

Fatty acids based biological membrane simple models

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It has been shown that many complex molecular structures of the living matter, as for example the biological membrane, pass through a mesomorphic (liquid-crystalline) state at a characteristic temperature. This state is involved in explaining the enhanced diffusivity of lipids and proteins and the membrane evolution during its interaction with some external stimuli. The present study presents experimental results and theoretical considerations concerning some simple membrane models, based on fatty acids and their mixtures with cholesterol (black lipid membranes, Langmuir-Blodgett membranes, thin films sandwiched between transparent conducting plates). Cholesterol modifies the liquid crystalline properties, the fluidity and permeability of the fatty acids. The experimental part contains investigations as microscopic aspect, answer to an external electric field, and nonlinear optical effects under laser action. Theoretical part presents basic considerations and modeling on the studied samples, in accordance with the experimental results. Concluding remarks are detailed exposed.

(Received September 25, 2007; accepted March 12, 2008)

Keywords: Biological membrane, Fatty acids, Cholesterol, Liquid crystals, Laser radiation

1. Introduction

Many researches in biology and biochemistry have emphasized deep implications of the liquid crystal (LC) state upon the changes in biological processes [1, 2], particularly processes that take place in the biological membrane (BM). Nowadays, BM is considered to be composed of a bilamellar lipid matrix with integrally or peripherally associated proteins. Fatty acids (FA), as components or forerunner of the BM, are involved, for example, in transmission of information across the membrane, their role being connected with the LC state they reached in some conditions (concentration in aqueous solutions, temperature variation and the speed of this variation, impurities, different physical fields etc.).

Our study is focused on thin systems of some FA and FA impurities with cholesterol (Ch). One can consider these systems as lipid membranes and ensembles of membranes (BLM-Black Lipid Membranes, LB-Langmuir Blodgett membranes and SC-sandwich cells). A space charge appears in these systems and it modifies with the Ch percentage and/or the application of an electric or/and optical field. Also changes of the refractive index of the samples are expected and really detected, by means of some nonlinear optical effects, induced with low power laser radiation.

2. Experimental

2.1 Black lipid membranes

We used the arachidonic acid (AA), an essential unsaturated FA (UFA), present in BM, having an important role in the metabolism of prostaglandins. Its saturated corresponding acid (SFA) is the arachidic acid (AR), found in animal fats and, in higher percentages in

vegetal fats. Between some temperature values, they are present the mesomorphic LC state (4-20 °C for AA and 73-76 °C for AR). AA has 4 double bounds (all-cis-5, 8,11,14-eicosatetraenoic) and AR has the chemical formula $\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$. At room temperature, the first one is a yellowish oleos liquid, and the other one - a white crystalline powder. Adding Ch in different percentages by weight made mixture samples. Very thin systems (BLM) manufactured by us, were subjected to some electrical measurements. From electrical point of view, these systems have a dielectric behavior [3].

The BLM have been created at room temperature over an orifice of 1- 1.6 mm² area, cut in a nylon sheet. This sheet separates two watery compartments equipped with two wolfram electrodes of 1.5 - 2 mm² area.

The membranes have been created in our laboratory by using Muller's brushing technique or by Szoba's dropper technique [4]. The solutions for creating membranes have been composed by AR and AA and their mixtures with Ch, irrespective of the weight percentages (25%; 50%; 75% cholesterol).

The membranes formation, finally having 10Å thickness, has been studied at the microscope. Electrical measurements have been done after the equilibrium had been reached. We have measured the dependence of the specific capacitance C of the membrane on an external potential applied over the membrane at 25°C and at a frequency of 474 Hz; V and the charge Q induced by Ch were determined in terms of the temperature and of the Ch percentage. (Fig. 1,2, 3, 4, respectively, for AR and mixtures).

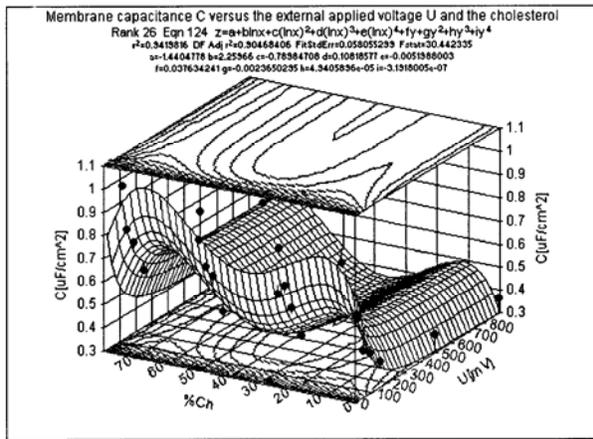


Fig. 1. Membrane capacitance C versus the external applied voltage U and cholesterol percentage.

