Study of Charged Particles Transport across Model and Real Phospholipid Bilayers

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Abstract: Air, waters and soils has being in increased levels contaminated with various metals, inorganic or organic compounds (partly products of human activity). To secure the normal processes in the living (plant or animal) cells, it is necessary to realize transport of various inorganic and organic compounds (nutrients, etc.), across the cell membrane into or out of the cells or various sub-cellular structures. Not only the useful and usual metabolic compounds are transported into the cells, across the membranes; however, the above mentioned undesired ions, compounds and particles, which are connected with pollution of human environment, are participated in the transporting processes. They are based on many principles, which we intend to study using electrochemical methods (electrochemical impedance spectroscopy, voltammetry, conductometry, patch-clamp techniques etc.). In this contribution, there are described the results of experiments realized on model of supported membranes and some ways of preparation of protoplasts, which would be suitable for patch clamp studies of the charged particles transport. The anodic stripping voltammetry, cyclic voltammetry and related methods were successfully applied for determination and characterization of heavy metal complexes with low-molecular-weight organic acids (LMWOAs) in soil solutions sampled from rhizosphere and bulk soil. All these experiments help us to explain the transporting processes of heavy metals across the real membranes of protoplasts.

Key-Words: Environment, Heavy metals, Charged particles, Phospholipid bilayers, Cell membrane, Electrochemistry, Voltammetry, Protoplasts, Ion channels, Ionophores.

1 Introduction

The pollution of human environment with various metals, inorganic or organic compounds (partly products of human activity) and its consequences belong to very frequently discussed and studied topics in many fields of sciences. Plenty of contributions presented on WSEAS conferences and published in WSEAS journals has been devoted to this topic, which were discussed from many different line of sight: characterization of contamination, mobility, bioavaibility and location of pollutants in various matrices (e.g. [1, 2]), mathematic descriptions of the state (e.g. [3, 4]), mathematical predictions and models of pollutions (e.g., [5, 6]), risk assessment for redevelopment of contaminated land at an old industrial sites (e.g., [7-11]), determination of pollutants (e.g., [12, 13]). Most of the applied approaches are macroscopic, i.e., they are dealing with general characterization of the state, without detail explanation of the processes connected with the environment of contamination. On the contrary, the approach which was applied in this paper is microscopic or almost "nanoscopic", because it is deals with processes, which occur on very thin biologic membrane (thickness of which amounts to about a few nanometers) [14].

Each real cell is surrounded by membrane, which are mainly composed of phospholipids. Additionally, they contain a wide variety of biological molecules,

primarily proteins and lipids, which take part in many cellular processes, such as ion channels, conductance, cell signaling etc. To elucidate such transport processes, synthetically prepared model phospholipid bilayers (PLBs) are utilized, in the form of black membranes (e.g., on porous materials), vesicles or supported membranes [15, 16], with ion channels incorporated (either artificially synthesized or obtained from real living cells, e.g. protoplasts).

The bilayer exists as a surface, at which the hydrophobic parts of phospholipids are protected from water, while the hydrophilic ones are in contact with the aqueous medium too. 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. 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. The rate of diffusion of organic molecules – nonelectrolytes – depends on their lipid-water distribution coefficient. The higher is the molecular solubility in fats, the faster is the diffusion rate across the membrane. Compounds insoluble in fats are transported across amphiphatic 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, which would not be otherwise able to pass across the bilayer.

