfide group, and covalently bound at the opposite end to the polar head of a PL molecule [17-19] (hydrophilic spacers (polyethyleneoxy [20] or peptide chains [21]) have been used frequently for such purposes too). Such films can be "completed" with complementary PL layer and such SPLB on the amalgam surface can serve for as model SPLB. The use of amalgam substrate seems to be especially advantageous, because many metals form amalgams with mercury. Hence the investigated metal ions

(Cd^{2+}, Pb^{2+}, Cu^{2+}, etc.) transported across the formed SPLB via an ion channel or by another transport mechanism, can yield a detectable voltammetric signal on such amalgam electrode, which simultaneously can be functional as the membrane support [5, 22]. Other possibility consists in formation of the SPLB in direct pores of a (e.g. polycarbonate) membrane [2, 5, 8, 22-24].

A new promising approach for the preparation of PL membranes seems to be the application of sol–gel technology [25], i.e., formation of SPLBs at the agar electrode (e.g., [13, 14, 23, 26]) . It is a very interesting and powerful modification of the classical sol–gel method (known from liquid – liquid interface, which is formed between two immiscible electrolyte solutions [27]). Introduction of the polymer gel electrode [28] has stimulated progress in electroanalysis at liquid|liquid interfaces. The gel can be prepared by mixing a suitable polymer (PVC, agar) with one of the electrolyte solutions at elevated temperature. After the mixture is cooled to room temperature in a suitable mould, the gel electrode is formed. According to our experience, it provides a variety of shapes, is easy to handle and can be easily renovated.

Various techniques have been applied to the study of the membrane formation and of the transporting processes, e.g., fluorescence microscopy [29], fluorescence lifetime correlation spectroscopy combined with lifetime tuning [30], combinations of fluorescence spectroscopy with *ab initio* calculations [31], solvent relaxation technique [32], or confocal fluorescence correlation spectroscopy [33].

In this paper are described the experiments realized with the substrate formed from agar or agarose. Agar consists of a mixture of agarose and agaropectin. Agarose is a linear polymer, made up of repeating monomeric units of agarobiose [34].

Electrochemical impedance spectroscopy (EIS) and voltammetric techniques have been frequently applied for characterization and modeling of electrochemical properties of PLBs and black lipid membranes (BLM) [35], similarly as of many other systems (e.g., [2, 8, 36]). Even today, it often provides the only non-invasive method for detailed structural-functional studies of these systems ([37] and the references therein). These techniques can help us to characterize the state and the changes of investigated SPLBs (e.g., steady state, changes after addition of the transported compound, destruction of the membrane).

The results of EIS are used for construction of equivalent electrical circuits (EEC). During the realization of the previously solved projects we suggested a few different EEC, which seem to be suitable for modeling of SPLBs. They are different for SPLBs formed on agar electrode, on amalgam electrode and on porous substrate [5, 8, 23]. The different components (capacitances, resistances etc.) can be added for better characterization of different investigated membranes and transporting processes. Agar substrate working electrode was characterized using EIS in [26] (applied frequency and voltage dependencies). This paper is more focused on the time stability of such electrodes and SPLBs, time changes of different parts of EECs and on the transport of hazardous ions species across the SPLBs.

2. EXPERIMENTAL

2.1 Preparation of Gel Electrode

The PLBs were formed by self-assembling at the surface of the agar or agarose electrode, respectively. Agar or agarose (both purchased from Sigma-Aldrich, Prague, Czech Republic) were warmed up on a water bath, at concentration 0.3 g in 15 ml of 3 M KCl. The Tygon tube (inner diameter 1.0 mm, outer diameter 2.0 mm) was immersed into the liquid gel. By shaking the tube, gel material rose up and an approximately 5 cm high compact column was formed. The silver/silver chloride wire (silver wire, diameter 0.4 mm, electroplated with silver chloride) was introduced into this column to represent the electrical contact. After cooling to room temperature, the preparation of the gel electrode was finished.

2.2 Preparation of Supported Phospholipid Bilayers

The end of the column (about 2-3 mm) was cut by a very sharp scalpel and such freshly prepared surface was immediately immersed into the solution of phospholipids in n-heptane (20 mg.mL^{-1}) or into the mixture of phospholipid with the selected ionophore, respectively. Before exposing to aqueous 0.1 M KCl, the column electrode was inserted into a plastic tube of 1 cm diameter of the same length as the Tygon tube (to protect the electrode surface from contact with other parts of the electrolytic cell).

To investigate the transport processes, the ionophore Calcimycin (Calcium Ionophore, Antibiotic A23187) was used (purchased from Sigma-Aldrich, > 98% (TLC) (**Figure 1**). The ionophore was added to the solution of the phospholipids before their application to the support. It has been proved as very suitable for transport of divalent cations (Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}). Moreover, it also had been described as a cadmium ionophore [8, 38, 39] and several stability constants have been determined for its 1:1 complexes with Ni^{2+}, Fe^{2+}, Zn^{2+} and some other ions [39].
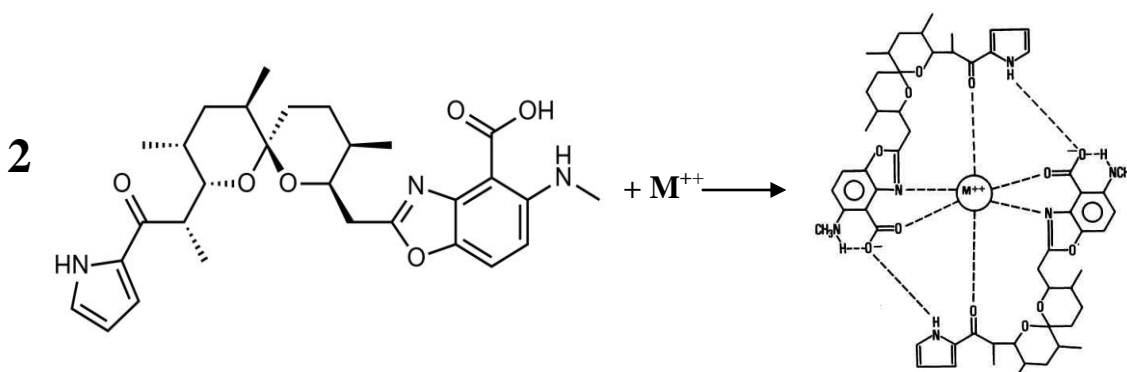
Figure 1. Structures of the A23187 ionophore (Calcimycin) and the way of its complexing with divalent cations (according to ref. [38]).

