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Electrochemical investigations of cholesterol enriched glassy carbon supported thin lipid films

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Abstract

The formation and study of stable cholesterol enriched thin lipid layers onto the surface of glassy carbon electrode is reported in this work. The method of formation relies on additional thinning of wetting films by electrostriction. Electrochemical techniques based on the concepts of impedance and voltammetry are used to explore the films' features. The impedance data reveal a substantial change of relaxation characteristics of the modified films. In this respect, opportunities for the evaluation of the films' stage based on the approximation with 'constant phase angle element' are discussed. The possible final structure of the films, as well as, their relevance for development of sensor elements are briefly viewed.

Keywords: Solid supported thin liquid films of lipids; Glassy carbon; Cholesterol; Impedance; Voltammetry

1. Introduction

The preparation of stable supported thin liquid layers of lipids by the thinning of wetting films [1,2] proved to be an adequate technique of lipid deposition alternative to other well-established methods. It was successfully demonstrated for glassy carbon and other supports. The electrical parameters of thus modified substrate surfaces were assayed in the light of their potential for sensor construction. Voltammetric and impedimetric studies of these and other similar systems revealed promising aspects in this direction [3]. The results from voltammetry suggested that solid supported lipid films are relatively compact and with good blocking properties to heterogeneous electron transfer between the electrode and the analyzed solution [3,4]. This makes them of special importance for the realization of sensors with electrochemical signal transduction.

Furthermore, the significant impact of the cholesterol contents on the structure and properties of biological membranes and their analogs is well-established [5]. As one of the common building species of the plasma membranes cholesterol affects in a great extent their fluidity, thus modulating such specific functions as transport and reception [6–8]. In the present study we have investigated the changes in solid supported films

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after addition of cholesterol to the forming solution of lipid, as well as their potency in the construction of sensitive interfaces. In this regard, some possibilities have already been disclosed [9]. Here an attempt is made for further clarification of the cholesterol effect on the relaxation parameters of modified thin lipid films.

2. Materials and methods

2.1. Formation of solid supported thin liquid films of lipids

The liquid films deposited onto the working electrode were prepared from natural lecithin (Bell Pharmacal Corp., USA), DLPC (L-α-phosphatidylcholine dilinoleoyl, SIGMA Chemical Comp., USA) and DPPC (L-α-phosphatidylcholine dipalmitoyl, SIGMA Chemical Comp.) dissolved in a mixture of 90%(volume) *n*-hexane (SIGMA Chemical Comp.) and 10% (volume) chloroform, to give the respective concentrations. Cholesterol (5-cholesten-3β-ol, $C_{27}H_{45}OH$, Kodak Comp., USA) was dissolved in the same solvent to a concentration of 10 mg/ml. This solution was mixed with the lecithin solution in different ratios to give the forming solution before each modified film preparation.

The fabrication of the solid supported films is achieved by a thinning of wetting layers of lipid solution with the set-up shown in Fig. 1. As previously described [2,4], the essence of the method is the preliminary formation of two interfaces (electrolyte/lipid solution and lipid solution/substrate) with self-assembled monolayers of lipid molecules adsorbed on the interphase boundaries. The process of thinning is initiated as the two interfaces are gradually brought into a contact by carefully pressing the substrate (working electrode) against the electrolyte/lipid meniscus. When an external DC potential is applied to the electrodes of the cell a mechanical compressing force arises in the solution. The phenomenon is known as electrostriction [10]. This compressing force overcomes the positive disjoining pressure [11] in the film and substantially promotes the thinning.

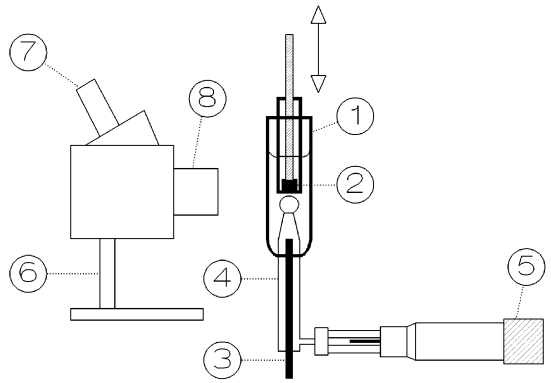

Fig. 1. Schematic view of the experimental set-up for generation and electrochemical studies of solid supported liquid films of lipids. (1) cuvet with forming solution of lipid; (2) solid support (working GCE); (3) Ag|AgCl reference electrode; (4) capillary with electrolyte (0.1 M KCl) for the formation of electrolyte/lipid meniscus; (5) microsyringe for the electrolyte; (6) binocular microscope; (7) eye-piece; (8) objective. The main parts are in different scale.

It should be said, that supported liquid films of lipids could also be prepared without application of external electric fields. In this case the process of spontaneous self-thinning leads to generation of stable, relatively 'thick' films with final equilibrium thicknesses of the order of 100–200 Å [2]. However, if external voltage is imposed to such film it undergoes an irreversible transition to a new stable state [9]. The numerous impedance data indicate a thickness on the order of 20 Å (for the case of plain lecithin and GC support) and a pretty high film resistance in this new final state. This clearly suggests that the film represents a monolayer of lipid molecules arranged onto the GC surface. Moreover, the removal of the external DC potential, after the film conversion to monolayer, does not change the thickness, which can be explained with the higher magnitude of the adhesion at distances closer to the substrate surface [7].

