

Interaction of thin wetting films of lecithin with some divalent cations

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Abstract

The impact of some divalent cations on the structure of a model membrane system, comprising wetting lipid films, is assayed in this work. The results from impedimetry suggest prominent structural changes upon the addition of the discussed ions to the electrolyte solution contacting the film. These changes are manifested by the increase of resistivity of the films as well as by the decrease of capacitance dispersion. In accordance with other data in the literature, manganese (Mn^{2+}) turned out to have an effect greater than those of Mg^{2+} and Ca^{2+} .

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1. Introduction

The great significance of divalent cations for the cell life cycle is out of the question. Especially, the impact on the membrane structure and functions is well established [1,2]. Besides their involvement in the attachment of peripheral peptides to the membrane, the potential of these species to disturb the lipid head group effective size, hence to induce perturbations in the bilayer arrangement, has also been outlined [3,4]. The active research of Langmuir–Blodgett (LB) monolayer and multilayer of fatty acids [5,6] and phospholipids [7,8] also suggested significant influence of divalent cations on the packing order and structure of the films. Thus, the importance of investigations in this direction with the aid of biomembrane analogs including spontaneously assembled solid-supported lipid films becomes clear [9–11].

Because of some advantages, such as durability, easy managing, simple technical appliance, etc., solid-supported lipid films turned out to be a very promising model membrane system [12,13]. Due to a specific way of preparation, they represent a good compromise between structural stability and flexibility, ensuring proper molecular dynamics.

Recently, the incorporation of Mn^{2+} in such type of films and its application for the mediation of redox processes in the electrolyte solution was reported [14]. In the present work, the interactions of Mn^{2+} and some other divalent cations (Mg^{2+} , Ca^{2+}) with wetting lipid films are explored.

2. Experimental

Supported lipid films employed in the work were obtained through the thinning of lipid solution deposited between an electrolyte phase and the working glassy carbon electrode (GCE) as described earlier [15]. They represent a particular branch of wetting films, constituting a three-phase system of the type solid–liquid–liquid. In other terms, it consists of an electrode, a supported lipid film and electrolyte solution. This system is asymmetrical in the sense that the lipid film is in contact with a solid phase from the one side and with a liquid from the other. Such architecture enables the analysis of the electrolyte/lipid interface by electrochemical methods.

The formation and study of the properties of GCE supported lipid films was accomplished by the aid of an electrochemical cell schematically shown in Fig. 1. The electrochemical cell was filled with 0.1 M KCl (Sigma, USA) as an electrolyte solution. GCE is used as working electrode and a single junction Ag/AgCl as reference

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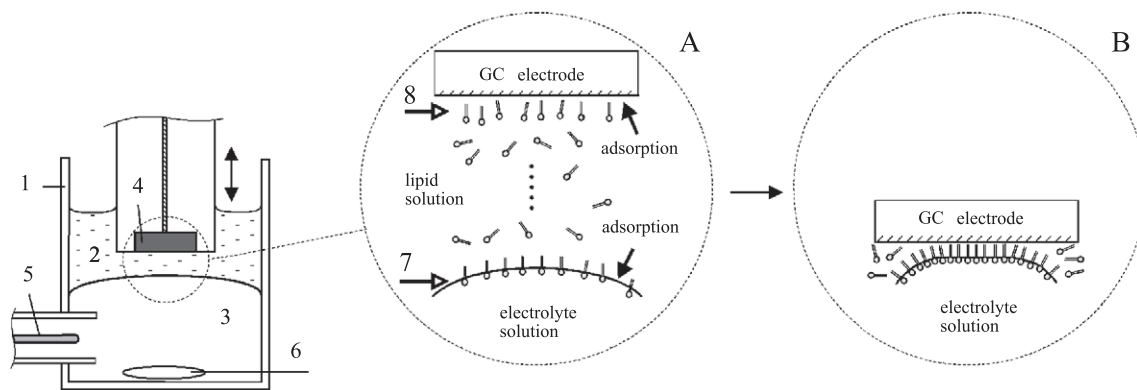


