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Electrochemical analysis of phase behavior of solid-supported lipid films. Influence of cholesterol

V. Kochev, L.Vladimirova- Mihaleva *, K. Bachev, M. Tanovska

Sofia University "St. Kliment Ohridski", Faculty of Physics, 5 James Bourchier Blvd. 1164 Sofia, Bulgaria

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ABSTRACT

Diverse techniques of electroanalytical chemistry have already earned the reputation of dependable tools for research of biomembranes and lipid model systems. Among them, Electrical Impedance Spectroscopy (EIS) seems to be one of the most promising with respect to interface studies. Here we have made an attempt to use EIS for evaluation of the effect of cholesterol on the phase behavior of supported liquid films of lecithin. Our results showed that cholesterol promoted tight packing of lipid molecules in the different regions of the film. These findings are in consonance with the widely accepted condensing role of cholesterol in lipid membranes.

1. Introduction

Due to their peculiar features including sensitivity, non-invasiveness, easy manipulation and inexpensiveness, the methods of analytical electrochemistry have long been recognized as extremely reliable in the field of model lipid membranes. Indeed, to date many routine tests on electrochemical basis have been developed and successfully used for investigation of different characteristics of various analogs of biomembranes [1]. In order to realize the great impact of electroanalytical approaches to Life Sciences it is only enough to mention the standard electrophysiology and patch-clamp techniques which are subject to improvements up to date [2–4]. Furthermore, measurements allowing to gather information not only for the conductance of the specimens, but also for their complex reactance gained a lot of attention at the very beginning [5,6]. Thus, Electrical Impedance Spectroscopy (EIS) of chemical and biological samples was born [7,8]. EIS is usually performed with the aid of time-dependent external potentials which cause polarization and relaxation in the samples, leading to changes in amplitude and phase of the measured response signal. Most commonly, sinusoidal AC voltage is applied to a simple electrochemical cell, consisting of a sample between two electrodes, and the curent passing through it is monitored. According to the curent changes an impedance of the cell, comprising a real and imaginary part, can be determined. As far as this complex variable can be measured at different frequencies, some kind of spectroscopy is actually realized in this way. However, the enormous complexity of living matter standards is a great obstacle on the way to complete an explicit generalized theory of bioimpedance [9]. Nevertheless, the use of EIS is broadening and many empirical models, describing semi quantitatively the main features of biomaterial under observation have been constructed for evaluation of different tissues changes under stress and normal conditions [10–12].

No doubt, biomembranes, with their morphological diversity, are namely those attributes that give the final shape of the cell and define it as the quant of living matter [13,14]. Although the native membranes exhibits myriads of forms and functions and their contents comprise thousands of polypeptides, lipids and sacharides, they share a common feature–the canvas of their architecture consists of bimolecular layer of lipid molecules. That cell membranes are arranged over lipid bilayer, became clear after a pioneering work of Gorter and Grendel [15] and in the early 70es Singer and Nicolson introduced their fluid mosaic model explaining how peptides and proteins are attached to it [16]. Furthermore, in the past few decades became evident that lipid bilayer itself is not a passive element of biomembranes, but governs many important cell processes through its physicochemical state [17]. In addition, new concepts for the 3D and 2D (lateral) organization of membranes flourished. Because of their intricate nature biomembranes are often studied via proper models, mimicking their structures and functions. The most popular of these model systems inevitably contain lipid bilayer and for the aim of our survey they could be divided into two major classes: "free

* Corresponding author. Tel.: +35928161317.
E-mail address: liliavladimirovamihaleva@gmail.com (L.V.- Mihaleva).

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standing" [1,18] and "supported" [19]. Of course, each of them is characterized with their advantages and drawbacks, which are mainly concerned with the great compromise between stability and fluidity [20].

In this work we have studied the main temperature transition gel–liquid-crystalline of supported lipid films and the condensing effect of cholesterol as revealed by specific changes in the electrical parameters of the films. In this regard, EIS turned out to be reliable method for investigation of the lipid films phase behavior. The obtained results undoubtedly suggested the ordering role of cholesterol in the films, which is in agreement with the present viewpoint widely described in the literature [17,21–23].

2. Materials and Methods

2.1. Solid supported liquid films of lipids

Soy-bean lecithin (Walmark, Czech Republic) was used without further purification as lipid material for preparation of the films in this study. Cholesterol (5-cholesten-3β-ol, $C_{27}H_{45}OH$, Sigma Chemical Co. USA) analytical grade, also was not purified additionally. Phospholipid dissolved in n-hexane and sterol dissolved in chloroform/methanol (9/1, vol/vol) were mixed in appropriate quantities to prepare stock solutions with desirable contents.

Gold plated silicon wafers (Microsens SA, Neuchâtel, Switzerland), shown in Fig. 2 were used as solid substrate. The simplest empirical method of preparation of lipid films, known as lipid painting, or paint brush technique, was applied to obtain supported films. Liquid material with different contents of lecithin and cholesterol was deposited directly onto the golden electrode surface, thus so called cast films were realized [24–26]. The two electrodes were then gently pressed one to another by a micrometer screw to a desirable distance between them (Fig. 1). In some cases Teflon spacer was used to define the distance.

