

FORMATION OF GLASSY CARBON SUPPORTED THIN LIQUID FILMS OF LECITHIN. REACTION TO ELECTROACTIVE SPECIES IN SOLUTION

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Abstract. A preparation of stable thin liquid films of lecithin on a highly polished glassy carbon support is reported. The electrical parameters of thus modified flat surface are voltammetrically studied. The evaluation of the organic phase thickness shows that under certain conditions a very compact monolayer of lipid molecules is obtained during the process of film thinning. The results from voltammetry suggest that such a solid supported lipid layer represents a barrier for the transport of charges between the electrolyte phase and the electrode which is of interest for the biosensor construction.

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1. Introduction

During the past few decades glassy carbon (GC) has found broad application in electrochemical analysis. Due to its peculiar features such as inertness, low porosity, good conductivity, etc [1] it was widely accepted as a promising material for the construction of indicator electrodes [2].

In a previous paper [3] discussing some new trends in the biosensor receptor part implementation, we emphasized the advantages offered by a two-dimensional (2D) design. Such a sensor construction can be realized employing

the properties of self-ordered layers of amphiphilic compounds. On the other hand, most of the macromolecules used in biosensor technology require proper local surroundings for their specific functions. Because of their proteinaceous nature such biomolecules like membrane receptors, enzymes, antibodies, channel proteins, etc, are known to be very sensitive to the media in which they are incorporated. That is why the natural lipids seem to be those species that in addition to their amphiphaticity offer a native microenvironment for the normal activity of biomolecules of sensor relevance. Lipid layers arranged by the Langmuir-Blodgett (LB) technique have already proved to be reliable structures in relation to the organization of biosensor detecting part [4-7]. Another approach is based on the formation of monolayers onto a solid substrate through the spontaneous adsorption from solution by a method known as self-assembly [8].

Recently we proposed a new technique of solid supported lipid bilayers assembling [9, 10]. It is closely connected with such phenomena like wetting of solids and liquid films thinning and relies completely on the self-organization of amphiphilic molecules. In comparison with usual bilayer lipid membranes (BLMs) between two aqueous solutions the supported lipid layer constitutes an asymmetrical system in the sense that the lipid film is in contact with a solid from the one side and with a liquid phase from the other. The main idea for building up such structures is to reinforce the film stability through the strong adhesion forces.

Furthermore, since no specific chemical interactions are involved in the generation of lipid layers by this technique there is no special requirements for derivatization of the substrate surface. Thus the choice of the solid substrate is not restricted to certain chemical species. Any sufficiently smooth surface can be used for that purpose, with the only requirement that the orientation of the lipid layer adsorbed to the substrate will depend on the surface hydrophilicity.

Logically, after successful formation of bilayer structures onto different hydrophilic substrates we have been looking for a material that will enable us to produce a supported monolayer of lipid molecules. In this regard, GC turned out to be one of the most suitable. Beside the above mentioned properties it also provides a sufficiently hydrophobic surface, that promotes an orientation of lipid molecules predominantly with hydrocarbon tails towards it.

2. Materials and Methods

2.1. The Electrochemical Cell Construction

The formation of supported liquid films was achieved by two methods described in detail elsewhere [11]. In both of them the working electrode surface and the

electrolyte/lipid solution phase boundary are gradually brought in contact with each other. The experimental setup is schematically shown in Fig. 1.

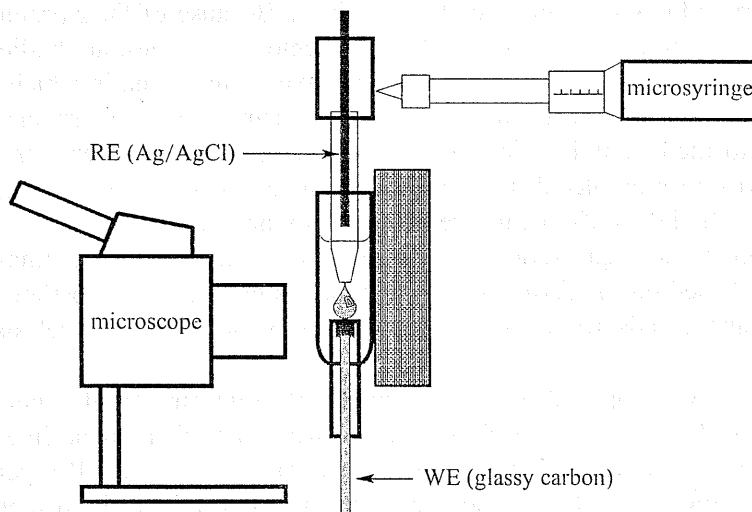
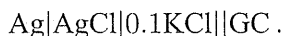
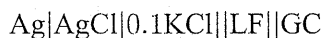