2.3 Apparatus

For quantification of the electrochemical impedances, the reference silver/silver chloride electrode (silver wire, diameter 1 mm, electroplated with silver chloride) was used. Platinum wire, diameter 1 mm, served as the auxiliary electrode. The measurements were realized using a CHI 650C Electrochemical Analyzer/Workstation, software CHI v. 8.1 (IJ Cambrija Scientific, Carms, UK).

2.4 Reagents and Materials

The 0.1 M KCl base electrolyte solutions were prepared from KCl Suprapur, purchased from Merck, Prague, Czech Republic. The p.a. solvents were obtained from Penta-Švec, Prague, Czech Republic. All the other chemicals used were of analytical grade. For all the measurements, deionized water from Milli-Q-Gradient, Millipore, Prague, Czech Republic (conductivity < 0.05 µS.cm^{-1}) was used. The AAS standard solution of Cd^{2+} and Pb^{2+} (1000 mg.L^{-1} in 2% HNO_3) were purchased from Analytica, Prague, Czech Republic.

Two types of phospholipids were used for the preparation of SPLBs: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (lecithin, DPPC, GPCho (16:0/16:0)) (Avanti Polar Lipids, Alabaster, USA) and Asolectin from soya beans (Sigma-Aldrich, Prague, Czech Republic) (a mixture, which comprises roughly equal proportions of lecithin (25 %), cephalin (phosphatidylethanolamine) and phosphatidylinositol along with minor amounts of other phospholipids and polar lipids; about 24 % saturated fatty acids, 14 % mono-unsaturated and 62 % poly-unsaturated fatty acids).

2.5 Electric equivalent circuits

Several types of electrical equivalent circuits (EEC) were tested and utilized for characterization of the formed SPLBs bilayers on the polymer surface and of the corresponding transporting processes.

The two simplest EECs (Figures 2A and 2B), composed of one resistor (R_s) in serial combination with parallel combination of a resistor (R_p) and a capacitance (C_p), were applicable for characterization of the free polycarbonate membranes [2, 8] and gel electrodes without covering by phospholipids [26].

The two EECs depicted in Figures 2C and 2D proved to be applicable for characterization of SPLBs formed in the pores of polycarbonate membranes [2, 8], of metal electroplated surfaces and of the surface of polymer electrode too. These circuits are similar to the simpler ones, but additionally, a parallel combination of one capacitor and one resistor was added to the first capacitor (series-connected) [2]. As well as in the case of the first two circuits, they differ mutually by a capacitance (C_s) included serially to the first resistor (R_1).

The last two EECs (depicted in Figures 2E and 2F) were used by us to characterize the SPLBs formed on the surface of polymer electrodes. These are composed of one resistor (R_s) in serial combination with two parallel combinations of resistors (R_{p1}, R_{p2}) and capacitances (C_{p1}, C_{p2}).

According to [26], the circuit 2F seems to be suitable for characterization of the SPLBs prepared in this way.

The pairs of EECs 2A and 2B; 2C and 2D, and 2E and 2F differ mutually by a capacitance (C_s) included serially to the first resistor.