2.2. The impedance spectroscopy: data acquisition and processing

As mentioned above, EIS is recognized as one of the most reliable techniques in the studies of solid/liquid interfaces. Moreover, it gives possibilities to interrogate the bulk phase between the electrodes either. In our case the electrochemical cell was of two-electrode type, comprising gold plated silicon wafers

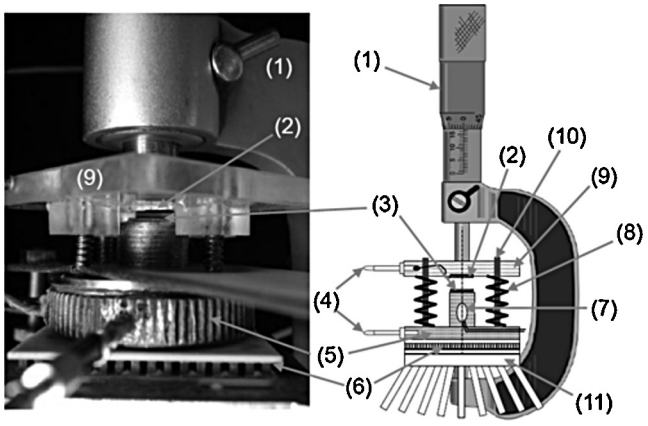

Fig. 1. Overall view (left) and schematic (right) of the experimental set-up: 1) micrometer screw; 2) upper electrode; 3) lower electrode; 4) gold plated pin connectors (type Amphenol Ø 1.5 mm) of the electrodes; 5) brass holder of the lower electrode; 6) Peltier element; 7) embedded thermo resistor; 8) spring; 9) Plexiglas holder of the upper electrode; 10) steel leading rods; 11) radiator of the Peltier element. Fan is not shown.

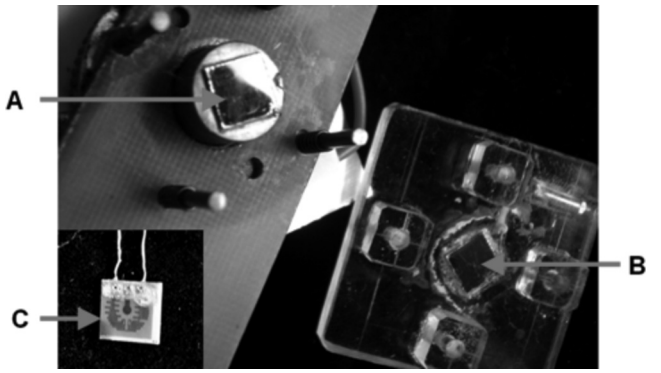

Fig. 2. Photographs of the lower (A) and upper (B) electrodes, and Pt thermometer (C) for calibration. Details are presented approximately in real dimensions.

and lipid solution between them (Fig. 2). All measurements of the cell impedance were done with the aid of Precision Component Analyzer 6425 (Farnell Instruments Ltd UK) which is functioning on the principle of auto balanced bridge. It can present the data as parallel resistor $R_p(f)$ and capacitance $C_p(f)$ which are functions of the frequency $f = \omega/2\pi$ in the range from 20 Hz to 200 kHz. Subsequently, these data were transformed into real (ReZ) and imaginary (ImZ) part of the impedance according to the theory of electrical circuits:

$$\mathrm{Re}Z = \frac{R_p}{1 + (\omega R_p C_p)^2} \quad (1)$$

$$\mathrm{Im}Z = \frac{-R_p(\omega R_p C_p)}{1 + (\omega R_p C_p)^2} \quad (2)$$

Furthermore, in order to interpret the behavior of a system under study, one has to build an electrochemical model, representing an equivalent circuit consisting of elements (in our case passive) reflecting the reaction of the different system's parts to the external AC field. It is well known, however, that because of the complex processes in biomaterials, the construction of circuits only from frequency independent elements is not always possible [7,27,28]. That is why, in our work we have used one of the most popular and simplest frequency dependent element, the so called Constant Phase angle Element, CPE [29], defined by the expression:

$$z_{\mathrm{CPE}} = A(i\omega)^{-\alpha} \quad (3)$$

Here a and A are frequency independent constants and obviously for $a = 0$, CPE will turn into an ideal resistor $R=A$, while for $a = 1$ it will represent an ideal capacitor $C = 1/A$. It is not difficult to realize, that thus defined CPE will show linear frequency dependence for $|Z_{\mathrm{CPE}}|$, $\mathrm{Re}Z_{\mathrm{CPE}}$ and $\mathrm{Im}Z_{\mathrm{CPE}}$ in logarithmic scale. Models constructed with the aid of CPE will be discussed below with respect to their competence to describe the features of supported films. It should be stressed, that to find an adequate model, describing sufficiently well the structures and processes in the explored system is not an easy task at all. The equivalent circuit itself can not say much about the real situation if some additional information concerning the physical meaning of the system is not available. So, the best models and their schemes should be referred to as a consequence of rational considerations of the basic physicochemical features of the films.

A very convenient way to analyze the connection between the parameters of complex impedance, characterizing chemical or bio interfaces is to express the data in the form of diagram in 3D space