The membrane phospholipids act as a solvent for membrane proteins. They create a suitable medium, in which the proteins can be active. The peptides, polypeptides and proteins are formed from 20 basic α-amino acids. The functional groups connected to the α-carbon atom of 6 of them are highly hydrophobic, several more are weakly hydrophobic and the rest are hydrophilic. The α-helical structure of proteins minimizes the hydrophilic character of the peptide bonds. Therefore, proteins can be amphipathic and can form an integral membrane component by having the hydrophilic groups arranged on the inner and outer surface of the membrane, connected by hydrophobic parts passing through lipophilic regions of the bilayer. The parts of proteins passing through the membrane contain most of the hydrophobic amino acids and occur in the α-helical arrangement or in the structure of the β-folded leaf. The number of various types of proteins in the membrane varies from 6 to 8 in the sarcoplasmatic reticulum to more than 100 in plasmatic membranes. Proteins act as enzymes, transporters, structural proteins, antigens (e.g., for histocompatibility) and as receptors of various molecules. Membranes and their components are dynamic structures. Lipids and proteins undergo metabolic reversal in the membrane, similarly as in other cell compartments. The rates of lipid and protein metabolisms may considerably differ. The membrane is considered to be of an asymmetric structure. That asymmetry may partly be ascribed to irregular representation of proteins in the membrane. The asymmetry between the inner and outer surfaces of the membrane (the transverse asymmetry) occurs on connecting saccharides to the proteins on the outer surface. Apart from this, specific enzymes are localized exclusively either on the outer or, on the contrary, on the inner side of the membrane, as, e.g., with the mitochondrial or with the plasmatic membranes. There can also be certain asymmetries between individual regions of the membrane. The membranes contain integral and peripheral proteins. The most of the membrane proteins are integral membrane components (they interact with phospholipids and tensides are needed for their solubilization). Those thoroughly studied [17] pass through the whole bilayer 5-10 mm thick.

2 Phospholipid Bilayers

By means of appropriate techniques, which are mentioned in this text, it is possible to prepare an artificial membrane system. In general, it consists of one or of a mixture of more phospholipids, either natural or artificially synthesized, which form spherical vesicules in the first step, and they are transformed into the phospholipid bilayers. The vesicules, covered by lipid bilayers, are called liposomes. For the study of membrane functions there are several advantages in using artificial membrane systems: A. The content of lipids in the membrane can be varied – this allows systematic research of the effect of changing lipid composition on a particular function. Therefore, e.g., vesicules can be prepared, consisting merely of phosphatidylcholine, or, on the contrary, of a known mixture of various phospholipids, glycolipids and cholesterol. Otherwise, the representation of individual fatty acids can be varied by utilizing synthetic lipids of known composition. The effect of fatty acids on several membrane functions can then be systematically studied. B. Certain purified membrane proteins or enzymes can be incorporated into the bilayers, following the factors essential for reconstructing function of the proteins. C. The medium of the systems can be strictly controlled and systematically varied (e.g., concentration of ions), or the systems can be exposed to the effect of well-known ligands, provided that the model PLB contain specific receptor proteins.

During the PLB formation, certain compounds can be incorporated into their interior, e.g., a drug or isolated

genes. In case of supported model PLBs the transport processes of various charged or uncharged particles can be elucidated or simulated. This procedure can prove positive in experiments with gene therapy too. Transport of some species across the membrane occurs via transmembrane channels. These are pore-like structures composed of proteins, providing selective pathways for passage of ions. The conducting channels for cations, about 5-8 nm in diameter, are negatively charged. Their permeability depends on the dimensions, the degree of hydration and the charge density of the ion. Specific channels have been described, controlled either by the voltage, or by the chemistry (Na^+, K^+, Cl^-, Ca^{2+}, …), the excitation acids, G-proteins and other factors. The transport systems can be described in terms of the number of the particles transported and of the direction of their motion, or from the point, whether the translocation aims towards equilibrium or away from it. Uniport systems translocate one type of solute in a certain direction. With cotransport systems, the transfer of one species depends on simultaneous or follow-up stoichiometric transfer of another species. Symport systems transport these species in the same direction. The proton saccharide transporter in bacteria, Na-saccharide transporters (glucose, mannose, etc.) and Na^+ amino acid transporters in mammalian cells can be given as examples. Antiport systems transfer two solutes in the opposite directions (e.g., Na^+ inside and Ca^{2+} out). Molecules, which cannot independently pass the lipid bilayer of the membrane, realize their transit in connection with the transfer protein. Two conditions must be met – facilitated diffusion and active transport, and highly specific transport systems. The facilitated diffusion and active transport have many features in common. Both these types involve transfer proteins and are specific for ions, saccharides and amino acids. The facilitated diffusion is explained by the ping-pong mechanism. Active transport differs from diffusion by transferring molecules away from thermodynamic equilibrium, i.e., it requires energy. This can be provided from ATP hydrolysis, from the motion of electrons, or from the light. The biological membrane contains a heterogeneous mixture of lipids differing from each other in their head-group structure, hydrocarbon chain length, the degree of unsaturation of the acyl chain, and the mode of attachment of the hydrocarbon chain [18]. Due to this complexity, it is difficult to ascertain the physical properties and functional roles of individual lipids and their mode of interaction with other lipids in natural membranes. Therefore, model systems consisting of phospholipids, have been a valuable tool in obtaining basic information on membrane lipid interactions.

