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Effects of divalent cations on the formation and structure of solid supported lipid films

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Abstract

The interaction of glassy carbon-supported thin wetting films of lecithin with some divalent cations is investigated by impedimetry and voltammetry. The influence of Ca^{2+}, Mg^{2+}, and Mn^{2+} on the film structure is explored in two different cases — the divalent cations are added to the electrolyte either before or after the formation of the film. When the film has been previously formed, the addition of divalent cations in millimolar concentrations leads to changes in the passive electrical parameters and the blocking properties of the films. On the one hand the dielectric properties of the film measured in 0.1 M KCl seem to improve after the interaction with divalent cations — the film capacitance decreases, the resistance and resistivity of the film increase. On the other hand the increase of the redox current in the presence of 1 mM $Fe(CN)_6^{3-/4-}$ in the electrolyte suggests the formation of some defects in the lipid structure of the film after the action of divalent cations. It is shown that the amount of these defects could be significantly decreased when the divalent cations are present in the electrolyte solution before the film formation. The effect of divalent cations on the film stability is tested by applying negative potential to the film. In 0.1 M KCl the films are not stable at potential of − 0.8 V (vs. Ag/AgCl) and are destroyed. The addition of divalent cations stabilizes the films and at certain millimolar concentrations the films remain intact after the action of the negative potential. The effect of Mn^{2+} is more pronounced, the Ca^{2+} and Mg^{2+} have smaller commensurate effect. It is proposed that the changes in the films' properties could be related with more tight packing of the lipid molecules with the divalent cations inserted in the film and that some defects could be opened during the rearrangement of the lipids when the film has been previously formed.

Keywords: Solid supported thin lipid films; Divalent cations; Voltammetry; Impedimetry; Film stability

1. Introduction

Solid supported lipid films have proved very useful both as biomembrane mimetic analogs and as a basis for electrode modifications in the construction of sensor devices [1,2]. The films are prepared in quite a different manner, including liposome spreading [3], self-assembly [4], Langmuir–Blodgett (LB) [5], transfer of lipid monolayers from air/water interface to the surface of HMDE (hanging mercury drop electrode) [6] or cast film technique [7]. An alternative method of formation is achieved through the thinning of wetting lipid layers on solid surfaces, submersed in electrolyte solution [8].

The impact of some divalent cations on the structure of a model membrane system has been investigated with several types of supported lipid films [5,9–16], comprising wetting lipid films [14,15]. Changes in the films' capacitance and resistance [12–16] and opening of defects in supported lipid films have been reported [5,9–11,15].

The present work represents further investigations of the effects caused by the divalent cations on the formation and structure of wetting lipid films.

The influence of the cations (Mn^{2+}, Mg^{2+}, Ca^{2+}) on the film structure is investigated in two different cases. In the first one the divalent cations are added to the electrolyte ambiance after the formation of the film. In the second one the addition is done before the film formation, which is a new type of experiment in comparison with our previous works [14,15]. In both cases the impedimetric and voltammetric results suggest significant influence of divalent cations on the packing order and strengthening of the lipid structure of the film. At the same time, concerning the opening of defects, the film response to the action of the divalent cations is found to be different in the two cases. In another series of experiments the strengthening of the

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lipid structure is additionally monitored by testing the stability of the films at negative potential.

2. Experimental

The techniques of formation of the wetting lipid films are described in details elsewhere [8,14,17]. The essence of the method is a preparation of two interfaces (solid/lipid and lipid/electrolyte) formed independently of each other. Their subsequent approach and contact lead up to a process of thinning and formation of a stable lipid film.

The electrochemical cell for development and investigation of the films was filled with an electrolyte solution of 0.1 M KCl (Potassium Chloride, SIGMA Chemical Comp., USA). The film-forming lipid solution containing natural lecithin (Bell Pharmacal Corp., USA) dissolved in *n*-hexane (SIGMA Chemical Comp., USA) with concentration 10 mg/ml was placed above the electrolyte. A glassy carbon working electrode (GCE) onto whose surface the films were prepared was immersed into the lipid solution. The diameter of the GCE was 3 mm. A single junction Ag|AgCl was used as reference electrode.