Each member (resistor, capacitance) of the circuits can be used for characterization of the system. Serial resistors (R_s) correspond to the resistance of the electrolyte, connectors, etc. Similarly, the serial capacitances (C_s) (Figures 2B, 2D, 2F) represent the capacitances of these parts of the tested cells. Parallel capacitors of the circuits (denoted as C_p, C_1, and C_{p1}) correspond to the parasitic capacitance of the membrane or of the agar electrode; parallel resistors to its resistance (R_p, R_2, and R_{p1}). The parallel combination of capacitances (denoted C_2) and resistors (denoted R_3) in the second pair of the circuits describe the electrical properties of the SPLBs formed in the pores of the supporting membranes (including ionophores and transport of metal cations) [2, 22, 24]. Similarly, the parallel combinations of the resistors and capacitances (R_{p2}, and C_{p2}) in the third pair of the EECs characterize the properties of SPLBs formed on the surface of the polymer electrodes.
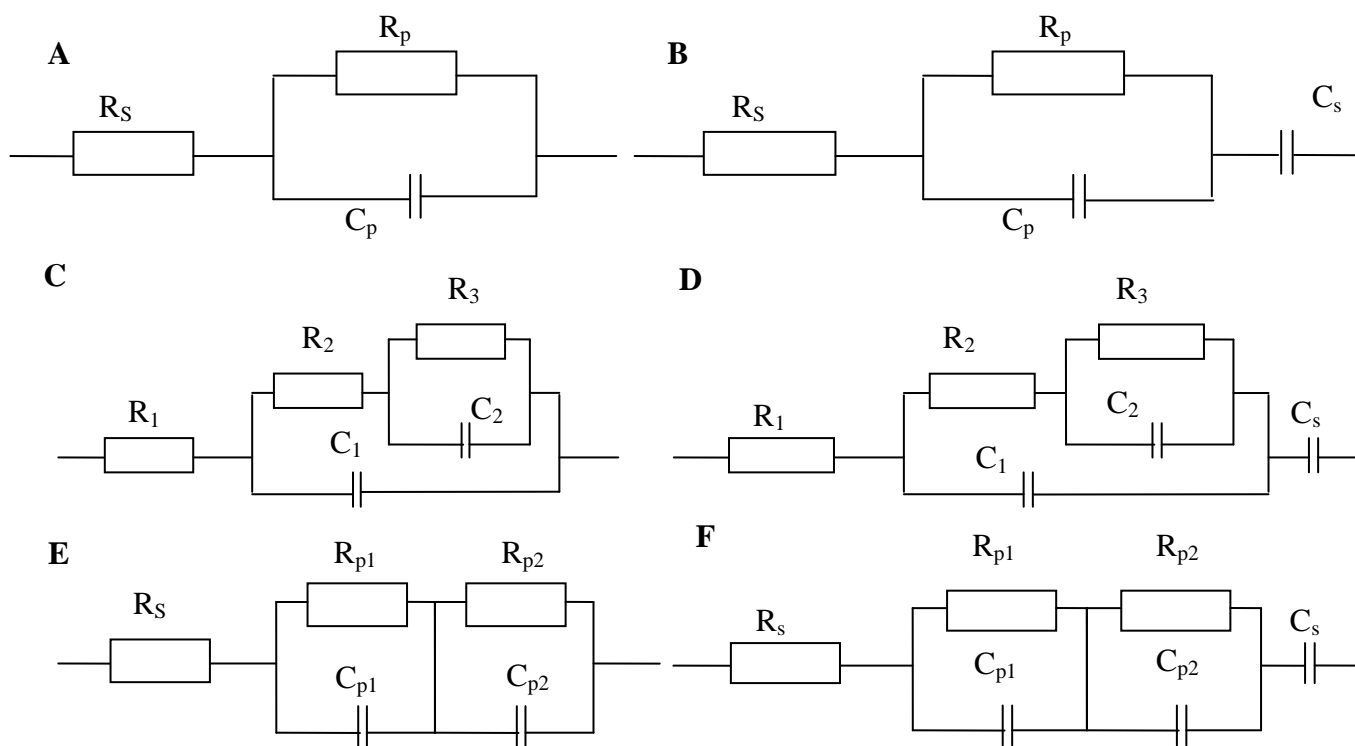
Figure 2. Electrical equivalent circuits used for characterization of SPLBs formed on polymer surfaces

3. RESULTS AND DISCUSSION

3.1 Characterization of s-BLP on gel support

Electrode with agar supported PLB was recommended for long term investigation [40]. In our EIS measurement (the dependence of the imaginary part (Z'') on the real part (Z') of impedance

recorded in 0.1 M KCl) the system provided satisfactory results in the frequency range of 0.1 – 1000 Hz, amplitude 0.005 V. Because we wanted to investigate the transporting processes under conditions very similar to those which are common in the real biological systems, the voltage -0.1 V has been used in all ESI-measurements described in this paper (this value is relatively close to the plant membrane potential). On the other hand, a shift to negative bias voltages could lead to a significant change of membrane resistance, possibly due to the increasing number of pores or defective structures in the lipid bilayer [26].

For the possibility of long term investigation, the length of agar column (i.e., agar layer between the end of contacting wire and the electrode surface) appeared to be crucial, and according to our experiments 1.5 – 2.0 cm long agar columns could be recommended.

As is depicted in Figure 3, for agar column length of about 1 cm, with prolonging time of experiment, the recorded curves are disturbed on the left shoulder, where a new hemi-circle rises up. This disturbance is probably caused by diffusion of ions from the contacting wire into the agar layer and the surface. After cutting the agar column to about 0.5 cm, similar time-impedance was recorded, but the disturbances on the left shoulder were recorded in shorter times. Generally, the shorter was the agar layer, the shorter was time in which this disturbance could be observed (in Figure 2 in about 1 hour).

Therefore, the electrodes with constant distances between the end of the electrical contact and the gel surface were fabricated. In order to avoid the possibility of contamination from previously realized experiments, new Tygon tube was used for fabrication of the gel electrode for each experiment.
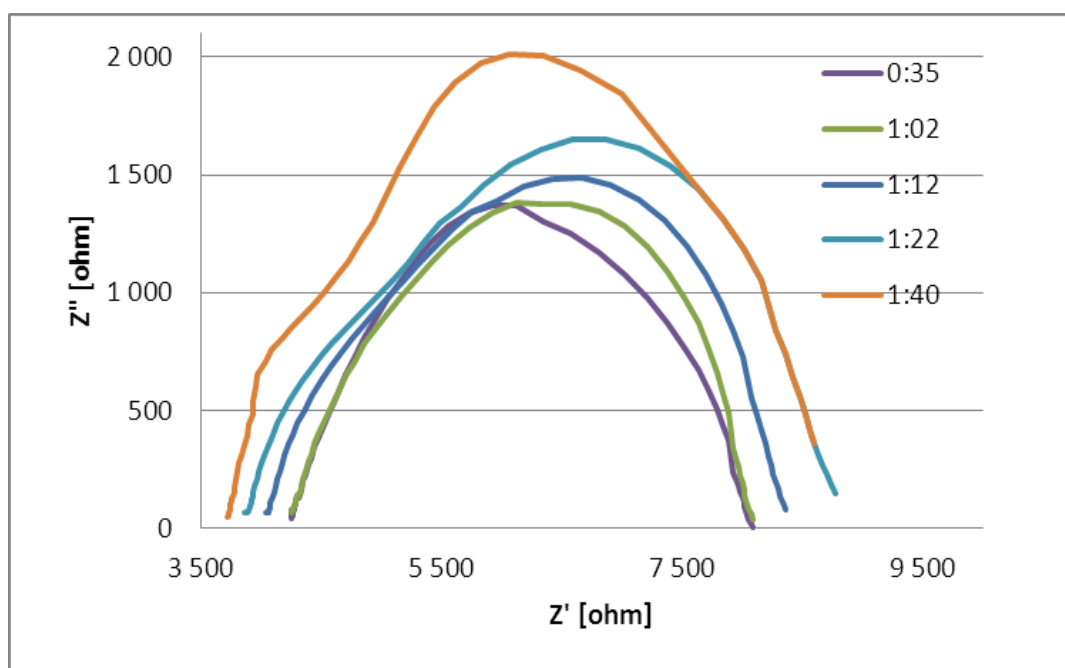