3 Investigation of Phospholipid Membranes

3.1 Applied Electrochemical Methods

Our research group exhibits a lot of experience with electrochemical methods (voltammetry, polarography, electrochemical impedance spectroscopy (EIS)), which are very suitable for determination and investigation of heavy metal ions ion (Cd^{2+}, Pb^{2+}, etc.), transport across the PLB membranes. The classical electrode used in polarography was dropping mercury electrode and in voltammetry the hanging mercury drop electrode (e.g., [19-26]). Although they are flat on atomic level, both of them are mechanically unstable. Therefore, the studies on solid electrodes were performed [27, 28]. Such electrodes without content of liquid mercury are more frequently used for voltammetric determination of metals and of many other various inorganic as well as organic, environmentally important compounds. Our research team deals with development and application of (silver, copper, etc.) solid amalgam electrodes (silver, graphite, gold, etc.) composite solid electrodes or solid amalgam composite electrodes (e.g., [28-43]). It is well known that black lipid membranes (BLM) [44] used as model membranes can also be very well characterized by electrochemical impedance spectroscopy (EIS) (e.g. [45]). In the past century, the impedance technique provided a means of characterizing the electrical properties of many systems. Even today, it often provides the only non-invasive method for detailed structural-functional studies of these systems ([46] and the references therein). In spite of a wide variety of experimental methods for the study of lipid bilayers, some long-lasting problems remain.

3.2 Apparatus

The electrochemical impedance spectroscopy measurements were realized using CHI 650C Electrochemical Analyzer/Workstation, Software: CHI v 8.1 (IJ Cambrija Scientific, USA) and Potentiostat No. 283 and FRA No. 1025, No. 5210 (Princeton applied research, USA). The electrochemical impedances were determined using silver/silver chloride electrodes (silver wire, diameter 1 mm, electroplated by silver chloride). Platinum wire, diameter 1 mm, served as the auxiliary electrode.

The voltammetric determinations of cadmium ions were carried out by the PC - controlled voltammetric analyzer ECO-TRIBO polarograph (Polaro-Sensors, Prague, Czech Republic), equipped with POLAR.PRO software v. 5.1 and with MultiElchem v. 2.1 software (J. Heyrovský Institute of Physical Chemistry of AS CR, v.v.i., Czech Republic). Pen-type HMDE [47] electrode

was used as the working electrode, Ag/AgCl/KCl(3 mol L^{-1}) electrode to which all potentials are referred to and platinum wire served as a counter electrode (both ED, Turnov, Czech Republic). For the determination of cadmium ions, the sample was acidified by addition HNO_3, Suprapur (Merck spol. s r. o., Czech Republic), to pH 1 and analyzed using anodic stripping differential pulse voltammetry (AS DPV) at conditions: E_a = -850 mV, E_{in} = -700 mV, E_{fin} = -200 mV, scan rate 10 $mV.s^{-1}$, pulse amplitude 50mV. A new drop was used for each record, measurement has been performed in nitrogen atmosphere. Some other types of working electrodes were tested for these determinations, e.g., silver solid amalgam electrodes (polished or mercury meniscus modified) (some determination realizable with this electrode are described e.g. in [28, 32, 48, 49]) or solid composite electrode (some determination realizable with this electrode are described e.g. in [28, 36-38, 50-52]). pH was measured by digital pH/mV meter MPH 61 with combined electrode TYPE 01-29 (all Monokrystaly, Turnov, Czech Republic).

3.3 Phospholipid Membrane Preparation

The simplest process of preparation of model PL membrane consists in formation of such a bilayer on some supporting material (supported phospholipid bilayers SPBL), e.g., on a metallic substrate (mercury [44], gold, or on some gel surface), or in the form of a self-supporting membrane, e.g., as a bubble at a Teflon cap [53], filling a small micro-holes in a plate [54], or in a membrane [55]. This, last mentioned way of preparation was used by our research group: the experiments with it will be described in this contribution. The selection of a proper solvent allows to obtain the membranes with the thickness and capacity values similar to those of membranes formed of monolayers [55].