The main stages in the film preparation process were: 1) formation of electrolyte–lipid interphase boundary; 2) electrode immersion in the lipid solution thus forming a solid/lipid interface; 3) incubation period necessary for the lipid adsorption on the two interfaces and for a reliable value of lipid interfacial concentration to be reached; 4) gradual approach of the electrode surface to the electrolyte/lipid solution interphase boundary by moving the support (GCE) and carefully pressing it against the meniscus; 5) process of spontaneous thinning. The thicknesses of these self-thinned films in most cases were of the order of 100–200 Å that was much bigger than a bilayer thickness. In order to obtain thinner films an electrical field was applied to the thick films. Using electrostriction we were able to prepare films with thickness of the order of monolayer (approx. 20 Å) [17]. The final stable state of the lipid films was achieved by electrostriction applying +1 V dc-voltage. The film covered the whole area of the GCE approximately for a few seconds. Then the dc-voltage was removed which did not change the state of the film.

Manganese chloride ($MnCl_2 \cdot 4H_2O$, Chimsnab, Bulgaria), as well as magnesium and calcium chloride ($MgCl_2 \cdot 6H_2O$, $CaCl_2$, MERCK, Germany), was added to the electrolyte for modification of the lipid films. Potassium ferricyanide/ferrocyanide ($K_{3/4}[Fe(CN)_6]$) was used to act as electroactive species whose redox reaction was monitored.

The electrochemical features of the films were investigated by cyclic voltammetry in a three-electrode cell with a platinum (Pt) wire as counter electrode. The electrochemical reactions were registered with the aid of polarograph (OH-105, Hungary). Impedimetric technique was used as an analytical tool for examination of films' behaviour in their different stages. The determination of the films' impedances was performed by the aid of lock-in nanovoltmeter UNIPAN, 232B (Poland) at zero potential vs. Ag/AgCl applying measuring signal with 50 mV ac-amplitude.

3. Results and discussion

In previous papers we reported impedimetric investigations of the reaction of glassy carbon-supported lecithin monolayers obtained by electrostriction upon the addition of divalent cations (Mn^{2+}, Mg^{2+}, Ca^{2+} in millimolar concentrations) in the electrolyte [14,15].

As a measure of the influence of divalent cations on the state of the films we used the changes in their equivalent parallel resistance R_p and capacitance C_p. R_p and C_p are evaluated at each frequency from the impedance of the film. Both R_p and C_p are frequency-dependent, but they are well approximated with power functions, which is the behaviour of a constant phase angle element (CPE). The frequency dispersion of the supported lipid films' electrical parameters is often reported [e.g. 1,4,18]. The concept of CPE is a convenient way to fit the experimental data, as well as, to monitor the state of the films by the power factor of the best fit functions [14,15].

The addition of divalent cations (Mn^{2+}, Mg^{2+}, Ca^{2+} in the range 1 mM–5 mM concentrations) to the electrolyte results in a readable change of the films' equivalent parallel resistance R_p and capacitance C_p for all frequencies. The resistance R_p increases and the capacitance C_p decreases. Moreover, evaluating the apparent film resistivity ρ from the experimental value of R_p and C_p we find for all frequencies an increase of ρ after the addition of divalent cations (for each type of cation — Mn^{2+}, Mg^{2+}, Ca^{2+}). There is a decrease of the capacitance dispersion as well, expressed by a decrease in the slope of the C_p fit, respectively the power factor of the best fit function for the treated films decreases [14,15].

Qualitatively, the three divalent cations (Mn^{2+}, Mg^{2+}, Ca^{2+}) invoke similar changes in the film's features. However, at a given concentration the Mn^{2+} disturbs the film's parameters in a greater extent, while the Mg^{2+} and Ca^{2+} have smaller commensurate effect, which is consistent with the results for the binding of divalent cations to phosphatidylcholine, decreasing in the sequence Mn^{2+}, Mg^{2+}, and Ca^{2+} [19].