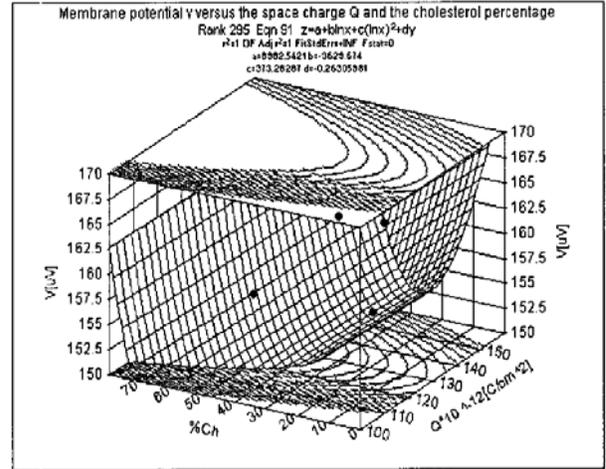


Fig. 4. Membrane potential V versus the space charge Q and the cholesterol percentage.

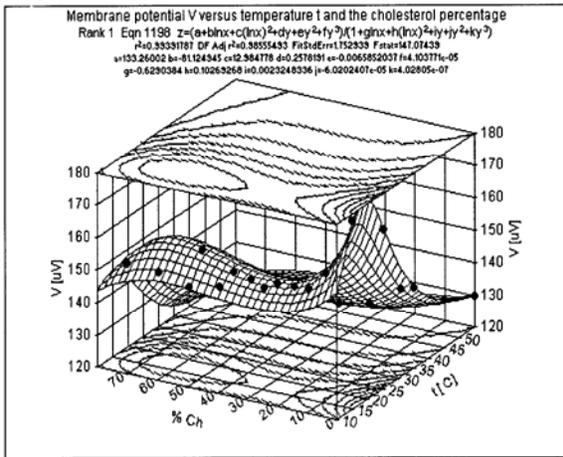


Fig. 2. Membrane potential V versus temperature t °C and the cholesterol percentage.

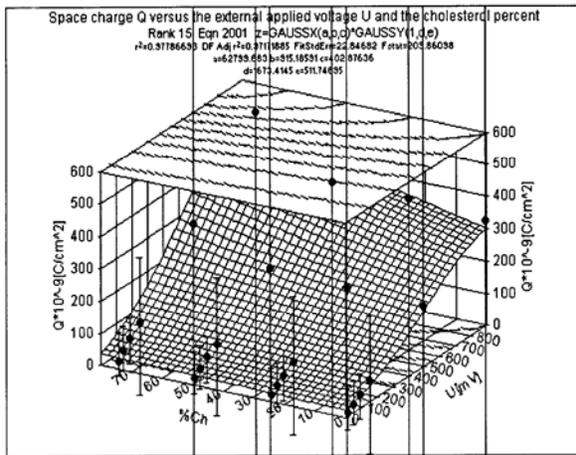


Fig. 3. Space charge Q versus the external applied voltage U and the cholesterol percentage. Prediction intervals are specified.

2.2 Sandwich cells

AA, RA, and mixtures were also sandwiched between two glass plates of transparent SnO_2 electrodes. The liquid samples filled the cells by capillarity; for the solid ones, the cells were first filled with grains of the substance and then, by thermal treatment, they became liquid and the unnecessary amount of substance has been removed until the required thickness was reached. The temperature has been controlled with a heating device described in³ and working between 4-150 °C. The cells were about 2 cm long, with 20 μm Mylar spacers at both ends. Microscopic, electric and optical laser measurements were performed.

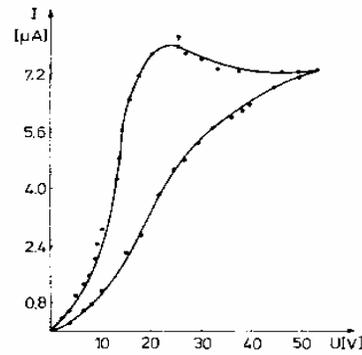


Fig. 5. Current intensity I through the sample versus applied voltage U in AA.

Electric measurements were conducted from which the electric conduction current I versus a d.c. applied voltage, obtained from a stabilized electrical source was determined. Experimental $I=I(U)$ curves have been plotted by a computer controlled installation. These systems provided a dielectric behavior, with a small conduction. Polarizing under action of an external electric field (induced polarization) is a main property of these dielectric materials [3]

Micro structural aspects, obtained with a polarizing microscope, showed smectic C thermotropic liquid crystal textures for all the FA and mixtures [3, 4, 5], between different temperature values, both before and after the SC sample submission to an electric field of $4 \times 10^4 V/m$.