Fig. 1. Experimental setup for the formation and observation of glassy carbon supported thin liquid films of lipids

The study of the electrochemical properties of the modified glassy carbon electrodes (GCEs) was accomplished by the aid of two types of cells. The first of them served for investigating the electrical double layer parameters of a plain freshly polished GCE. It consisted of a working GCE, 0.1 mol/l solution of KCl as electrolyte and single junction Ag/AgCl reference electrode



In the second type, at same other conditions, a thin lipid film was formed onto the surface of the working GCE, so the cell can be represented as



where LF is the liquid film of lipid molecules.

2.2. The Working Electrode Conditioning

The construction of the GCE used in this work was previously described in detail [11]. It includes as the main part commercially available GC material (Chemtronics International Inc., Ann Arbor, MI, USA) that was fabricated into cylindrical form with diameter of 3 mm giving a 0.0707 cm² circular surface

area. Additionally the GC surface was conditioned by manual grinding and fine polishing as mentioned in [11].

For the sake of a better performance the working surface of the GCE was renewed before each measurement as follows. First it was polished with the finest grade ($<0.03 \mu\text{m}$) polishing powder. Then it was carefully cleaned with commercially available detergent solution and washed extensively with double distilled deionized water. No electrochemical pretreatment was used to avoid any surface roughening which is unfavorable for our purposes.

2.3. Forming Solution and Reagents

The electrochemical cell was filled with 0.1 M KCl (Potassium Chloride, SIGMA Chemical Comp., USA) as electrolyte solution. The liquid films deposited onto the working electrode were prepared from lipids as follows. Natural lecithin (L- α -Phosphatidylcholine, Bell Pharmacal Corp., USA) was dissolved in *n*-hexane (SIGMA Chemical Comp., USA) to give a concentration of 20 mg/ml and was stored in a refrigerator as a stock solution. The lipid film forming solutions were being prepared daily by dilution with *n*-hexane.

Potassium ferricyanide $\text{K}_3\text{Fe}(\text{CN})_6$ at a final concentration of 1 mM (Fluka Chemie AG, Switzerland) and ascorbic acid $\text{C}_6\text{H}_8\text{O}_6$ at the same concentration (Pharmachim, Bulgaria) were used as electroactive species added to the electrolyte.

2.4. Cyclic Voltammetry Technique

In the present work voltammetry was used as an analytical tool for the determination of charge exchange between the GCE and the solution. The measurements were made with commercial polarograph (OH-105, Hungary). The sweep rates as well as the potential range are indicated separately in each figure and discussed in the text.

3. Results and Discussion

3.1. Kinetics of Thin Lipid Film Formation

The formation and examination of stable solid supported thin liquid films of lipids is the essence of this work. As it was earlier described [11] these films are made by successive thinning of "thick" layers of lipid material deposited directly onto the solid substrate (GC in this case). Further evolution of such a film could bring it to an ordered molecular structure of a bilayer or monolayer type. Bearing in mind the hydrophobicity of the freshly polished GC surface it is possible to "arrange" the supported liquid film in such a manner, so that it

would represent a monolayer of molecules contacting the phase of electrolyte with their polar heads.

It should be noted, that the most important among the factors affecting the process of thinning is the incubation time following the formation of electrolyte/lipid solution interface. This appeared to be an absolutely necessary period during which adsorption of lipid molecules to the interface is taking place. The incubation period allows a reliable value of lipid surface concentration to be reached. This is a very important prerequisite for the generation of a stable film [12].