2.2. Electrochemical technique

The electrochemical parameters of the films are studied in a two-electrode cell filled with 0.1 M KCl (Potassium Chloride, SIGMA Chemical Comp.) as electrolyte solution. Glassy carbon elec-

trode (GCE) is used as working electrode and a single junction Ag|AgCl as reference electrode, so the electrochemical cell can be represented as:

Ag|AgCl|0.1 KCl|LF|GC

where LF is the liquid film of lipid molecules formed onto the surface of the working GCE. The cell construction is given in Refs. [2,4].

Commercially available GCE for electrochemical analysis is playing the role of a rigid support for the liquid films. The working area of the electrode is 0.0707 cm^2. The GCE surface was conditioned by fine polishing and cleaning before each measurement. No other pretreatments of the electrode have been done.

Voltammetry is 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. Ascorbic acid ($C_6H_8O_6$, Pharmachim, Bulgaria) at concentration of 1 mM is used as electroactive species added to the electrolyte. The measurements are made with commercial polarograph (OH-105, Hungary). The sweep rates as well as the potential range are indicated in the figures and discussed in the text.

2.3. Impedance measurements and equivalent circuits

In the present work impedimetric technique is used as an analytical tool for examination of the films in their different stages. The impedance is measured by the aid of a WAYNE KERR 6425 multi-bridge. The set-up, method of measurement and calculations are described in more details elsewhere [1,2].

A circuit with equivalent parallel resistance $R_p(f)$ and capacitance $C_p(f)$ is ascribed to the experimentally obtained impedance of the film Z_f at each frequency f (Fig. 2a). For different types of films Z_f is approximated with a model combining frequency-independent parallel resistance R_0 and capacitance C_0 with constant phase angle elements (CPE) as shown in Fig. 2b.

A lot of data from impedimetric analyses suggest that, with respect to their relaxation characteristics, the solid supported lipid films can be well represented by the model comprising the so

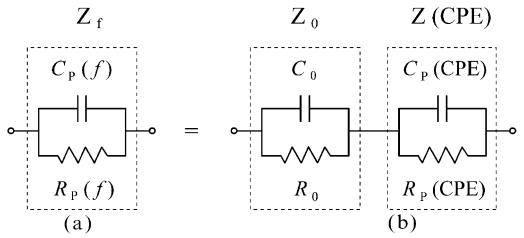

Fig. 2. Equivalent circuits of the supported lipid films. (a) $R_p(f)$ and $C_p(f)$ are the experimentally obtained equivalent frequency-dependent parallel resistance and capacitance; (b) the most common model used to fit the experimental data; $Z_f = Z_0 + Z(\mathrm{CPE})$; R_0 and C_0 are frequency-independent parallel resistance and capacitance; $R_p(\mathrm{CPE})$ and $C_p(\mathrm{CPE})$ are the parameters of the CPE.

called CPE [12]. Essential for a CPE is the identical dependence of the real and imaginary parts on the frequency, hence the impedance diagram (ImZ vs. ReZ) represents a line. The impedance of the CPE is given by

$$Z(\mathrm{CPE}) = A(j\omega)^{-a} \tag{1}$$

where $\omega = 2\pi f$ is the radial 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 the apparent equivalent parallel capacitance and resistance are:

$$C_p(\mathrm{CPE}) = A^{-1} \sin(a\pi/2)\omega^{a-1} \tag{2}$$

$$R_p(\mathrm{CPE}) = A \cos^{-1}(a\pi/2)\omega^{-a} \tag{3}$$

Physically, on molecular level, the CPE behaviour has been most often associated with a constant dissipation of energy during the charge motion within the dielectric upon the action of external alternating field. However, still there is no exact theory on the question. Nevertheless, in the case of supported films the concept of CPE is very useful, at least over the range of relatively low frequencies used in our work.

The respective films' thicknesses have been obtained by the next simplified relation approximating the lipid film with a planar capacitor [13]:

$$C_p\left[\mu F/\mathrm{cm}^2\right] = 8.85\frac{\varepsilon_l}{h[\text{Å}]} \tag{4}$$