Figure 3. Time dependence [h:mm] of the imaginary part (Z'') on the real part (Z') of impedance over a frequency range of 0.1 – 1000 Hz for the agar layer formed in the Tygon tube (amplitude 0.005 V, E=-0.1 V) recorded in 0.1 M KCl.

In case of gel electrode without s-PLB the simplest circuits, depicted in Figures 2A and 2B were used for construction of the EECs. The capacitor R_s corresponded to the resistance of the electrolyte, connectors, wires, etc. Its value was proportional to the length of the gel column in the electrode and it was practically independent of the material used (agar or agarose). Similarly, the serial capacitances (C_s) (Figures 2B) represented the capacitances of these parts of the tested cells. The values of R_s were constant or they changed linearly in time. On the other hand the registered changes of the mentioned capacitance C_s have been time independent and could not be used for characterization of the system.

Parallel capacitor of the circuits (denoted as C_p) corresponded to the parasitic capacitance of the agar surface and the parallel resistor (R_p) to its resistance.

The scheme of the electrochemical cell, used in our experiments, is depicted in Figure 4. Because Tygon tube exhibit very plastic properties under laboratory temperature, it is very complicated to fix it in a constant position in an electrochemical cell. Therefore, for better protection of the SPLB formed on the gel surface from mechanical contact with other electrodes and other parts of the cell, the Tygon tube was inserted into a polyethylene tube of about 1 cm (inner diameter). The bottom end of this tube could be covered by a frit, but the transported ions could be adsorbed on its surface and its cleaning could be very complicated. Therefore, it is recommended to leave the bottom open.
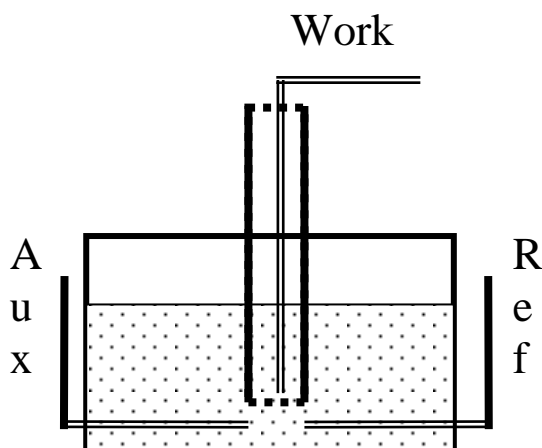

Figure 4. Electrochemical cell with column gel electrode. Work – gel electrode covered by phospholipid bilayer, Aux – auxiliary electrode (platinum wire), Ref – reference electrode (silver wire covered by silver chloride).

The studied SPLBs have been formed on the surface of the gel column in correspondence with the procedure described in chapter "Experiment" of this paper. Their stabilities have been characterized using EECs too. We tested all four variants of ECCs depicted in Figures 2C, 2D, 2E, and 2F, because in the literature it is possible to find attempts to characterize such systems for the mentioned purposes (e.g., [26]).

As it is possible to see from Figure 5, the resistances, evaluated according to the EECs 2E were constant (R_s, R_{p2}) almost "immediately" (i.e., in about 10 minutes) after immersing of the electrode into the testing solution of 0.1 M KCl under potential of -0.1 V. The other resistance R_{p1} was slightly increasing in time, but the slope of the time dependence was very low. Similar results were observed in case of evaluation of the resistances according to the EECs depicted in Figures 2C-D, and 2E-F. These trends have not even been disturbed by the addition of metallic ions Pb^{2+} and Cd^{2+} to the supporting electrolyte of 0.1 M KCl (see Figure 5). After removing of the lecithin bilayer, generally a SPLB, the R_s resistance decreased and the "parallel" resistance increased. It is necessary to take into account the fact that the resistance of the absolute value of the resistors is strongly affected by the presence of accidentally formed pores, across which the conducting ions could be transported. Therefore, it can be concluded that the capacitances incorporated in the different circuits are much more suitable for characterization of the electrodes and of transport of metallic cations, respectively.
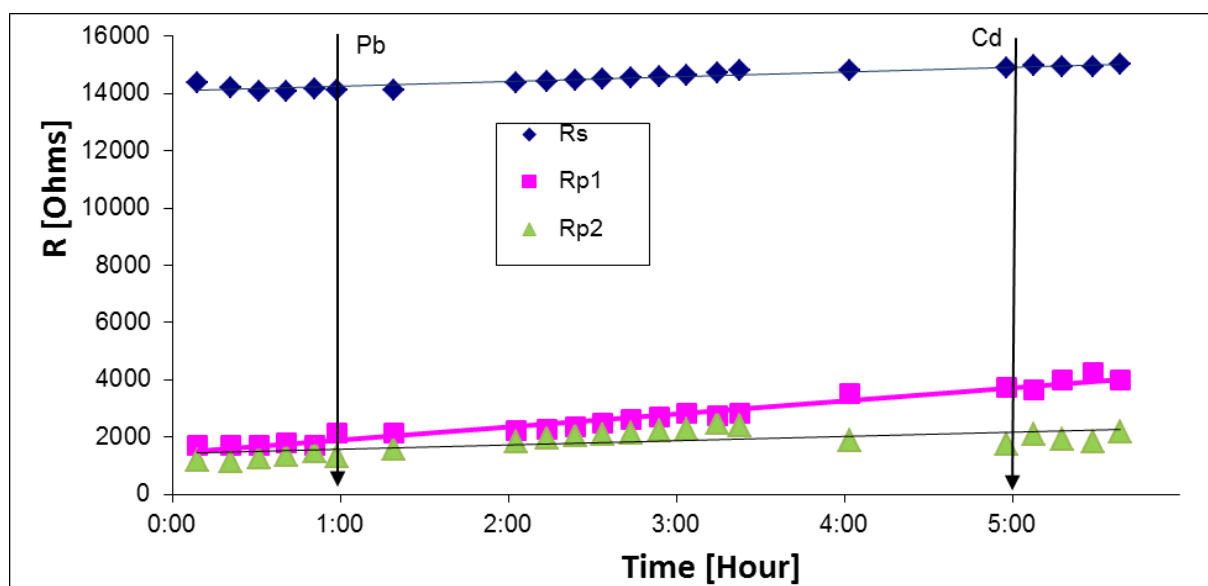
Figure 5. Changes of resistances of the lecithin SPLB (with addition of calcimycin ionophore) formed on the surface of the agar electrode, evaluated according to the EEC **Figure 2E and 2F**, 0.1M KCl, E=-0.1 V.