The influence of divalent cations on the structure of the films can be tested with the aid of different electroactive probes. It is known from the literature that the inclusion of divalent cations increases the probability for opening of channel-like defects in supported lipid layers and this effect can be monitored with electroactive marker ions. Such effects were observed for films obtained by different techniques from various types of lipids and on various supports [5,9–11].

In Fig. 1 cyclic voltammograms of 1 mM $K_{3/4}[Fe(CN)_6]$ on bare GCE and on lecithin film-modified GCE at different concentrations of Mg^{2+} in the ambient electrolyte solution are presented. The results are in consistence with the above-mentioned works. The increase of the redox currents with the raise of Mg^{2+} concentrations suggests a larger $Fe(CN)_6^{3-/4-}$ penetration into the film, indicating the occurrence of some defects in the lipid structure of the film.

The above discussed impedance results and the presented voltammetric results might seem contradictory. On the one hand the impedance data suggest improvement of the dielectric property and on the other hand the redox current increases after the action of divalent cations.

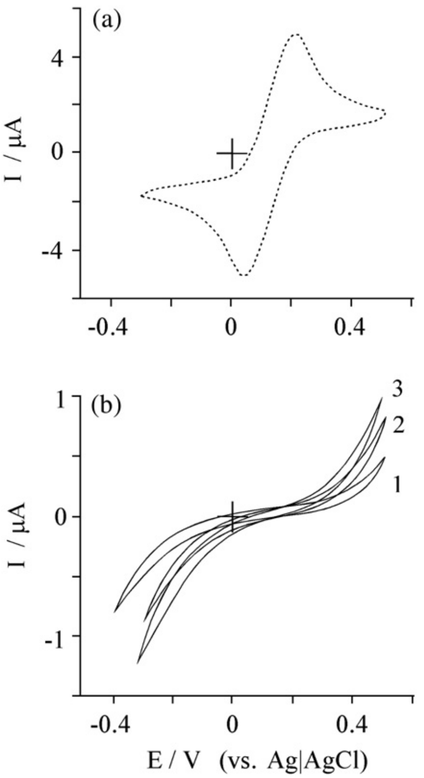

Fig. 1. Cyclic voltammograms of 1 mM $Fe(CN)_6^{3-/4-}$: (a) in 0.1 M KCl on bare uncovered GCE; (b) curve 1 — in 0.1 M KCl on lipid film coated GCE; curve 2 — in 0.1 M KCl on lipid film coated GCE after addition of 1 mM Mg^{2+} in the electrolyte solution; curve 3 — in 0.1 M KCl on lipid film coated GCE after addition of 3 mM Mg^{2+} in the electrolyte solution. Scan rate 2 V/min.

A plausible explanation of the presented results can be associated with a notion for some "rigidification" in the films' molecular ordering, more tightly packed lateral structure and hampered molecular mobility within the film as a consequence of the interaction of the divalent cations with the phospholipid headgroups. The presence of divalent cations in the polar region of the phospholipids causes changes of the conformation, hydration and effective size of the headgroups [20–22], as well as, an increase of the acyl chain packing [20,23] and rigidification of the membrane [22,23].

This picture is in accordance with the impedance data. The decrease of C_p is consistent with the suggested more tightly packed lipid structure of the film. Similar effect of Ca^{2+} on the film capacitance has been reported for lipid monolayers transferred on Hg surface and this effect has been attributed to condensation of the lipid monolayer [16]. The observed increase of R_p is also expected in regard to the suggested increased lipid packing. However, the increase of R_p does not coincide with the observed increase in the charge transfer current (Fig. 1). This discrepancy could be interpreted taking into account the CPE-like impedance behaviour of the film. The discussed R_p is the apparent equivalent resistance of the film and is obtained by using a simple RC-parallel model for the film impedance. This R_p is connected with the overall processes of energy dissipation during the measuring ac-cycle at the given frequency. In our case the impedance is obtained in the absence of electroactive species in the electrolyte contacting the film. Thus the apparent equivalent resistance R_p and respectively the overall energy dissipation might be dominated by processes other than an ionic current through defects within the film and charge transfer at bare film-free sites of the electrode surface. At the same time both C_p and R_p exhibit power-law frequency dependence indicating that the film has the properties of a CPE. Indeed, the CPE whose impedance is $Z_{CPE}=A(i\omega)^{-a}$ could be presented as combination of parallel resistance R_{CPE} and capacitance C_{CPE} that are both frequency-dependent: $R_{CPE}\sim\omega^{-a}$ and $C_{CPE}\sim\omega^{1-a}$ (here A and a are the parameters of the CPE, i is the imaginary unit and ω is the angular frequency — for details see Refs. [14,15]). The apparent resistance R_{CPE} reveals the CPE intrinsic feature of dissipating energy during the ac-cycle.