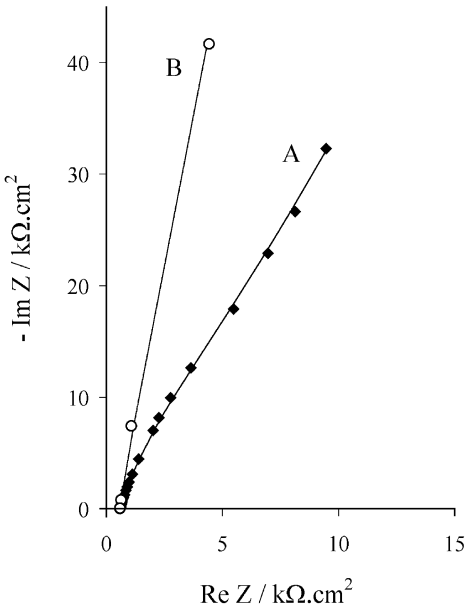

Fig. 3. Impedance diagrams of plain lecithin film (A) and cholesterol modified film (B). The modified film is generated from a solution with lipid/cholesterol ratio of 1:1 (wt/wt). Experimental data for the modified film (open circles) are obtained at several frequencies: 20, 120, 1200, 12 000, 60 000 Hz. The curve for the plain lecithin film (A) is the best fit approximating the film with a circuit combining frequency-independent parallel resistance and capacitance with CPE connected to them in series ($Z_f = Z_0 + Z(\mathrm{CPE})$). The best fit for the modified film (B) is obtained with frequency-dependent (CPE) parallel resistance and capacitance ($Z_f = Z(\mathrm{CPE})$). The two films are with approximately the same thickness (100 Å).

here C_p is the specific capacitance of the film, h is its thickness and $\varepsilon_l \approx 2.1$ [14] is the dielectric constant of the hydrophobic core of lipid tails mixed with organic solvent and eventually cholesterol.

3. Results

3.1. Impedimetric analysis

In these experiments impedance dispersion is used to differentiate the behaviour of films prepared from plain lipids and those modified with cholesterol. The results shown in Fig. 3 are obtained with a plain lecithin film (A) and a modified film (B) generated from a solution in which the ratio lipid:cholesterol is 1:1 (wt/wt). The figure represents the widely used impedance diagrams (ImZ vs. ReZ) of the two films.

The curve for the plain lecithin film (Fig. 3A) is the best fit approximating the film with the model shown in Fig. 2b. Such combination turned out to be useful for the description of the relatively 'thick' (100–200 Å) films. As it was shown [2], an impedance diagram of such a film can be regarded as superposition of readily distinguishable parts ascribed to the different film regions. According to these considerations, films possessing unstructured regions (in depth) will always show the prominent segment of the semicircle inherent for the bulk lipid solution featuring frequency-independent R_0 and C_0. On their parts, the ordered regions with more pronounced molecular constraints are represented by a 'linear' CPE behaviour. In this respect, it is evident that for the cholesterol-containing film (Fig. 3B) the semicircle part of the diagram is completely absent. That is why in this case the film impedance is approximated only with CPE consisting of frequency-dependent parallel resistance $R_p(\mathrm{CPE})$ and capacitance $C_p(\mathrm{CPE})$. The values of the power factor $a-1$ are -0.09 for the plain lecithin film and -0.03 for the cholesterol enriched film respectively.

In Fig. 4 a comparison between the dispersion of the equivalent capacitances $C_p(f)$ of the two films is made. The smaller slope in the presence of cholesterol is evident. This is connected with a change in relaxation characteristics of the film in direction of dielectric loss decrease.

In order to examine how the impedance dispersion is influenced by the type of phospholipid species experiments were carried out with three different lipids (DPPC, DLPC and lecithin) and with a lecithin/cholesterol mixture. The results are shown as histograms of the parameter $a-1$ in Fig. 5. They are averaged from measurements of several films for each species (indicated in the figure caption) over a range of frequencies from 7.5 Hz to 60 kHz.

Fig. 5 clearly suggests, that the films from unsaturated phospholipid (DLPC) as well as the cholesterol enriched films exhibit lower dispersion.

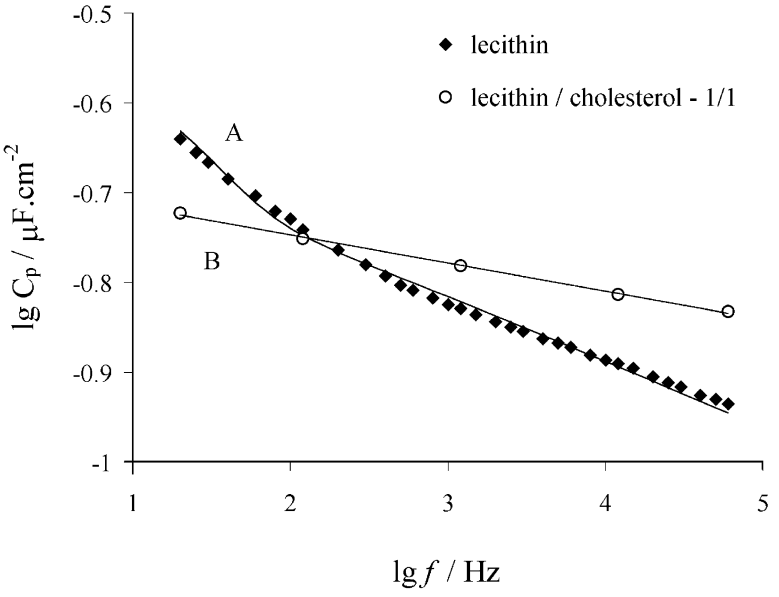

Fig. 4. Logarithmic scale of the capacitance $C_p(f)$ dispersion of the two films from Fig. 3. (A) film without cholesterol; (B) film with 1:1 (wt:wt) lipid/cholesterol ratio. Points indicate the experimental data. Lines represent the fits with the model shown in Fig. 2b.