Two types of phospholipids were used for preparation of PLB on porous membranes: First, the attention has been devoted to the construction of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and di-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine. The capacity of membranes increases with time after the start of bilayer formation, until a steady-state value attained about 10-20 min. later [53, 55]. Therefore, it is reasonable to start the measurements 20-30 min. after applying of the phospholipid solution on the membrane [55]. In our experiments, there were the PLBs formed as self-assembled in the holes of the Isopore™ Membrane Filters (Millipore, USA) polycarbonate, hydrophilic, 2.0 or 8.0 µm, supporting membrane thickness: 7-22 µm, by injection of 10 µL of phospholipid solutions in heptane on the membrane surface. These membranes (supports) were glued on the plastic cup(s) of the polypropylene electrochemical cell (s.c. "Insert") (Fig1. A) or inserted between compartments of the glass electrochemical "U-Cell" (Fig1. B).

To investigate the transport processes, some ionophore (e.g. Valinomycin and Calcimycin) can be added to the solution of phospholipids. This can either support (decrease the Gibbs transport energy) the transport of ions or compounds in the form of a complex or form an ion channel (working on the cage principle) applicable for similar purposes.

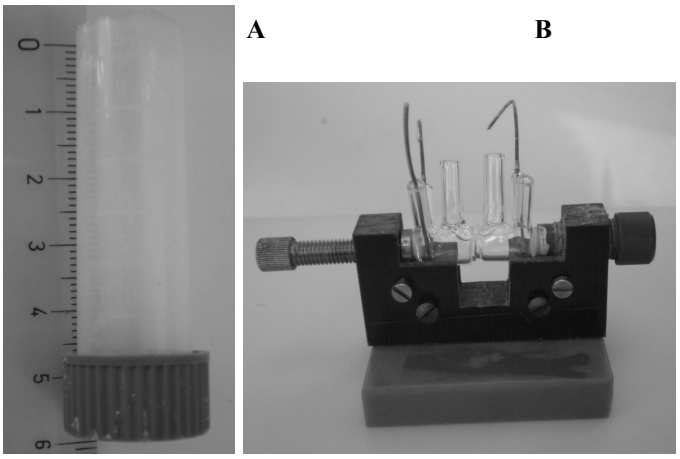

Fig. 1: Electrochemical cells used by investigation of transporting processes across the SPLBs: A-"Insert"; B-"U-Cell".

First of applied ionophores – Valinomycin (Figure 2) is a peptide actively transporting ions through phospholipid bilayer and is highly selective for potassium ion. It is a dodecadepsipeptide, made of twelve alternating amino acids and esters, which form a macrocyclic molecule (the cycle consists of three times repeated sequence of L-valine - D-hydroxyisovaleric acid - D-valine and L-lactic acid). It is gained usually from the cells of several Streptomyces strains (e.g., "S. tsusimaensies"), and therefore it belongs to the natural ionophores.

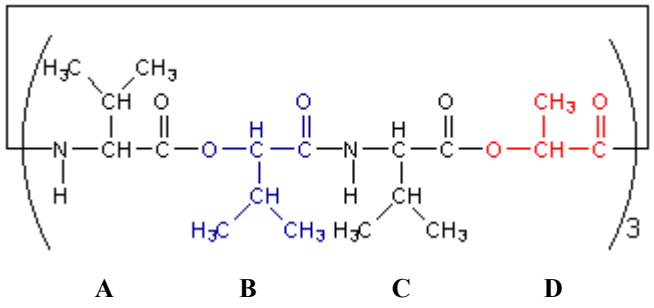

Fig. 2: . Structure of the ionophore Valinomycin ("A"-L-valine; "B" - D-hydroxyisovaleric acid; "C" - D-valine; "D" - L-lactic acid).