In the process of thinning the excess of lipid material is extruded towards the film periphery. As the electrolyte/forming solution interface is approaching the GCE surface at distances on the order of microns the thick film becomes laterally inhomogeneous. This results in a prominent interference pattern of colour Newton rings which can be readily observed. Thus the film thinning can be directly monitored under a microscope in reflected light mode by the changes in the interference pattern. The evolution of this pattern during the process of thinning can be seen in Fig. 2 where four successive states of the film are shown.

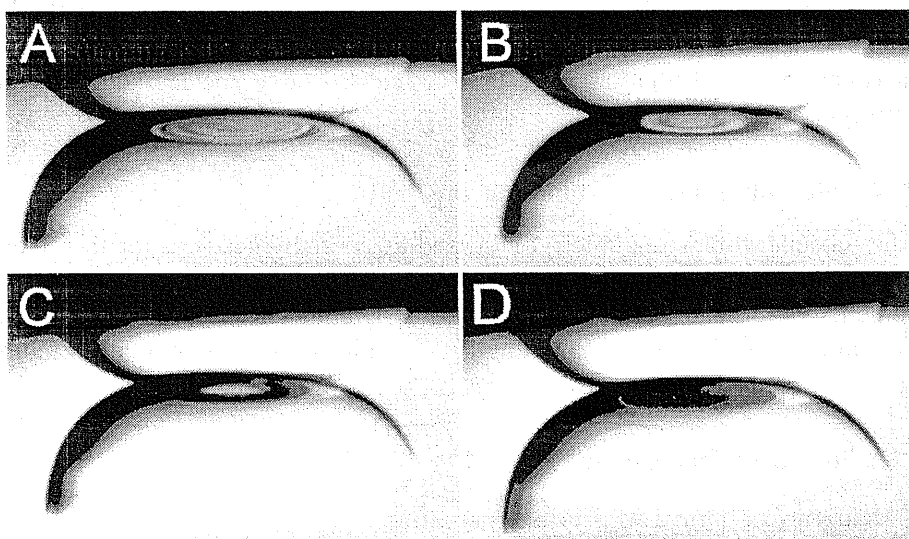


Fig. 2. Four successive stages of film thinning. “Drop approaching the substrate surface” method of formation

(A) and (B) initial stages of *thick* film with readily observed interference patterns; (C) stage with a *lens* of forming solution in the center of the film; (D) stage of *black* film

On attaining a thickness of 100–200 Å the film is already so thin, that it

reflects almost no light and looks dark under observation. Nevertheless, after this stage there is still some light reflected by the substrate that cannot be completely eliminated (Fig. 2D). By analogy with the BLMs this stage can be regarded as a "black" film. Further the thinning continues with light reflected entirely from the polished GCE surface.

3.2. Electron Exchange with Redox Species in the Electrolyte Solution. Blocking Properties

In order to be of interest for the construction of sensors with an electrochemical signal transduction, supported liquid films should represent a high enough barrier for charge exchange between the electrode and the analyzed solution. As to the ions and larger electroactive species it is well established that symmetrical bimolecular films of LB type or BLM show poor permeability. Ionic currents through the bilayer hydrophobic core are highly restricted [13]. Self-assembly monolayers of lipids chemisorbed onto gold also have been reported to possess pronounced insulating properties [8].

In a previous study [10] with supported bilayers we did not observe penetration of ions through the films to the working electrode. The main reason pointed out was the lack of electrolyte phase between the layer and the substrate. In the present work we did not find sufficient electron transfer either. This is clear from the cyclic voltammograms shown in Fig. 3.