3.2. Electrostriction treatments

Unlike the plain lecithin films, however, those enriched with cholesterol fail to develop a monolayer under electrostriction up to 1.5 V. This is demonstrated in Fig. 6 where the behaviour of the two types of films is shown. The figure illustrates the dependence of the films' thickness on the applied external voltage. It represents the disjoining pressure isotherm $\Pi(h)$ [15] when equilibration of Π with the pressure caused by electrostriction is achieved, i.e. $\Pi = P = \varepsilon_l \varepsilon_0 U^2 / 2h^2$ [10]. Here U is the external DC potential; h is the film thickness; ε_l is the dielectric constant of the film; ε_0 (8.8542×10^{-12} F/m) is the permittivity of free space.

The film of plain lecithin (Fig. 6a, closed circles) is preliminary generated by a spontaneous thinning and its equilibrium thickness before electrostriction is 200 Å. The application of an increasing external DC potential causes the increase of the disjoining pressure and additional gradual thinning of the film to 60 Å. This process is reversible and the film returns back to its initial state with

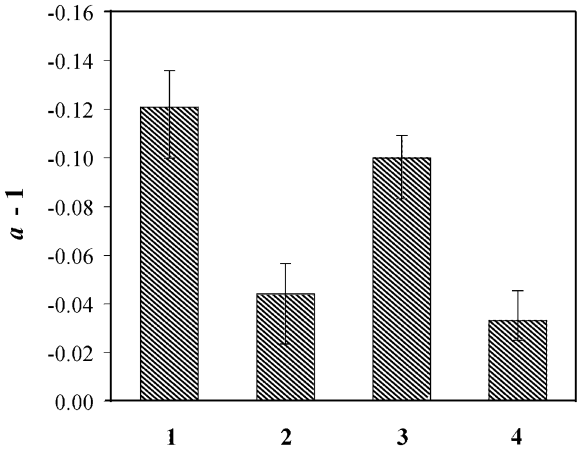

Fig. 5. The dependence of the impedance dispersion on the type of lipid. (1) DPPC, (2) DLPC, (3) lecithin, (4) lecithin/cholesterol mixture. On the ordinate the value of the parameter $a-1$ is given. The results are averaged from measurements of four films for each species over a range of frequencies from 7.5 Hz to 60 kHz.

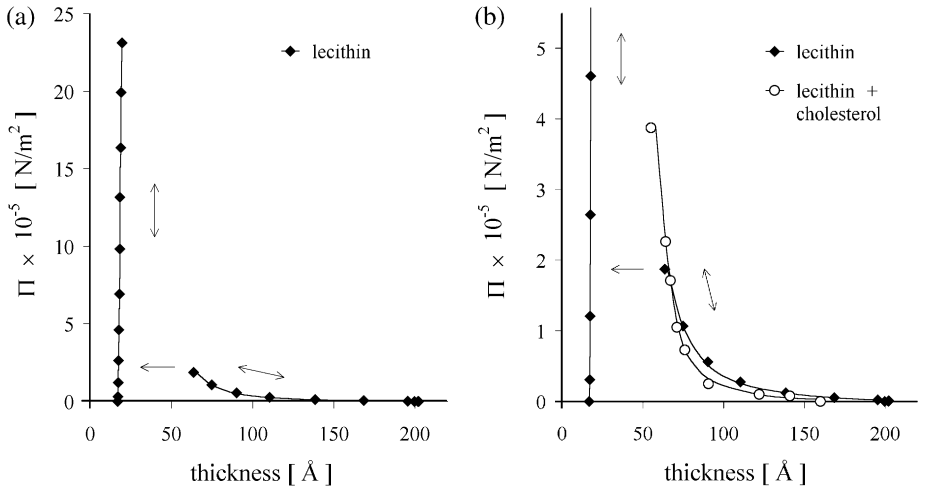

Fig. 6. Isotherms of the disjoining pressure $\Pi(h)$ (equal to the pressure caused by electrostriction $P=\varepsilon_l\varepsilon_0U^2/2h^2$). (a) Isotherm obtained with a supported film of plain lecithin (5 mg/ml) during its evolution to the monolayer stage; (b) enlarged view of the reversible part of the isotherm from (a). Open circles denotes isotherms of cholesterol containing films which has been preliminary develop to an equilibrium thickness of 200 Å; U is the external DC potential; h is the film thickness; $\varepsilon_l=2.1$ is the dielectric constant of the film; $\varepsilon_0=8.8542\times10^{-12}$ F/m is the permittivity of free space.

the decrease of the applied voltage passing through the same points without any visible hysteresis. However, the compression of the film by electrostriction below 60 Å results in irreversible 'jump' to 20 Å where its thickness is already independent of the voltage. The sharp change in the thickness indicates structural rearrangement to monolayer [16].