Time of reaching the steady state, judged according to the capacitances, is much longer as in case of resistances. In case of capacitances characterizing gel layer (C_2 in Figures. 2C, 2D, C_{p1} in Figures 2E, 2F) as well as in case of capacitances characterizing SPLB (C_3 in Figures 2C, 2D, C_{p2} in Figures 2E, 2F) it takes about 50-60 min. after immersing of the electrode into the supporting electrolyte, 0.1 M KCl). During this time the capacitances increase. which phenomenon can be explained as thinning of the membrane [41]. Similar effect was observed in case of SPLBs formed in pores of the polycarbonate membranes ([3, 8, 22, 24]).

The shapes and values of resistances and capacitances included in EECs were similar for both types of EECs. (Figures 2C-D, and 2E-F). The Chi2 errors were of the same order. Nevertheless, the

serial combination of two parallel RC circuits corresponds more to the real structure of an agar layer, on surface of which a SPLBL is formed. Similarly as in case of the free gel layer, the registered changes of the capacitance C_s have been time independent and could not be used for characterization of the system in any of the mentioned EEC. Hence, it was decided to use the 2C and 2E types of EECs for characterization of the electrode systems in the following experiments.

It was found that the results achieved using SPLBs prepared from asolectin were similar to those formed from lecithin. This conclusion is not surprising, because asolectin is composed of phosphatidylcholine a phosphatidylethanolamine mainly. As it was stated in our recently published papers [2, 8, 22, 24], the results achieved using both these types of phospholipids by formation of the SPLBs in polycarbonate membrane pores are very similar. It is probable that the membrane composed of different types of phospholipids will not be as compact as the membrane composed of uni-type phospholipid. The presence of unsaturated fatty acids can cause disturbances in its homogeneity. On the other hand, in correspondence with our results, these disturbances can be "repaired" by a proper combination of different phospholipids, which form the practically compact SPLB on the gel surface. Consequently, the electrical parameters of EECs of such compact monolayer are very similar to those formed from lecithin only.

The basic information on the influence of DC voltage on the formation of a stable SPL membrane on the electrode surface was investigated in [26]. It was concluded that the hydrogel support is sufficiently stable in the applied range of bias voltages (-0.4, +0.4V). These values are similar to those reached by us for SPLBs formed in the pores of the polycarbonate membrane [22]. Insertion of higher voltage than ±0.5 V can cause an irreversible destruction of the SPLB.

3.2 Transport of heavy metals across the SPLBs

The main attention of our research was aimed at investigation and description of the transport of divalent cadmium and lead cations across the SPLBs. Transport of these ions was realized using addition of ionophore calcimycin to the phospholipid solution, before its placing on the gel surface. Because its main role consists in transport of divalent cations, the presence of monovalent cations (H^+, K^+) does not affect its function.

The transporting processes were studied using EIS as well as cyclic voltammetry.

In the first set of experiments, it was confirmed that practically negligible amount of Pb^{2+} and Cd^{2+} cations is transported across the SPLBs, without addition of this ionophore (independently of the type of the used PLs).

As it was stated above, the applicability of resistances in EECs is, owing to their small sensitivity, very featureless. The utilization of capacitances is much more promising. The solution of investigated cations was added to the solution of the supporting electrolyte after formation of the SPLBs, which was characterized by reaching the steady state of the recorded capacitances (about 40 - 60 minutes after immersing of the electrode into the supporting electrolyte).

In correspondence with theoretical presumptions, the capacitances (more precisely, their values and trends of their time changes) characterizing agar (agarose) layers (C_1 in Figures 2C, 2D and C_{p1} in

Figures 2E, 2F) remain unchanged after additions of lead and cadmium cations to the supporting electrolyte 0.1 M KCl solution.

On the contrary (and similarly as in case of the SPLBs formed in polycarbonate membrane pores), the capacitances corresponding to the SPBLs (C_2 in Figure 2C and C_{p2} in Figure 2E), decreased immediately after addition of lead ions and subsequently their values increased slightly in time.