All these considerations give insight to an interpretation that in the absence of redox current the measured R_p is dominated by the R_{CPE} manifestation of the CPE of the films and respectively that the main part of the energy dissipation is accomplished by the processes that are responsible for the CPE behaviour. In general, the observed increase of the resistance R_p and especially the increase of the resistivity ρ for all frequencies reflect a decrease in the overall dissipation processes after the action of divalent cations. Moreover the decrease of the capacitance dispersion expressed by the decreased power factor, $(1-a)$, indicates directly an enlargement of the ratio of the energy stored to the energy lost, suggesting a diminished role of the dissipative processes in treated films. It is a characteristic of the CPE that the ratio of the energy stored to the energy lost is constant and is quantitavely expressed by the parameter a (no energy is dissipated if $a=1$, and if $a=0$ the CPE acts as a pure resistive element [24]).

It should be noted that the CPE impedance of an electrode could be related to a variety of different geometric, physical and chemical effects, alone or in combination [24,25]. In the case of lipid film-modified electrodes the CPE impedance has been associated most often with inhomogeneity and disorder in the lipid film structure. Diao et al. [26] used the CPE-parameter, a, to estimate the degree of disorder in a thiol monolayer structure. The closer the parameter a value approaches to 1, the greater the order in the structure. Whitehouse et al. [27] proposed a model that concerns with two types of power-law frequency dependencies — one, arising from nonideality or "roughness" of the interface between the lipid-coated electrode and the electrolyte solution, and another, related to distribution of the relaxation time constants of dipole or charge movements within the dielectric core of the lipid layer. Wiegand et al. [28] assume a model with distribution of the time constants related to structural heterogeneities perpendicular to the plane of the lipid layer. These interpretations support the suggestion that, in our case, the changes in the impedance parameters are caused by more uniform molecular ordering in the film structure and increased lipid packing after the action of the divalent cations.

At the same time the rigidification in the lipid structure could be the reason for an opening of defects which increases the charge transfer current in the presence of electroactive species. Such defects may occur at the boundary of coexisting domains in different phase state, the liquid crystalline and the gel phase, or the liquid-expanded and the liquid-condensed phase [11]. Another possibible explanation is that during the rearrangement of the film molecules the increased packing in one region leaves the neighbour region without enough lipid material. That is, in the case of supported films, if the film tends to increase its thickness it must decrease its area thus leaving uncovered electrode regions.

In order to check this assumption we performed experiments with addition of divalent cations in the electrolyte before the contact of the two interfaces and the formation of the film (before stage 4 described in the Experimental section) thus allowing the lipid molecules to rearrange freely at the electrolyte/organic solution interface. After such film is formed its blocking properties are almost the same as the untreated with divalent cations films. Results obtained with Mg^{2+} are presented in Fig. 2. The redox current (curve 1, a) is much smaller in comparison with the same quantity Mg^{2+}, acting on previously formed film (curve 2, a). The voltammogram (curve 1, b) is almost the same as the voltammogram obtained without any Mg^{2+} (curve 3, b). Apparently, when the film is formed in the presence of previously added Mg^{2+} the number of defects is no bigger than in the film formed over a pure 0.1 M KCl solution.