Films prepared by the same as above-mentioned procedure, but from forming solution containing cholesterol and phospholipid (Fig. 6b, open circles) show different features in comparison with plain lecithin films. As seen, their disjoining pressures fit the reversible part of the isotherm, but the increase of the potential up to 1.5 V cannot decrease the thickness below 60 Å, i.e. does not cause a transition to monolayer.

It should be noted additionally that in accordance with thermodynamic considerations of wetting films (given as a main condition for the film stability ($\partial\Pi/\partial h<0$; [15,17]), it is clear that the isotherms for both types of films consist only of stable branches.

Here an interesting observation is worth mentioning. After the electrostriction treatment of a cholesterol enriched film has been accomplished, a pronounced amount of cholesterol material turns out to be deposited onto the GCE surface. This is evidenced by enhanced contact angles for electrolyte solutions, as well as, by the hampered redox reactions at 'plain' GCE when the latter is transferred to a new measuring cell (the results not shown). Moreover, in this case it is impossible to thoroughly clean the GCE surface with solvents in which the cholesterol is fairly well soluble. Detergents are also ineffective and polishing pretreatment is necessary to completely remove the cholesterol remnants.

3.3. Voltammetric studies

With the application of linear sweep voltammetry (LSV) an attempt was made to assess in some extent the structural changes in the films upon modification with cholesterol. The results are shown in Fig. 7 where representative voltammograms of ascorbic acid (1 mM in 0.1 M KCl) obtained with GCE covered with different type of films are given. A voltammogram (curve 1, Fig. 7a) with the oxidative peak of ascorbic acid at uncovered working GCE is shown for comparison.

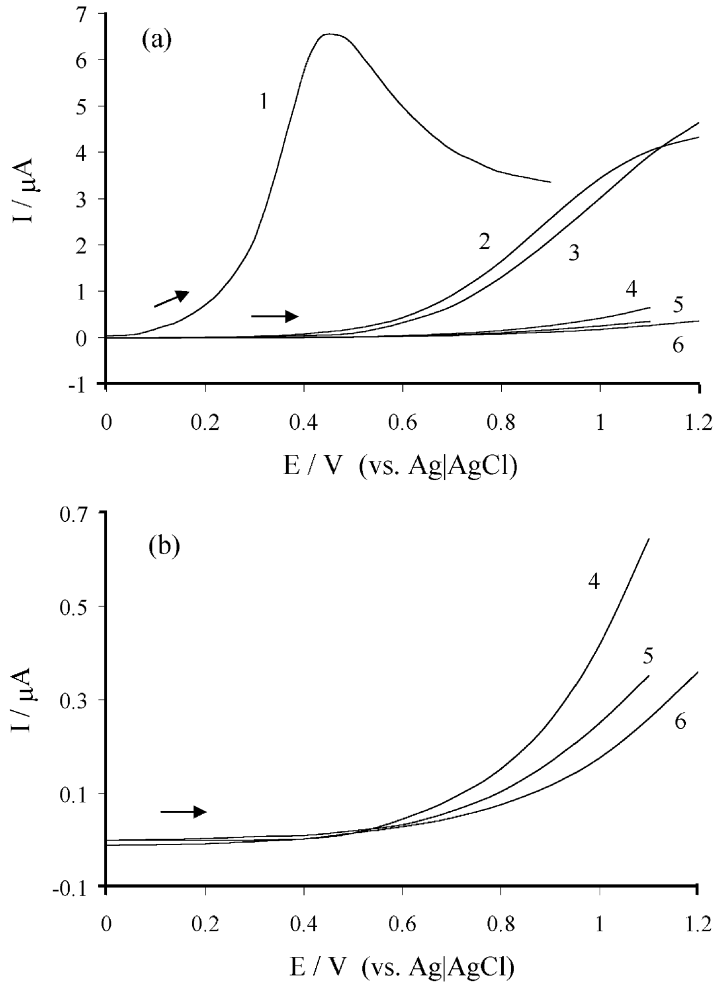

Fig. 7. (a, b) Representative voltammograms of ascorbic acid (1 mM in 0.1 M KCl) obtained with GCE covered with different type of films; curve 1—voltammogram of the oxidative peak of ascorbic acid at uncovered working GCE; curve 2—voltammogram of the ascorbic acid oxidation at GCE covered with monolayer of DLPC; curve 3—voltammogram of the ascorbic acid oxidation at GCE covered with monolayer of lecithin; curve 4—voltammogram of the ascorbic acid oxidation at GCE covered with 'thick' (100 Å) film of DLPC; curve 5—GCE covered with 'thick' (100 Å) film of pure lecithin; curve 6—voltammogram of the ascorbic acid oxidation at GCE covered with 'thick' (100 Å) film generated from a mixture of lecithin/cholesterol (1:1 weight ratio). Scan rate 2 V/min.