Calcimycin (known as well under names Calcium Ionophore, Antibiotic A23187), is a highly selective natural ionophore for divalent cations [56]. Ion transport by A23187 is mediated by a dimeric form of the molecule that complexes the metal cation. Relative stabilities of the complexes formed are Mn^{2+}> Ca2+ ≈ Mg^{2+} > Sr^{2+} > Ba^{2}+. A23187 was described also as a cadmium ionophore [57, 58] and several 1:1 complexes stability constants were determined for metal ions as Ni2+, Fe2+, Zn2+ and others [58].

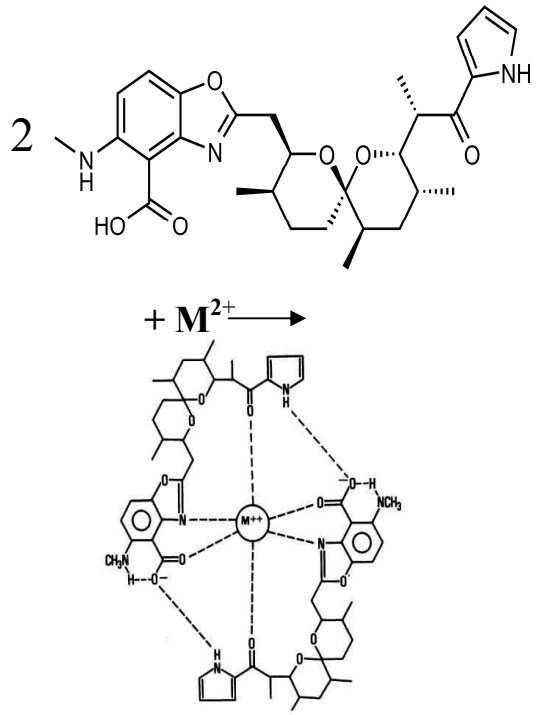

Fig. 3. Structure of the ionophore Calcimycin and the way of its complexing with divalent cations (According to the ref. [57]).

The thickness, stability and the transporting parameters across PLBs depend on the way of their preparation, on temperature, pH and on applied potential.

3. 4 Equivalent circuits

Two types of equivalent circuits were used for characterization of SPLBs. The first one was applicable for characterization of the free polycarbonate membranes (Figure 4A). The other one was more suitable for characterization of SPLBs formed on polycarbonate membrane (Figure 4B).

3.5 Sol–gel Technology of Phospholipid Membrane Preparation

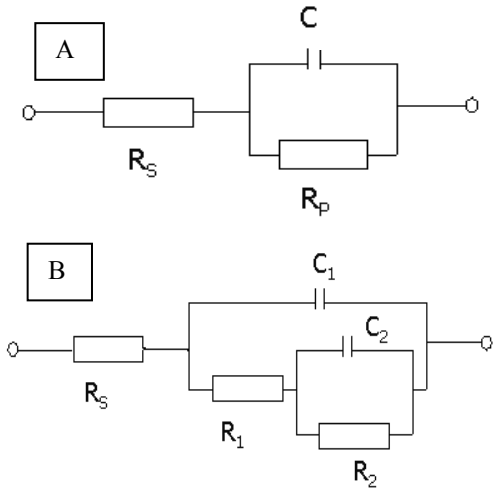

Fig. 4. Equivalent circuits used for characterization of the PLBs.

One of new promising approaches for the preparation of phospholipid membranes seems to be the application of sol–gel technology [59]. It is a very interesting and powerful modification of the classical sol–gel method that performs the operations required in a single phase, which comes from studies of interfaces between two immiscible electrolyte solutions (ITIES) (e.g., [60]). A scheme of the cell was described in [61]. The preparation of phospholipid membranes on the surface of agar-substrates has been tested in the framework of our research. Introduction of the polymer gel electrode [62] 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 an elevated temperature. After cooling the mixture to room temperature in a suitable mould, the gel electrode is formed. It assumes a variety of shapes and it is easy to handle. Polarization measurements at the polymer gel|liquid boundaries can be employed for analysis of the behavior of ISE's, or for direct determination [63] or continuous monitoring [64] of ion concentrations. However, no systematic research has so far been done on the adsorption of phospholipids on the polymer gel electrode. The introduction of agar gel also will improve mechanical stability of the liquid|liquid interface. This effect will simplify the use of the confocal fluorescence correlation spectroscopy (PCS) for *a)* detection of labeled molecules penetrating the adsorbed phospholipid monolayer and *b)* determination of the lateral lipid diffusion coefficients. The latter procedure has been recently reported for a phospholipid monolayer at the liquid|liquid interface [65]. One of the aims of our investigations is the preparation of supported phospholipid bilayers (SPBs) on an agar gel electrode.