Fig. 1. The experimental set-up and the method of preparation of solid-supported liquid films of lipids. (1) Cuvette; (2) forming solution of lipid; (3) electrolyte (0.1M KCl); (4) solid support (working glassy carbon electrode, GCE); (5) Ag/AgCl reference electrode; (6) magnetic stirrer. (A) Formation of two interfaces with adsorbed lipid molecules: (7) electrolyte/lipid solution interphase boundary; (8) lipid solution/substrate (GCE) interphase boundary. (B) GC-supported lipid monolayer obtained by the approach and contact of the two interfaces and subsequent process of film thinning.

electrode. The working area of the electrode was 7.07 mm². The liquid films deposited onto the working electrode were prepared from forming solution containing natural lecithin (Bell Pharmacal, USA) dissolved in *n*-hexane (Sigma) with concentration 10 mg/ml. 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. Thus, uniform homogeneous films of the monolayer type were produced. The techniques of formation as well as the structural characteristics of the films exhibiting the features of monomolecular arrangement in the final stable state are described in details elsewhere [16].

Divalent cations (Mn²⁺, Mg²⁺, Ca²⁺) were added to the electrolyte contacting the film as small portions of MnCl₂, MgCl₂, CaCl₂ solutions to give final concentration of 1 mM.

The electrical parameters of the films were obtained by the aid of lock-in nanovoltmeter UNIPAN, 232B (Poland) at zero potential vs. Ag/AgCl applying measuring signal with 50 mV ac amplitude.

Cyclic voltammetry was employed in this study for determination of charge transfer between the GCE and the solution, hence giving some information for the packing order in the films. Sodium ascorbate (C₆H₇NaO₆) and equimolar mixture of potassium ferricyanide (K₃[Fe(CN)₆]) and potassium ferrocyanide (K₄[Fe(CN)₆]) were used as electroactive species added to the electrolyte. The measurements were made with commercial polarograph (OH-105, Hungary).

3. Results and discussion

The films prepared by the described above technique exhibit certain blocking properties to the heterogeneous electron transfer [15] suggesting a proper covering of the

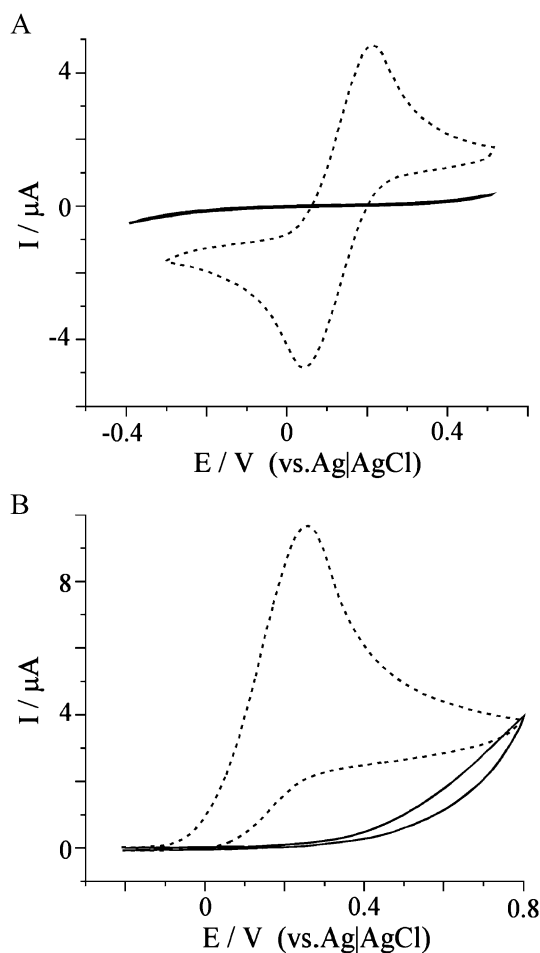


Fig. 2. Cyclic voltammograms of (A) 1 mM Fe(CN)₆^{3-/4-} in 0.1 M KCl and (B) 1 mM sodium ascorbate (C₆H₇NaO₆) in 0.1 M KCl. Dashed lines: bare uncovered GCE; solid lines: GCE covered with a thin lecithin film; scan rate 2 V/min.