Similar behavior has been observed after the second addition of heavy metal ions (Cd^{2+} ions, about 5 hours after immersion of the electrode into the supporting electrolyte): the capacitances decreased again and then follow the started trend. (Figure 6).
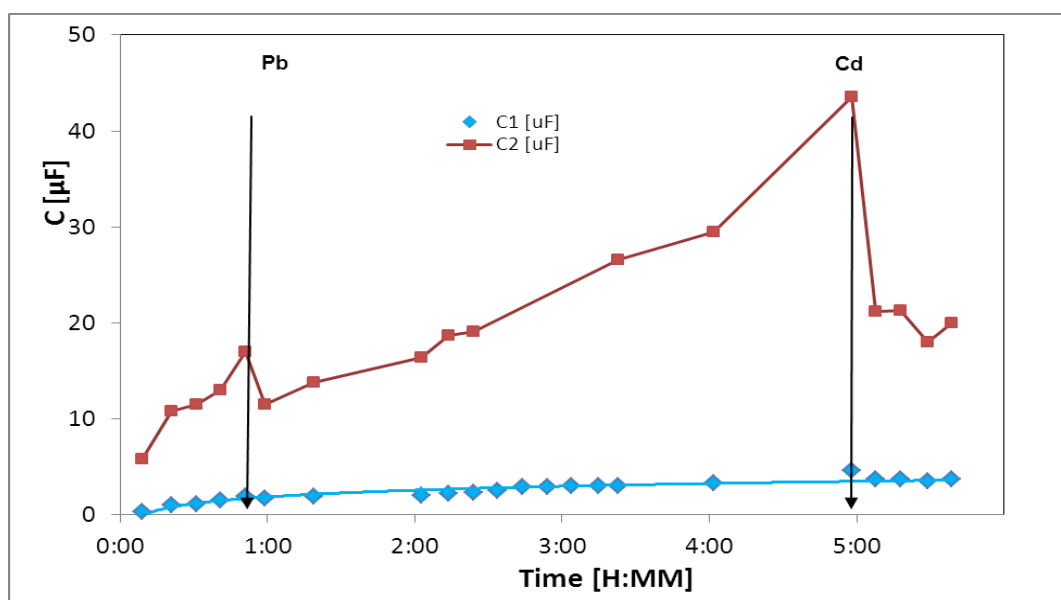
Figure 6. Changes of capacitances of the lecithin SPLB (with addition of calcimycin ionophore) formed on the surface of the agar electrode, evaluated according to the EECs Figures 2C and 2D, 0.1M KCl, E=-0.1 V.

The decrease of the capacitances is easily explainable. The transporting process of divalent cations Cd^{2+} or Pb^{2+} can be seen as the electrical current of charged particles, which are passing across the SPLB. This current decreases the registered capacitances. This effect was observed in the case of SPLBs formed on the porous membrane in previews experiments too [8, 24]. The surface of the gel electrode (of the SPLB membrane respectively) is about by 1 order lower in comparison with SPLBs formed on the porous membrane. Therefore, the registered capacitance decrease is much less pronounced.

Using the EIS it was proved that the SPLBs prepared from both phospholipids are almost equivalent (from the point of view of the transporting processes). Similarly, the results were almost independent of the type of the gel used (agar or agarose).

3.3 Cyclic voltammetry

EIS is powerful tool for SPLB characterization, but it cannot be used for qualitative analysis of the solution. For these purposes, the voltammetric techniques are much more suitable. Anodic stripping voltammetry would enable the accumulation of the metals on the electrode surface, but the SPLB on the electrode surface do not enable the reverse dissolution into the supporting electrolyte. Therefore, the anodic DC voltammetry was used.

Because the experiments were realized in KCl solution, the used potential range is limited by reduction of silver cations. During the reduction of lead and cadmium respectively, the monolayers of these metals on the solid surface of the working (e.g. silver, gold, silver composite) electrode are first formed. This process is represented by so called monolayer peaks, which are situated more positively than the reversible Nernst potential. Bulk depositions of these metals are realized by 150-300 mV more negatively. This phenomenon is called underpotential deposition (UPD) effect [42-47]. The peak corresponding to the monolayer adsorption/dissolution is fully described by two parameters: the potential shift (ΔE_p) and the half-peak width ($\delta_{1/2}$). This means that the monolayer deposition occurs before the bulk deposition starts. On the other hand, during the anodic polarization, the dissolution of the bulk takes place before the monolayer dissolution [42, 43, 48-52].
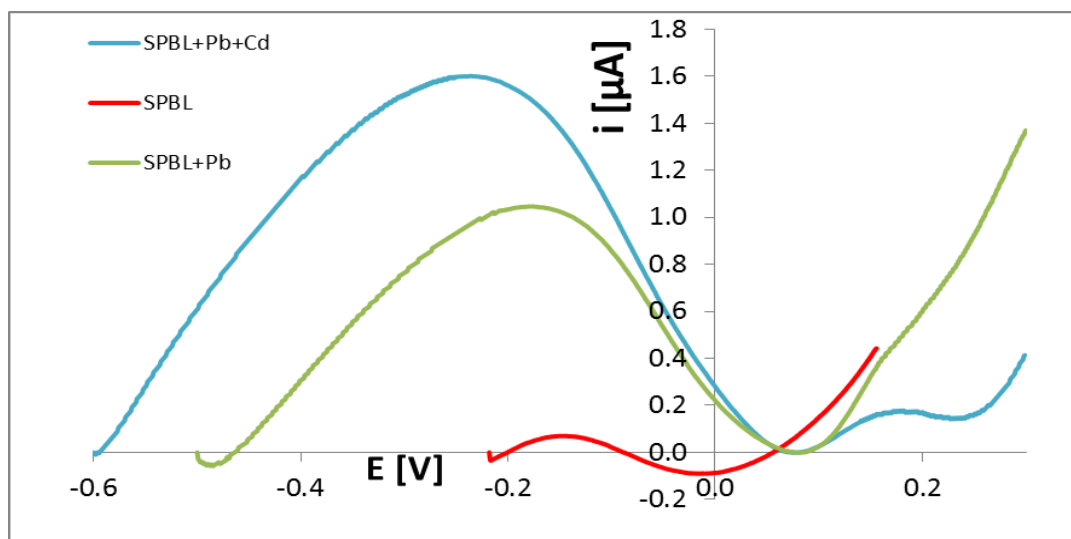
Figure 7. DC voltammogram of 50 µg.L^{-1} Pb^{2+} and 150 µg.L^{-1} Cd^{2+} in 0.1 M KCl registered using agar gel electrode covered by lecithin SPLB with addition of calcimycin (v=100 mVs^{-1}, linear base line correction).