SPBs have now been applied to biosensors, micro- and nano-structures, blood-compatible surfaces, medical implant devices, and to production of catalytic interfaces [66]. Many further applications have been proposed or are currently under study. Systematic studies of the mechanisms of SPB formation, such as the conditions for fusion of adsorbed vesicles, have only been performed over the last few years. In the suggested agar-gel supported PB's, the agar gel is supposed to serve as a new type of "soft polymer cushion" [66].

4 Results and discussion

4.1 Electrochemical characterization of the SPLBs

The capacity of phospholipid bilayer increases after exposition to the aqueous phase with time. This process can be explained by thinning of the phospholipid membrane [67]. The changes in real and imaginary parts of impedances were recorded and they can be used for characterization of the state of the membrane (formation or its destruction). In Figure 5, this process is demonstrated by dependence cotg(Phase) vs. $w^{0.5}$ in measurement with "U-cell".

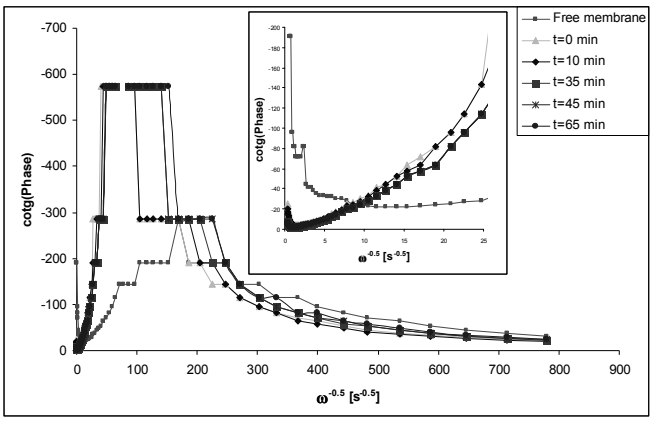

Fig. 5. Dependence of cotg(Phase) on $\omega^{0.5}$ in measurement with "U-cell" in time over a frequency range 0.1 – 1000 Hz for a membrane made from phosphatidylcholine in "U-cell" (amplitude 0.005 V, E = -0.1 V).

Similar behavior was observed at measurement with Insert- cell. At both cases, steady-state is reached after 40-60 min. from exposition to aqueous phase.

4.2 The effect of applied voltage on the SPLBs

The effect of applied DC voltage on SPLB at its steady state can be seen from Fig. 6. The records were realized in steady state of the membrane existence, i.e., after about 1 hour after immersion into aqueous KCl. There was no substantial difference in behavior of SPBL prepared from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine monohydrate or 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine. With the application of potential (Figure 6), the increase of capacitance has been observed (with the exception of potential -0.4 V), but changes were reversible (dotted lines represent the values, which would be recorded under voltage -0.1 V). This confirms that SPLB is not damaged, similarly as has been described for s-BLMS on the tip of Teflon coated silver electrode [68]. Very interesting results were recorded under application of the voltage 0 V.