electrode surface. This is clearly demonstrated in the presence of hydrophilic redox probes in the solution. Cyclic voltammograms of $\text{Fe}(\text{CN})_6^{3-/4-}$ and sodium ascorbate are given for demonstration in Fig. 2. In the presence of lipid film covering the GCE (solid lines) no redox peak currents are observed, indicating kinetically limited reactions. The current increase at high overpotentials could be attributed to the redox reactions of the electroactive probes outside the film surface. The blocking properties of the films are treated in more details in Refs. [12,14,15].

The impedance of supported liquid films is a convenient physical quantity characterizing their condition. In this work, the changes in the equivalent parallel resistance R and capacitance C are used as a measure of the influence of divalent cations on the state of the films. The results are shown in Fig. 3 where the experimental values of R and C at several frequencies are presented.

First, the frequency dependence of both R and C should be noted. Other authors also reported frequency dependence of the electrical parameters of solid-supported lipid films (e.g., Refs. [17,18]). In our case, both R and C are well approximated with power functions [19]. For the description of such film features, an equivalent electrical model comprising constant phase angle elements (CPE) was previously [19,20] proposed. CPE is often used for fitting the imped-

ance of different types of supported lipid films [21–23]. The impedance of the CPE is given by:

$$Z_{\text{CPE}} = A(j2\pi f)^{-a} \quad (1)$$

where $j = (-1)^{1/2}$, f is the frequency and A and a are the parameters characterizing the CPE. For ideal capacitance $a = 1$; $A = C^{-1}$. For ideal resistance $a = 0$; $A = R$. At intermediate values of the parameter a , the impedance Z_{CPE} can be represented as combination of apparent equivalent parallel capacitance (C_{CPE}) and resistance (R_{CPE}) according to the relation:

$$\frac{1}{Z_{\text{CPE}}} = \frac{1}{R_{\text{CPE}}} + j2\pi f C_{\text{CPE}} \quad (2)$$

From Eqs. (1) and (2) and using the equation $j^a = \cos(a\pi/2) + j \sin(a\pi/2)$, the expressions for C_{CPE} and R_{CPE} can be found:

$$C_{\text{CPE}} = \frac{\sin(a\pi/2)}{A} (2\pi f)^{a-1} \quad (3)$$

$$R_{\text{CPE}} = \frac{A}{\cos(a\pi/2)} (2\pi f)^{-a} \quad (4)$$

As can be seen, both C_{CPE} and R_{CPE} are power functions of the frequency f , decreasing with the increase of f .