Deposition of lipid films on the GCE surface results in a notable change of the parameters of the heterogeneous electron transfer. The curves 2 and 3 (Fig. 7a) represent ascorbic acid oxidation at the electrolyte/lipid interface in the case of DLPC and lecithin monolayers respectively. The curves 4 and 5 (Fig. 7b) are obtained with 'thick' (100 Å) films of DLPC and pure lecithin, respectively. The curve 6 (Fig. 7b) is obtained with a 'thick' (100 Å) film of lecithin/cholesterol (1:1 weight ratio). The shift of the peak current potential of ascorbic acid oxidation to more positive values for the different films is remarkably clear.

As demonstrated earlier [4], the relatively large molecule of the ascorbic acid does not penetrate the hydrophobic core of the film even at monolayer

stage. Due to the film oleophilic barrier, the electron transfer is highly restricted and the reaction is kinetically limited, hence giving a shift in the potentials.

For the aim of our survey, more interesting is the fact that the thick films from unsaturated lipid DLPC (curve 4, Fig. 7b), from pure lecithin (curve 5, Fig. 7b) and from lecithin/cholesterol mixture (curve 6, Fig. 7b) show almost identical electron blocking properties.

4. Discussion

Thus far a lot of investigations has been done on the cholesterol–phospholipid relations in natural and model membranes. Different techniques for the assessment of the cholesterol localization in the bilayer, including X-ray and neutron diffraction [18,19], NMR studies [20], impedimetric analysis [21], differential scanning calorimetry and fluorescence [22], has been employed. Although there is no complete consonance in details, it is clear that the cholesterol positioning is determined in a great extent by the specific hydrophilic lipophilic balance as well as by steric effects associated with the rigid sterol moiety. Currently, the most commonly accepted model represents the cholesterol molecules embedded between the phospholipid acyl chains with the hydroxyl groups penetrating more or less deeply into the hydrophilic zone of the bilayer.

In spite of the difference in the structures of glassy carbon supported films and bilayer membranes the observed features of the cholesterol modified films are in a good agreement with the above scheme. As revealed by the impedimetric analysis, cholesterol-modified films show decreased dispersion which can be connected with an enhanced 'elasticity' of the molecular interactions involving the acyl chains. This is in accordance with earlier observations of decreased number of gauche configurations in DPPC [20,23]. Furthermore, based on a model composed of circuits of frequency-independent elements [24,25], Karolis et al. [26] have shown a decreased capacitance dispersion in cholesterol containing planar bilayer lipid membranes proposing a respective structural scheme. Our results (Fig. 4) are very similar to those reported in Ref. [26]. In addition, Fig. 5 shows comparable dispersion between the cholesterol enriched films and the films from unsaturated phospholipid (DLPC) suggesting the involvement of acyl chain configurations in the mechanism of film relaxation. An assumption can be made in this direction that a decrease in the degree of freedom of intramolecular movements in the hydrophobic part of the film results in a dielectric loss decrease, hence giving lower dispersion. Respectively, the CPE parameter $a-1$ can serve as a quantitative measure of the increased 'rigidity' of the film structure.

The relatively higher fluidity of the solid supported liquid films of lipids have been pointed out as one of their attributes providing an important difference compared to other techniques of lipid deposition such as chemisorbed molecular films or Langmuir–Blodgett (LB) layers [2]. The inability of cholesterol modified films to undergo the transition to monolayer stage under electrostriction (Fig. 6b) should be ascribed to a decreased lateral mobility and lowered potency for molecular rearrangements. Thus, the normal drainage is hampered preventing the further film thinning. This is not so strange and experimental evidences concerning the drastic effect of the cholesterol on the order parameters of the lipid hydrocarbon chains disturbing their interactions and fluidity can be found in Refs. [20,22,23,27–29].

Based on the presented impedance and electrostriction data, a general scheme for the solid supported films modified with cholesterol could be assumed. It is in compliance with the main features of the aforementioned conventional model and takes into account the structure of the 'thick' films as earlier proposed [2]. Characteristic for such films is the presence of a homogeneous 'bulk' phase between the adsorbed monolayers (Fig. 8a). Under electrostriction, this phase is gradually compressed and eventually structured to the stage shown in Fig. 8b.

Essential for the scheme (represented in Fig. 8) is the location of the rigid sterol rings integrated into the phospholipid acyl chains region. Certainly, such location results in a strengthening of the film structure with increased molecular constraints. On the other hand, it is impossible with the present

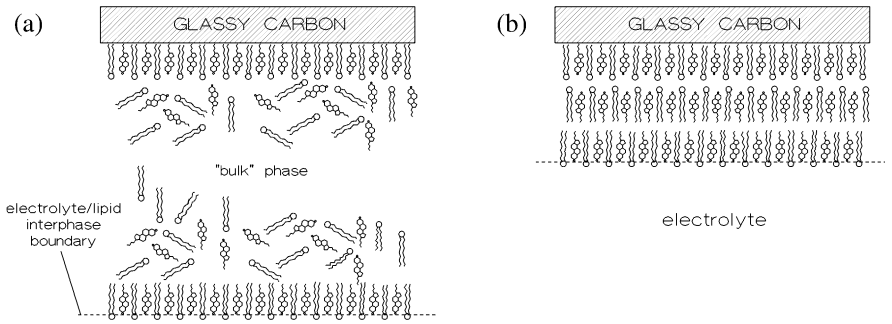

Fig. 8. Schematic presentation of a possible molecular structure of a 'thick' GCE supported lipid film modified with cholesterol; (a) before electrostriction; (b) under external potential of 1.5 V. The shape and size of the molecules are not strictly indicated. The molecules of the solvent are not shown.

experimental technique to determine the exact cholesterol/lipid molar ratio in the monolayer at the electrolyte/lipid interface. Nevertheless, in our opinion, this scheme could serve as a general view of the molecular arrangement of the cholesterol enriched lipid films formed onto the GCE surface.