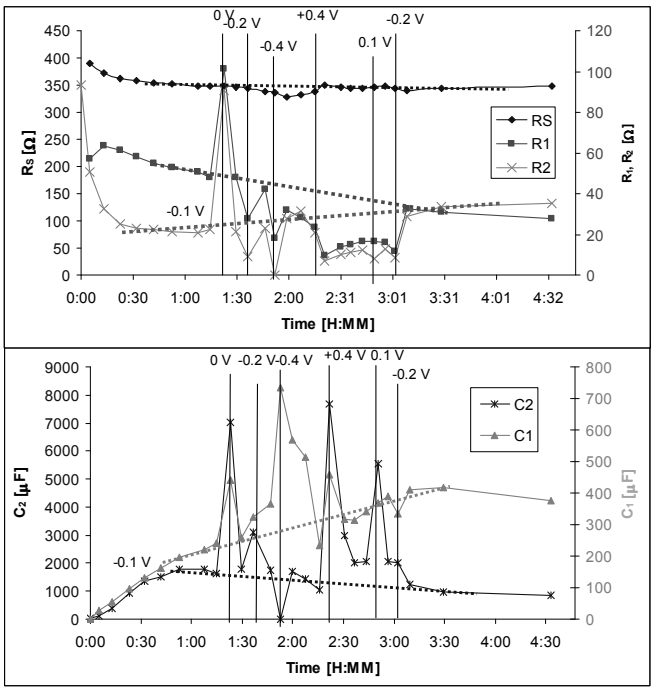

Fig. 6. The application on DC voltage on SPLB prepared from 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine. Capacitance and resistance values were calculated according Fig 4B.

4.3 Transport of cations across the polycarbonate membrane

Two ionophores were utilized for transport of cations: Valinomycin and Calcimycin. The first one was used for the starting experiments with transported calcium and potassium. It was proved that using Valinomycin, only monovalent cations are transported across the supported PL membranes [55].

The following group of experiments were realized with ionophore A23187 (Calcimycin), which is normally used as calcium ionophore, but the application for Cd^{2+} uptake by metal-sorbing vesicles prepared from phosphatidylcholine doped with A23187 has been described [56, 68]. Whereas there chelating agent encapsulated in the vesicle interior provided the driving force, only the concentration gradient was applied in our

experiment (2.10^{-4} M Cd^{2+} in electrolyte 1 and 0 Cd^{2+} in electrolyte 2 in experimental cells depicted in the Fig. 1). Solution of cadmium ions was applied to electrolyte 1 again when SPBLs on polycarbonate support was in steady condition and exactly 1 hour from the application, amount of cadmium in electrolyte 2 was determined by anodic stripping voltammetry. Results (in Table 1) show enhanced amount of transported Cd^{2+} in the presence of Ca^{2+}.

Table 1. Transport of Cd^{2+} ions across the lecithin SPLB. Total amount of Cd^{2+} in Electrolyte 1 (100 %) was 20 μg.

Conditions	**Elyte 1**	**Elyte 2**	**E [V]**	**transported mass of Cd^{2+}**	**% of transported Cd^{2+}**
Free membrane	0.1M KCl	0.1M KCl	-0.1	5.746	**28.73**
Lecithin + Calcimycin	0.1M KCl	0.1M KCl	-0.1	0.002	**0.01**
Lecithin + Calcimycin	0.1M $CaCl_2$	0.1M $CaCl_2$	-0.1	0.070	**0.35**
Lecithin + Calcimycin	0.1M $CaCl_2$	0.1M KCl	-0.1	0.028	**0.14**

This observation is different from usually described Ca influence on cadmium uptake [69], but is in correspondence with experiments on tobacco containing transporter LCT1 [70]. There cadmium ions transport was enhanced or deceased at certain calcium concentrations. No similar experiments were performed with A 23187 and therefore, more detailed dependence of amount of transported cadmium on the calcium concentration in solution is necessary.

4.4 Complexes of Pb and Cd Complexes with Low Molecular Weight Organic Acids

Under current knowledge [71, 72] metals in environmental systems have not exist as ions, but mostly as complexes with low molecular weight organic acids (LMWOAs). Prior to study the transport these complexes cross the membrane is essential to investigate the structure of the complexes. The potential risk of soil contamination with heavy metals and possibilities of phytoextraction in relation with solubilisation of LMWOAs was investigated using anodic stripping voltammetry and diffusive gradient in thin film technique [71, 73-75]. The Cd and Pb complexes with oxalic (OA) and citric acid (CA) were detected in model and soil solutions using cyclic and stripping voltammetry. A mixed complex consisting of Cd, Pb and OA was found. For mixed complex formation, the presence of $PbOH^+$ species and Cd^{2+} in OA solution was supportive. Only the "simple" complexes of CA with Pb and Cd were found in the model solution [73].