As it is depicted in Figure 7, only one peak corresponding to the lead and cadmium dissolution was observed on our silver electrode connected with SPLB on gel support. Most probably it was the monolayer peak, because the amount of heavy metal cations in solution was low (lead 50 µg.L^{-1}, cadmium 150 µg.L^{-1}) and only part [8] of this could be transported across the SPLB with calcimycin. This conclusion (existence of the monolayer signal), can be confirmed by position of the peak corresponding to lead oxidation (cf. [42, 44, 50, 51, 53]) in KCl solution with added lead cations

(50 μg.L^{-1}). After addition of cadmium cations (150 μg.L^{-1}) to the supporting electrolyte with lead cations (50 μg.L^{-1}), the originally registered anodic peak increased, and the peak potential was shifted to more negative potentials. In correspondence with literature (e.g., [42, 44]), the sensitivity to lead is much higher than the sensitivity to cadmium. The signals of both metals could not be separated from each other. This phenomenon could be explained by higher scan rate applied, which was necessary to use in connection with low signals of small transported amounts of heavy metals.

4. CONCLUSION

In correspondence with the earlier published results [2, 8, 9, 24] and with the results published in this contribution, it can be concluded that the model membranes in the form of SPLBs on an agar or an agarose surface can be used for simulation of real cell membranes. The SPLB can be considered as completely formed in about 40-60 minutes after application of phospholipids on the support. The values of its capacitances increase after the SPLB exposure to the aqueous phase till steady state is reached. Application of voltage equal or higher than ±0.5 V can destroy the consistency of the SPLB irreversibly. The results are almost independent of the phospholipid used, i.e., the results achieved using DPPC are almost equivalent to those achieved using asolectin.

The parameters of different EECs, achieved using EIS, can characterize the systems formed. Two of them were tested to be used for characterization of the gel electrode covered by phospholipids. The first was composed of a serial combination of two parallel RC (resistance - capacitance) circuits; the other one of two mutually inserted such parallel RC circuits.

It was proved that the transporting processes are relatively complicated, because they can be affected by many parameters (e.g., pH, applied voltage, composition of the intracellular and extracellular solutions, and presence of other cations).

Lead and cadmium cations, transported across the SPLB built on the surface of the gel electrode, can be detected using DC voltammetry, however with low specificity.

References

1. S. Clemens, *Planta,* 212 (2001) 475
2. T. Navratil, I. Sestakova, J. Jaklova Dytrtova, M. Jakl, and V. Marecek, *WSEAS Trans. Environ. Develop.,* 6 (2010) 208
3. T. Navratil, I. Sestakova, J. Jaklova Dytrtova, M. Jakl, and V. Marecek, in *7th WSEAS International Conference on Environment, Ecosystems and Development* (M. Otesteanu, S. Celikyay, N. Mastorakis, S. Lache, and F. K. Benra, eds.), World Scientific and Engineering Acad. and Soc., Puerto de la Cruz, SPAIN (2009), p. 212
4. S. Clemens, *J. Plant Physiol.,* 163 (2006) 319