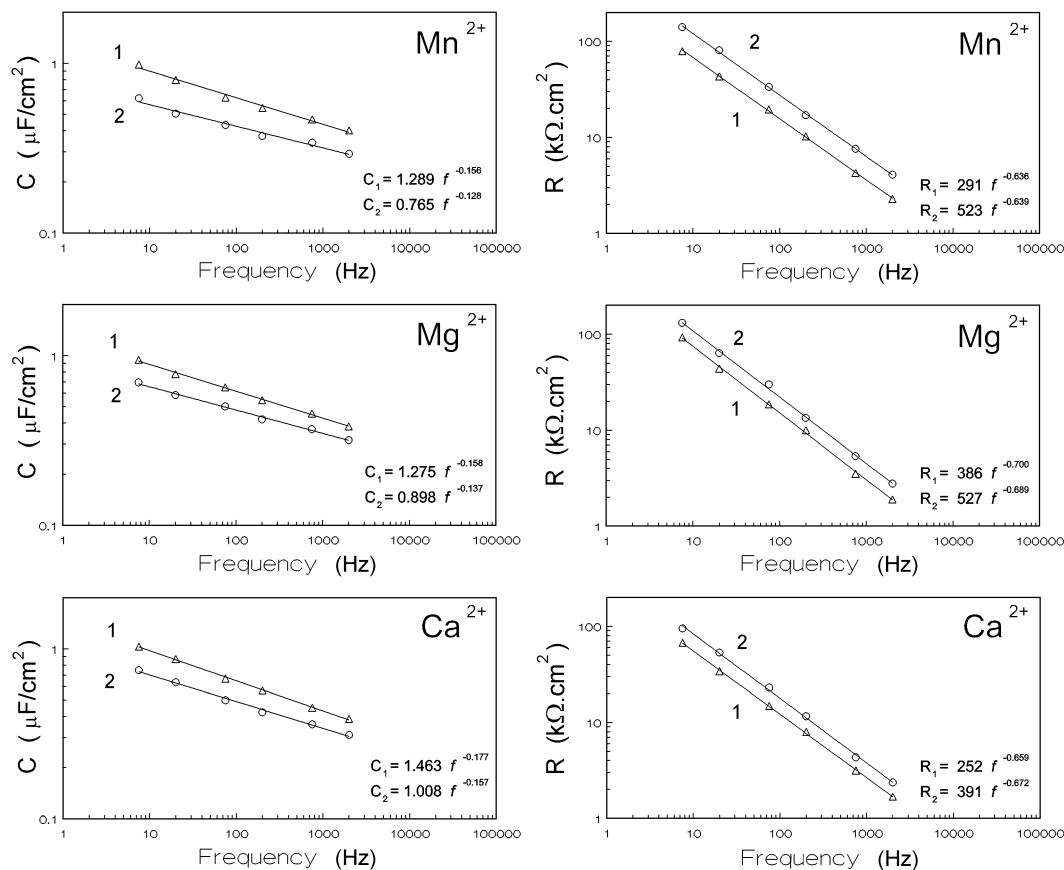


Fig. 3. Equivalent parallel capacitance (C) and resistance (R) of solid-supported lipid film before (curves 1) and after addition (curves 2) of 1 mM divalent cations (Mn^{2+} , Mg^{2+} , Ca^{2+}) to the electrolyte solution. The markers represent the experimental data, the lines are best fits.

Table 1

Relative changes in equivalent capacitance (C), resistance (R), resistivity (ρ), and capacitance dispersion ($a - 1$) after addition of 1 mM divalent cations (Mn^{2+} , Mg^{2+} , Ca^{2+}) to the electrolyte solution

Cation (1 mM)	$\Delta C/C_1$ (%)	$\Delta R/R_1$ (%)	$\Delta \rho/\rho_1$ (%)	$\Delta(a-1)/$ (a_1-1) (%)
Mn^{2+}	– 31.8	76.5	20.4	– 18.4
Mg^{2+}	– 22.1	44.6	12.7	– 13.3
Ca^{2+}	– 24.1	46.2	10.9	– 11.3

$\Delta C = C_2 - C_1$; $\Delta R = R_2 - R_1$; $\Delta \rho = \rho_2 - \rho_1$; $\Delta(a-1) = (a_2 - 1) - (a_1 - 1)$. The values are averages of the data for all frequencies.