Moreover a further increase of divalent cation concentration has now a very little effect. In Fig. 3 the values of the current at a potential of 400 mV during the positive scan of the voltammogram are plotted. In the case of films formed in the absence of divalent cations their addition results in significant increase of the current. The reaction of the film is most prominent for the first portion of 1 mM Ca^{2+} and reaches saturation at 3 mM Ca^{2+}. The shape of curve 1, as well as, the saturation concentration is quite similar to the concentration dependencies obtained by other authors [5,9]. This concentration pattern is similar to the well-known Langmuir-type adsorption isotherms of divalent cations bound to phosphatidylcholine bilayers [29]. There is also a correspondence between this dependence (curve 1) and the concentration dependencies of the film capacitance decrease and the resistance increase, reported in our previous work [15]. When the film is formed in the presence of 1 mM Ca^{2+}, the subsequent addition of Ca^{2+} has little impact on the film (curve 2). At each concentration the current is much smaller with respect to the other film. There is a slight current increase with the rise of the Ca^{2+} quantity from 1 mM to 5 mM but even at the highest concentration the respective current does not exceed the current obtained without any Ca^{2+} for the other film (the first point of curve 1).

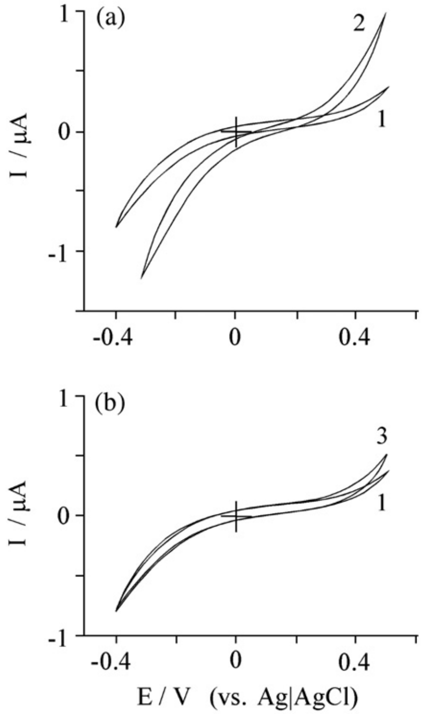

Fig. 2. Cyclic voltammograms of 1 mM $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl on lipid film coated GCE: (a) curve 1 — in the presence of 3 mM Mg^{2+} added before the film formation; curve 2 — in the presence of 3 mM Mg^{2+} added after the film formation; (b) curve 1 — in the presence of 3 mM Mg^{2+} added before the film formation; curve 3 — without divalent cations in the electrolyte solution. Scan rate 2 V/min.

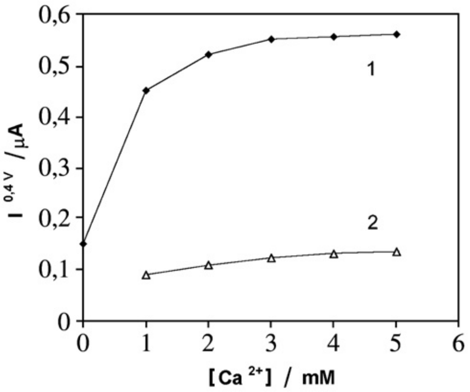

Fig. 3. Dependence of the oxidation current at 0.4 V on the Ca^{2+} amount during the positive scan of cyclic voltammograms of 1 mM $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl on lipid film coated GCE: curve 1 — film formed without initial amount of divalent cations: curve 2 — film formed in the presence of 1 mM Ca^{2+}.

These results suggest that the state and the behaviour of the films depend not only on the divalent cations concentration but also on whether the cations act on the lipid/electrolyte solution surface before or after the film formation. There is no reason to expect the lipid–cations interaction to be different for the two cases. In our opinion these results support the proposed mechanism of structural changes in the films after the action of the divalent cations. Namely, this is the increased packing order of the lipid molecules. The lipid–cation interaction itself is not a direct reason for the observed defects in the films. It is rather the reorganization in the entire lipid film structure caused by the “condensation” of the lipid molecules, which is the reason. When the divalent cations interact with the lipids before the film formation the lipids reorganization on the lipid/electrolyte solution surface could be accompanied by additional adsorption of lipids from the film-forming lipid solution. The possibility for such additional adsorption assures a continuous arrangement of lipid molecules and defect-free film structure even after the interaction with the divalent cations.