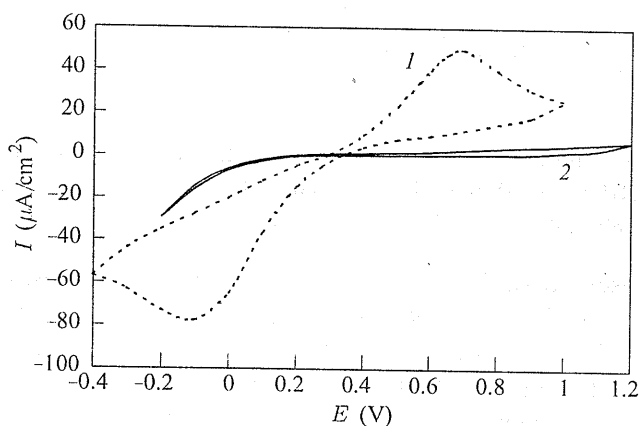


Fig. 3. Voltammograms of bare GCE in solution of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl (---) and GCE covered with thin liquid film of lipid in the same solution (—). Scan rate 0.5 V/min

We have used $Fe(CN)_6^{3-}$ as electroactive species to examine the electron exchange with the solution. As it is seen, with uncovered GCE (curve 1)

the specific peaks of redox currents are observed in the voltammogram. The formation of a thin lipid film onto GCE results in a drastic decrease of these currents in the range of positive potentials (curve 2). The substantial current for potentials more negative than -100 mV might be caused by local reversible ruptures due to electric breakdown in the film. In our opinion, as a whole the results presented in Fig. 3 support the idea to use the liquid films' blocking properties for modifications of solid electrodes.

In accordance with the above mentioned are the results obtained with an electroactive substance with the larger molecule ascorbic acid. The data are shown in Fig. 4 where with dashed line the current vs potential dependence of plain GCE is plotted. The solid curve represents the behaviour of an electrode modified with a thin lipid film with the same parameters as in the previous case.

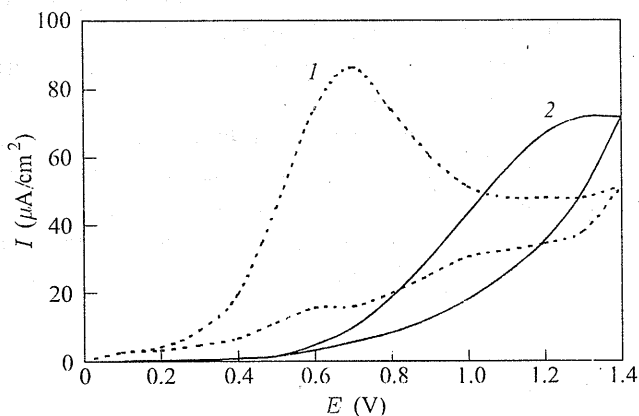


Fig. 4. Voltammograms of bare GCE in solution of 1 mM ascorbic acid in 0.1 M KCl (---) and GCE covered with thin liquid film of lipid in the same solution (—). Scan rate 2 V/min

A readily observed shift of maximal current peak to the positive potentials obviously can be ascribed to a decrease in the rate constant of the redox reaction. This means that the electron transport is highly restricted and the reaction becomes kinetically limited. If the reaction was diffusion limited, i. e. if the electroactive species were able to penetrate the film and react on the very surface of the electrode then there would not be any shift of the current peak position but only a decrease in the height of the peak. On the other hand the current passing through the film at more positive potentials can be explained by the electrons tunneling the film. This is in accordance with the data of other authors where the examination of electron transfer through self-assembly layers has been made [14, 15].

4. Conclusions

As it can be seen from the results presented here and previous data [11] glassy carbon (GC) can be a quite suitable material as a substrate in the generation of stable solid supported thin liquid films. These films (in our case of lipids) are formed directly onto a freshly polished GC surface. In the process of thinning these self-assembled molecular structures are developed to the final state of a monolayer of lipid molecules [11]. In such monolayer the amphiphiles are oriented with the tails to the substrate and with their hydrophilic heads to the electrolyte solution.

To our mind the results in this study suggest that GCEs in conjunction with supported lipid monolayers seem to be very promising as solid state electrodes for analytical purposes. The cyclic voltammetry data show undoubtedly that the monolayer of lipids built up by the technique described above is compact and has good blocking properties. The lipid layer in these sensors can be modified in different manner. As the film serves like a media for various substances it can be doped with ion-specific molecules (e. g. crown ethers) or with macromolecules of biological relevance like enzymes, antibodies, channel peptides etc. In our view, this can result in a development of a novel type of sensors with electrochemical signal transduction and receptor part organized close to a 2D design.

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