The experimental data of the films C and of R represented in Fig. 3 are approximated with power functions that appear as line in the logarithmic scale. Both C and R decrease with the frequency according to the CPE behaviour. At lower frequencies, however, the values of C (approximately $1 \mu\text{F}/\text{cm}^2$) and R (approximately $100 \text{ k}\Omega \text{ cm}^2$) are in reasonable agreement with those reported for other supported lipid films [23–26]. Typical values of the electrical resistance of solid-supported membranes are from 1 to $1000 \text{ k}\Omega \text{ cm}^2$ [21,23,24]. The capacitance of supported bilayer membranes obtained from lipids dissolved in n -alkanes is 0.3 – $0.6 \mu\text{F}/\text{cm}^2$ [25]. Monolayers usually exhibit capacitance in the range 1 – $2 \mu\text{F}/\text{cm}^2$ depending on the support and the technique of preparation. For example, the capacitance of self-assembled alkanethiol monolayers on gold is approximately $1 \mu\text{F}/\text{cm}^2$ [25], while for phospholipid monolayers transferred from an air/electrolyte solution interface on mercury support, C is in the range of 1.6 – $1.8 \mu\text{F}/\text{cm}^2$ [26].

Concerning the interaction of the film with the divalent cations the following can be stressed out. The addition of divalent cations (Mn^{2+} , Mg^{2+} , Ca^{2+}) to the electrolyte contacting the film results in a readable change of C and R for all frequencies (Fig. 3). The increase of the resistance and the decrease of the capacitance are clearly observed. The changes in the slope of the C suggest a decrease of the capacitance dispersion as well. According to the fit, the absolute value of the capacitance power factor ($a - 1$) for treated films turns out to be with 10 – 20% smaller. On the other hand, the difference in the action of the three investigated ions is quite prominent. While Mn^{2+} has greater impact, the effects of Mg^{2+} and Ca^{2+} are commensurable. In order to demonstrate these properties of the disturbed films more clearly, a table (Table 1) with the relative changes in the R , C , resistivity (ρ), and capacitance dispersion ($a - 1$) is given. The effective resistivity (ρ) is estimated for each frequency according to the relation $\rho = R/h$, where h is the apparent film thickness determined from the experimental values of C using the equation for planar capacitor [27]:

$$C[\mu\text{F}/\text{cm}^2] = 8.85 \frac{\varepsilon_l}{h[\text{\AA}]} \quad (5)$$

Here, $\varepsilon_l \approx 2.1$ is the dielectric constant and h is the thickness of the hydrophobic region of the film.

The considerable increase of the effective films resistivity upon the addition of Mn^{2+} can be stressed out. As known, this parameter is independent on the film thickness, which allows a perturbation in the molecular ordering of the films to be supposed. Such hypothesis is in consonance with the changes of R and C . The decrease in C , although prominent, is not large enough to suggest phase transition from a lamellar to a hexagonal H_{II} phase. A plausible explanation of the presented results can be associated with a notion for more tightly packed lateral structure and hampered molecular mobility within the film. This situation definitely would lead to lower capacitance dispersion as well [20]. There is a good agreement in the literature about the localization of divalent cations in lipid bilayers. The binding with the phosphate groups is widely accepted [28]. The presence of cations in the polar region of the phospholipids causes an inevitable change of the form and effective size of hydrated head groups, hence generating the discussed effect. In this respect, an interconnection of lipid molecules via divalent cations proposed by some authors [29] is also worth mentioning.

Finally, it should be noted that the addition of cations of some transition metals, including Mn^{2+} , to the electrolyte around a bilayer lipid membrane (BLM) causes a drastic increase of its resistance [27]. Furthermore, earlier investigations of cations adsorption to phospholipid bilayers revealed the following sequence in their binding: Mn^{2+} , Mg^{2+} , Ca^{2+} [30]. In our opinion, these facts are in accordance with the above-reported results.

4. Conclusions

The glassy carbon-supported lipid films are sensitive to the presence of divalent cations in the electrolyte ambience. Their dielectric properties are significantly improved by the addition of these species. The films under consideration exhibit features similar to other conventional artificial membrane analogs. Thus, they can serve as a reliable model system in the investigation of the mode of action of different membrane active substances as well as play the role of sensitive interfaces in the sensor construction.

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