Finally, we tested directly the suggested strengthening of the lipid structure by applying a negative potential to the films. We

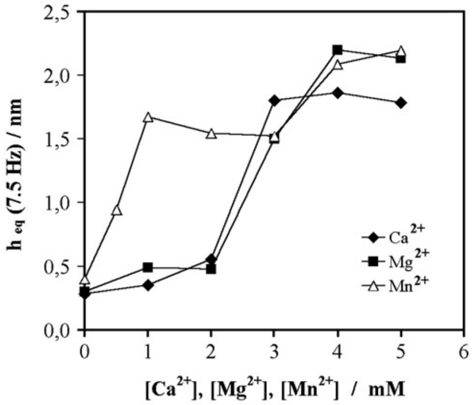

Fig. 4. Apparent film thickness at 7.5 Hz after application of −0.8 V to the films at different concentrations of divalent cations (Mn^{2+}, Mg^{2+}, Ca^{2+}) added to the electrolyte solution before the film formation.

chose a potential of −0.8 V that destroys the films at normal conditions. The state of the film is monitored by its equivalent thickness evaluated from the film capacitance (Fig. 4).

From these results it is evident first that the films formed without divalent cations in the solution cannot stand the negative potential. The evaluated thicknesses of a few angstroms suggest the lack of intact film on the electrode surface. At a certain quantity of divalent cations in the electrolyte depending on the type of the ion the films remain stable with a thickness of the order of monolayer. When the films are stabilized their thickness is similar for the three different ions but different concentrations of Mn^{2+}, $Ca^{2+}$and Mg^{2+} are necessary to provoke the stabilization. With Mn^{2+} it is 1 mM that is sufficient, while for Ca^{2+} and Mg^{2+} at least 3 mM are necessary. This is in accordance with our previously reported results that the impact on the lipid structure is more pronounced for Mn^{2+} while the Ca^{2+} and the Mg^{2+} have smaller commensurate effect [14,15].

In our opinion these results deserve additional emphasizing. Structural transitions in supported films at negative potentials have been reported in the literature [6,16,30–33] for different films on different supports. Bizzotto et al. [33] described these processes as a consequence of potential-dependent competition for adsorption onto the electrode by the film's organic molecules and the solvent (water). Scanning the potential in the negative direction the film becomes first increasingly hydrated and porated. At more negative potentials a reorientation of lipid–water structures may occur, and finally, the organic molecules become desorbed because water is preferred on the electrode surface [27,33]. In some cases these structural transitions are reversible, while in other cases the negative potential provokes irreversible changes in the structure of the films. In general the first transition processes in lipid films onto solid electrodes become at potentials more negative than −0.4/−0.6 V [31,32]. In our experiments, the potential of −0.8 V provokes irreversible perturbation in the films' lipid structure in pure electrolyte solution. The addition of divalent cations has a stabilizing effect and prevents the films from this irreversible perturbation. It should be noted that a stabilization effect of Ca^{2+} on phospholipids monolayers onto Hg surface has been reported in [16,33]. The results presented here give us motivation to suggest that small amounts of divalent cations in the electrolyte solution allow the formation of films with more tightly packed lipid structure and that such films have an extended electric potential range of stability. Such property of the films could be useful in various fields of the investigations and applications of model membrane systems.

4. Conclusions

The presented impedimetric and voltammetric results suggest significant influence of Mn^{2+}, Mg^{2+}, and Ca^{2+} on the packing order of the lipid structure of the glassy carbon-supported films.

In general, the incorporation of divalent cations within the films results in strengthening of the structure and improvement of the dielectric properties of the films. However, the state and behaviour of the films depend on the moment of addition of the divalent cations — before or after the film formation. When divalent cations act on previously formed films the reorganization of the lipid structure may cause some defects in the films. This can be avoided if the divalent cations are added before the formation of the film. In the presence of divalent cations in the electrolyte solution the electric potential range of stability of the films could be extended as shown at negative potentials.

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