4.5 Preparation of Protoplast from Plant Cells

The other different approach to the study of ion transport consists in preparation of protoplast, e.g., from plant cells, and in investigation transporting processes using patch clamp techniques. We prepared the protoplasts from Pea leaves and from Thlaspi caerulescens (leaves or roots). For these purposes different protocols were applied ([76, 77]): application of enzymes only or of enzymes in combination with osmotic removing of the cell wall. Such way prepared protoplast can be investigated using e.g. "whole cell", "perforated" patch" or "isolated patch" technique. Using a sharp pipette and pulling it is possible to prepare the real PLB with ion channels, which can be incorporated in model PLB membranes.

4.6 Analysis of Heavy Metals in Soil Solution

The potential risk of soil contamination with heavy metals and possibilities of phytoextraction in relation with solubilization of heavy metals using low molecular weight organic acids have been studied by authors of this manuscript [73, 78]. However the problem with reliable in situ determination of defined heavy metals forms (in the meaning of a real molecule or ion) does not exist. The LMWOAs outside the plant and phytochelatins inside the plant play an important role in the puzzle of questions. Namely oxalic acid participates on mineral mobilization, metal and/or nutrition uptake, oxidation processes, and others [79]. The stability constants of metal complexes with oxalic acid are relatively high [80], due to oxalic acid is predictable as possible complexant. Oxalic acid is the most abundant low-weight-molecular organic acid in obtained soil solution from smith willow and alpine penny-cress and creates a variety of complexes [73, 75, 78, 81, 82].

The Cd and Pb complexes with oxalic acid (OA) were detected in model solution using differential pulse anodic stripping voltammetry and differential pulse cathodic stripping voltammetry. Recording of the voltammograms was carried out by means of classical assembly of three electrodes, using HMDE as a working electrode, $Ag/AgCl/KCl_{sat.}$ as a reference electrode and Pt as an auxiliary electrode. The pH of the model solutions were adjusted to pH 7 with sodium hydroxide.

A mixed complex consisting of Cd, Pb and OA was found, its peak potential varies from -582 to -542.5 mV (vs. $Ag/AgCl/KCl_{sat.}$) and depends on the

Pb:Cd or Cd:Pb ratio. The "single" complexes of OA with Pb and Cd (Pb-OA and Cd-OA) are constrained on specific conditions. The existence of all focused metal complexes is confined to neutral or weakly acidic ambient. In acidic ambient (pH 2) in model and soil solution does not exist any Pb or Cd complexes, all Cd and Pb were presented in free ionic forms.

We can summarize our results and express our conclusion that citric acid in neutral pH creates with Pb and Cd only "simple" complexes. On the other hand, apart from "simple" complexes of oxalic acid with Pb or with Cd (when only one metal is present in solution), mixed complex of oxalic acid with Pb and Cd (Cd-OA-Pb) in neutral pH range was found. The formation of Cd-OA-Pb complex is supported by the existence of $PbOH^+$ species in neutral or weakly basic milieu (pH 7.0-7.5). The peak potential of this complex depends on the Pb:Cd or Cd:Pb ratio. The mixed complex stability is higher than the stability of simple complexes of Cd or Pb with oxalic acid. The environmental importance of the mixed complex is supported by its existence in real soil solutions obtained from willow and penny-cress planted in contaminated Fluvisol [73]. On the base of our experiments with model solutions consisted from cadmium and lead nitrate and/or chloride with oxalic acid and cadmium nitrate, lead nitrate and oxalic acid we can suppose that more complicated complexes of these metals with oxalic acid are present (except monomer, dimmers and trimmers as well).

5 Conclusion

The starting experiments realized with supported phospholipid bilayers helped to improve the ways of their preparation and to find the possibilities of their characterization. The suitable measuring techniques were developed and the measuring analytical cells for these purposes were constructed. We can conclude that successful incorporation of ionophore Valinomycin and Calcimycin was confirmed. Stability of SPBL allowed to study the influence of applied DC voltage and to quantify transport of cadmium ions through SPBL with Calcimycin. Enhanced transport of cadmium ions in the presence of Ca^{2+} has been observed.

Leaves and roots from Thlaspi caerulescens were tested as source of protoplasts. As models of complexes occurring in real soils, the complexes of lead and cadmium with two low molecular weight organic acids - oxalic (OA) and citric acid (CA) were studied.

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