Interestingly, the results from LSV suggest that the change in the structure and packing order caused by the cholesterol does not alter too much the direct electron transfer through the hydrophobic part of the film. It is manifested by equally enhanced electron blocking properties for the films obtained from different forming solutions but with similar equilibrium thicknesses (curves 4, 5, 6 Fig. 7b). Thus, it seems reasonable that the electrical conductance of the film (respectively R_p, ascribed on the base of an CPE impedance model) is only connected with the overall processes of energy dissipation but not with an ionic current within the film. On the contrary, the heterogeneous electron transfer revealed by the LSV data turns out to be almost independent of the film structure. This item obviously needs further efforts for the elucidation of the exact mechanisms of the charge transport through the film.

As far as the pure cholesterol is not able to generate stable bilayer structures, the question of its highest amounts present in the membranes is also challenging. The discussed solid supported films are enriched to a relatively high molar ratio ($\approx$65%) of cholesterol/lipid. This value is lower but very close to the upper limit of the solubilization of cholesterol into lipid bilayers recently determined by Huang et al. [30]. It should be stressed, however, that higher cholesterol contents in the 'thick' (100–200 Å) films are possible because of their specific structure comprising homogeneous phase of 'bulk' lipid solution except the monolayer at the electrolyte/lipid interface. In this regard it sounds plausible the excess of cholesterol from the monolayer region to be extruded into the 'bulk' phase in the vicinity of the GCE surface. Actually, this can explain the observed cholesterol remains onto the GCE after electrostriction treatments.

For the purposes of sensor construction the modification of solid supported liquid films of lipids with cholesterol is favorable because of several reasons. First of all, the consolidation of the molecular structure of the film increases the stability of the sensitive interface. Second, the incorporated cholesterol could serve as 'receptor' via specific interactions with some membrane active substances like saponin [9]. Thus, a selective molecular recognition is transformed into changes of macroscopic parameters of the film which can be readily monitored. Third, the decreased dispersion in the presence of cholesterol helps to avoid some uncertainties connected with the evaluation of the film thickness.

At the end of the discussion, we have to say that the presented results of course, are with no pretension to exhaust the question of cholesterol/lipid relations in solid supported films prepared by

the technique of thinning of wetting lipid layers. Further investigations (most probably engaging other experimental methods) are obviously necessary to clarify in more detail the exact molecular structure and interactions within the cholesterol modified films.

5. Conclusions

The cholesterol modification of solid supported liquid films of lipids results in readable changes of their electrochemical characteristics associated with effects similar to those reported for various biological and artificial lipid membrane structures. The data from impedance and voltammetric studies are consistent with the contemporary schemes for the incorporation and mode of action of cholesterol into the lipid bilayer. On the other hand, the films under consideration here possess some advantages such as durability, easy managing, low cost and simple technical appliance in comparison with other membrane analogs. Particularly, interesting seems the possibility to enrich the films to relatively high cholesterol/lipid molar ratio, which is a difficult task with other bilayer lipid systems. Thus it becomes clear that these films are relevant model system, which can be successfully used in the exploration of different cholesterol/lipid relations. This could promote the research of cholesterol interactions with membrane active substances, as well as, of the cholesterol dynamics within the lipid phase.

References

[1] M. Karabaliev, V. Kochev, Glassy carbon as a solid substrate for thin liquid film based sensors, Anal. Lab. 2 (1993) 88–97.

[2] M. Karabaliev, V. Kochev, Probing the 'interfacial' and 'bulk' characteristics of solid supported thin liquid films, Mater. Sci. Eng. C 14 (2001) 11–16.

[3] D. Jiang, P. Diao, R. Tong, D. Gu, B. Zhong, Ca^{2+} induced $Fe(CN)_6^{3-/4-}$ electron transfer at Pt supported BLM electrode, Bioelectrchem. Bioenerg. 44 (1998) 285–288.

[4] M. Karabaliev, V. Kochev, Some electrochemical features of glassy carbon supported thin liquid films of lecithin, Electrochem. Commun. 3 (2001) 367–371.

[5] R.B. Gennis, Biomembranes: Molecular Structure and Function, Springer, New York, 1989.

[6] M. Shinitzky, Membrane fluidity and cellular functions, in: M. Shinitzky (Ed.), Physiology of Membrane Fluidity, vol. 1, CRC Press, Boca Raton, 1984, pp. 1–51.