5. T. Navratil, I. Sestakova, and V. Marecek, *Int. J. Energy Environ.,* 5 (2011) 337
6. I.Sestakova, J. Jaklova Dytrtova, M. Jakl, and T. Navratil, *Int. J. Energy Environ.,* 5 (2011) 347
7. F. Thevenod, *BioMetals,* 23 (2010) 857
8. T. Navratil, I. Sestakova, K. Stulik, and V. Marecek, *Electroanalysis,* 22 (2010) 2043
9. I.Sestakova, J. Jaklova Dytrtova, M. Jakl, and T. Navratil, in *Development, Energy, Environment, Economics (DEEE '10)* (V. Mladenov, K. Psarris, N. Mastorakis, A. Caballero, and G. Vachtsevanos, eds.), Puerto de la Cruz (2010), p. 186
10. R. K. Murray, K. D. Granner, P. A. Mayes, and V. W. Rodwell, *Harper's Biochemistry*, Appleton and Lange, Stamford (1996)
11. A. Kotyk, *Chem. Listy,* 97 (2003) 37
12. M. Naumowicz, A. D. Petelska, and Z. A. Figaszewski, *Electrochim. Acta,* 51 (2006) 5024
13. M. Legin, G. Laputkova, J. Sabo, and L. Vojcikova, *Physiol. Res.,* 56 (2007) S85
14. J. Sabo, A. Ottova, G. Laputkova, M. Legin, L. Vojcikova, and H. T. Tien, *Thin Solid Films,* 306 (1997) 112
15. J. Jaklova Dytrtova, M. Jakl, I. Sestakova, E.-L. Zins, D. Schroder, and T. Navratil, *Anal. Chim. Acta,* 693 (2011) 100
16. H. T. Tien, Z. Salamon, D. L. Guo, and A. Ottovaleitmannova, in *Active Materials and Adaptive Structures* (G. J. Knowles, ed.), Iop Publishing Ltd, Bristol (1992), p. 27
17. B. Yosypchuk and V. Marecek, *J. Electroanal. Chem.,* 653 (2011) 7
18. H. Lang, C. Duschl, and H. Vogel, *Langmuir,* 10 (1994) 197
19. B. Sustrova, K. Stulik, V. Marecek, and P. Janda, *Electroanalysis,* 22 (2010) 2051
20. L. M. Williams, S. D. Evans, T. M. Flynn, A. Marsh, P. F. Knowles, R. J. Bushby, and N. Boden, *Langmuir,* 13 (1997) 751
21. C. Peggion, F. Formaggio, C. Toniolo, L. Becucci, M. R. Moncelli, and R. Guidelli, *Langmuir,* 17 (2001) 6585
22. T. Navratil, I. Sestakova, and V. Marecek, in *Development, Energy, Environment, Economics (DEEE '10)* (V. Mladenov, K. Psarris, N. Mastorakis, A. Caballero, and G. Vachtsevanos, eds.), Puerto de la Cruz (2010), p. 192
23. T. Navratil, I. Sestakova, and V. Marecek, in *Modern Electrochemical Methods XXXI* (J. Barek and T. Navratil, eds.), BEST Servis, Jetrichovice (2011), p. 91
24. T. Navratil, I. Sestakova, V. Marecek, and K. Stulik, in *Modern Electrochemical Methods XXX* (J. Barek and T. Navratil, eds.), BEST Servis, Jetrichovice (2010), p. 119
25. H. Janchenova, K. Stulik, and V. Marecek, *J. Electroanal. Chem.,* 591 (2006) 41
26. G. Laputkova, M. Legin, and J. Sabo, *Chem. Listy,* 104 (2010) 353
27. Z. Samec and T. Kakiuchi, eds., *Advances in Electrochemistry and Electrochemical Science*, Verlag Chemie, Weinheim (1995)
28. T. Osakai, T. Kakutani, and M. Senda, *Bunseki Kagaku,* 33 (1984) E371
29. H. Hoffmannova, M. Hof, and P. Krtil, *J. Electroanal. Chem.,* 588 (2006) 296
30. A.Benda, V. Fagul'ova, A. Deyneka, J. Enderlein, and M. Hof, *Langmuir,* 22 (2006) 9580
31. J. Sykora, P. Slavicek, P. Jungwirth, J. Barucha, and M. Hof, *J. Phys. Chem. B,* 111 (2007) 5869
32. K. Rieber, J. Sykora, A. Olzynska, R. Jelinek, G. Cevc, and M. Hof, *Biochim. Biophys. Acta-Biomembr.,* 1768 (2007) 1050
33. A.Benda, M. Benes, V. Marecek, A. Lhotsky, W. T. Hermens, and M. Hof, *Langmuir,* 19 (2003) 4120
34. P. W. Williams and G. O. Phillips, *Handbook of hydrocolloids*, Woodhead, Cambridge (2000)
35. L. Becucci, M. R. Moncelli, and R. Guidelli, *Langmuir,* 19 (2003) 3386
36. F. Bordi, C. Cametti, and A. Gliozzi, *Bioelectrochem.,* 57 (2002) 39
37. H. G. L. Coster, T. C. Chilcott, and A. C. F. Coster, *Bioelectrochem. Bioenerg.,* 40 (1996) 79
38. B. J. Abbott, D. S. Fukuda, D. E. Dorman, J. L. Occolowitz, M. Debono, and L. Farhner, *Antimicrob. Agents and Chemother.,* 16 (1979) 808

39. J. H. Vanzanten and H. G. Monbouquette, *Biotechnol. Prog.,* 8 (1992) 546
40. X. D. Lu, A. L. Ottova, and H. T. Tien, *Bioelectrochem. Bioenerg.,* 39 (1996) 285
41. M. Ikematsu, M. Iseki, Y. Sugiyama, and A. Mizukami, *J. Electroanal. Chem. ,* 403 (1996) 61
42. T. Navratil, S. Sebkova, and M. Kopanica, *Anal. Bioanal. Chem.,* 379 (2004) 294
43. S. Sebkova, T. Navratil, and M. Kopanica, *Anal. Lett.,* 36 (2003) 2767
44. E. Kirowa-Eisner, Y. Bonfil, D. Tzur, and E. Gileadi, *J. Electroanal. Chem.,* 552 (2003) 171
45. D. M. Kolb, in *Advanced in electrochemistry and electrochemical engineering*, Vol. 11 (H. Gerischer and C. W. Tobias, eds.), Wiley, New York (1978), p. 125
46. S. Sebkova, T. Navratil, and M. Kopanica, *Anal. Lett.,* 38 (2005) 1747
47. T. Navratil, J. Barek, and S. Fasinova-Sebkova, *Electroanalysis,* 21 (2009) 309
48. T. Navratil, J. Barek, and M. Kopanica, *Collect. Czech. Chem. Commun.,* 74 (2009) 1807
49. T. Navratil and M. Kopanica, *Crit. Rev. Anal. Chem.,* 32 (2002) 153
50. T. Navratil and M. Kopanica, *Chem. Listy,* 96 (2002) 111
51. T. Navratil and J. Barek, *Crit. Rev. Anal. Chem.,* 39 (2009) 131
52. S. Sebkova, T. Navratil, and M. Kopanica, *Anal. Lett.,* 37 (2004) 603
53. Y. Bonfil and E. Kirowa-Eisner, *Anal. Chim. Acta,* 457 (2002) 285