[7] M. Shinitzky, Membrane fluidity and receptor function, in: M. Kates, L.A. Manson (Eds.), Biomembranes, vol. 12, Membrane Fluidity, Plenum Press, New York, London, 1984, pp. 585–601, Chapter 20.

[8] G.H. Addona, H. Sandermann, M.A. Kloczewiak, S.S. Husein, K.W. Miller, Where does cholesterol act during activation of the nicotinic acetylcholine receptor?, Biochim. Biophys. Acta 1370 (1998) 299–309.

[9] M. Karabaliev, Formation and investigation of solid supported thin liquid films of lipids. Possibilities for development of sensor elements. Ph.D. Thesis, University of Sofia, 2001.

[10] S.G. Kalashnikov, Electricity, Nauka, Moscow, 1977, p. 144.

[11] (a) B.V. Derjaguin, N.V. Churaev, V.M. Muller, Surface Forces, Nauka, Moscow, 1985
(b) B.V. Derjaguin, N.V. Churaev, V.M. Muller, Surface Forces, Plenum Press, New York, London, 1987.

[12] C. Miller, P. Cuendet, M. Grätzel, K^+ sensitive bilayer supporting electrodes, J. Electroanal. Chem. 278 (1990) 175–192.

[13] P.M. Krugliakov, U.G. Rovin, Physical Chemistry of Black Hydrocarbon Films. Bimolecular Lipid Membranes, Nauka, Moscow, 1978, p. 33, Chapter I.12.

[14] R. Benz, O. Fröhlich, P. Läuger, M. Montal, Electrical capacity of black lipid films and of lipid bilayers made from monolayers, Biochim. Biophys. Acta 394 (1975) 323–334.

[15] B.V. Derjaguin, N.V. Churaev, Wetting Films, Nauka, Moscow, 1984.

[16] M. Karabaliev, V. Kochev, Preparation of extremely thin solid supported films of lipids by the method of electrostriction. Coll. Surf. B, in press.

[17] N.V. Churaev, Wetting films and wetting, Rev. Phys. Appl. 23 (1988) 975–987.

[18] L.P. Franks, Structural analysis of hydrated egg lecithin and cholesterol bilayers. I. X-ray diffraction, J. Mol. Biol. 100 (1976) 345–358.

[19] D.L. Worcester, L.P. Franks, Structural analysis of hydrated egg lecithin and cholesterol bilayers. II. Neutron diffraction, J. Mol. Biol. 100 (1976) 359–378.

[20] E. Oldfield, D. Chapman, Dynamics of lipids in membranes: heterogeneity and the role of cholesterol, FEBS Lett. 23 (1972) 285–297.

[21] R.G. Ashcroft, H.G.L. Coster, D.R. Laver, J.R. Smith, The effects of cholesterol inclusion on the molecular organisation of bimolecular lipid membranes, Biochim. Biophys. Acta 730 (1983) 231–238.

[22] S. Mabrey, P.L. Mateo, J.M. Sturtevant, High-sensitivity scanning calorimetric study of mixtures of cholesterol with dimyristoyl- and dipalmitoylphosphatidylcholines, Biochemistry 17 (1978) 2464–2468.

[23] M. Cortijo, A. Alonzo, J.C. Gomez-Fernandez, D. Chapman, Intrinsic protein–lipid interactions: infrared spectroscopic studies of Gramicidin A, Bacteriorhodopsin and Ca^{2+}-ATPase in biomembranes and reconstituted systems, J. Mol. Biol. 157 (1982) 597–618.

[24] H.G.L. Coster, J.R. Smith, The molecular organisation of bimolecular lipid membranes. A study of the low frequency Maxwell–Wagner impedance dispersion, Biochim. Biophys. Acta 373 (1974) 151–164.

[25] D.R. Laver, J.R. Smith, H.G.L. Coster, The thickness of the hydrophobic and polar regions of glycerol monooleate bilayers determined from the frequency-dependence of bilayer capacitance, Biochim. Biophys. Acta 772 (1984) 1–9.

[26] C. Karolis, H.G.L. Coster, T.C. Chilcott, K.D. Barrow, Differential effects of cholesterol and oxidised-cholesterol in egg lecithin bilayers, Biochim. Biophys. Acta 1368 (1998) 247–255.

[27] K.W. Butler, I.C.P. Smith, H. Schneider, Sterol structure and ordering effects in spin-labelled phospholipid multibilayer structures, Biochim. Biophys. Acta 219 (1970) 514–517.

[28] R.A. Demel, B. de Kruyff, The functions of sterols in membranes, Biochim. Biophys. Acta 457 (1976) 109–132.

[29] O.P. Bondar, E.S. Rowe, Role of cholesterol in the modulation of interdigitation in phosphatidylethanols, Biochim. Biophys. Acta 1370 (1998) 207–217.

[30] J. Huang, J.T. Buboltz, G.W. Feigenson, Maximum solubility of cholesterol in phosphatidylcholine and phosphatidylethanolamine bilayers, Biochim. Biophys. Acta 1417 (1999) 89–100.