The hysteretic curves of the current versus the applied voltage on the AA and mixtures SC, $I=I(U)$, showed a nonlinear dielectric feature and a negative resistance (Fig. 5), displaced to smaller voltage values when cholesterol was added.

The dependences $I=I(t)$ (see, for example Fig. 6), when a step voltage U has been applied in AA and mixtures and then removed, showed a decreasing of the current, typical for the strong dielectric materials.

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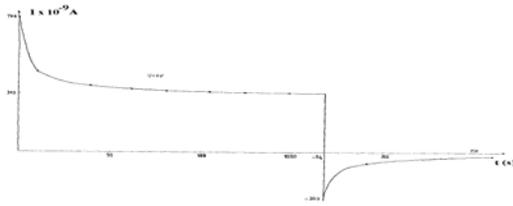


Fig. 6. Current intensity I through the sample versus time t in AA .

These remarks are confirmed by the samples behavior in laser field, which is possible and relevant, since SC is transparent, with very low intensity losses for the laser light. A nonlinear dependence of the emergent optical power versus the incident one was noticed (Fig. 7) by using a He-Ne laser (wavelength of 6328 Å and 50 mW maximum optical power). A “lens-like” nonlinear effect, due to the external self-focusing of the laser beam, and a typical ring pattern in far field was also emphasized, which demonstrates the nonlinear behavior of the samples [5].

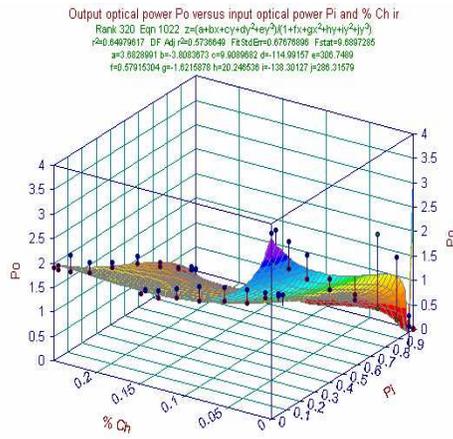


Fig. 7. The output power P_o versus input power P and Ch percentage for RA mixtures.

The diffraction efficiency is illustrated in Fig. 8 for AA is influenced by Ch amount in mixture.

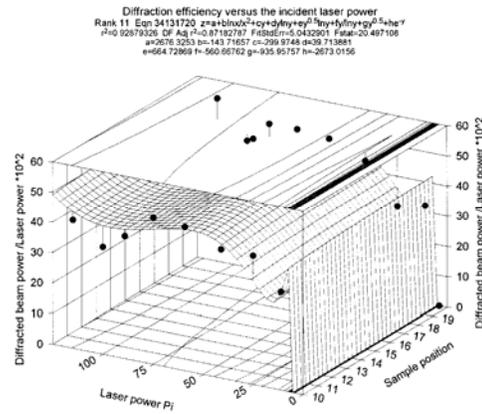


Fig. 8. Diffraction efficiency versus the incident laser power and sample position.

2.3 Langmuir- Blodgett membranes

LB membranes study provides supplementary information on the BM mechanisms, which completes the image realized by the previous studies on BLM and SC. These results could be enough for starting a study of some BM simple models, based on the LB films and on the movements within the BM [6]. This has been done by using the arachidic acid AR and the chromophore diI-C18 (Fluka). Samples preparation is computer controlled; experimental data are also computer processed by using a special program based on Matlab. This program creates a superposition of the fluorescence image and the topographic image of the sample in 3D.

The LB membranes were created by depositing the substance on a glass plate, in movement with 0.1 mm/s, in a special cuve with pure water [7]. The molecular arrangement and density is in function of the superficial pressure of the liquid. In our experiment this was of 20 mN/m. The pressure is correlated with the right – left displacement of the plate (Fig. 9). The process is computer controlled.

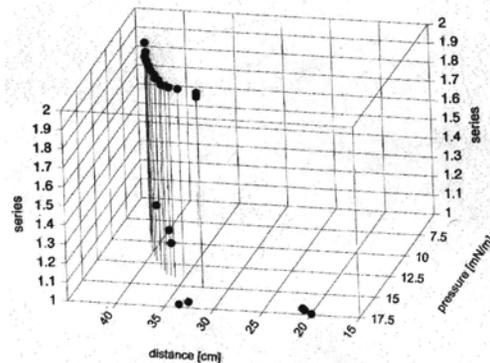


Fig. 9. Dependence of measured pressure on the distance during sample preparation.

For studying LB we used an experimental setup with a Near-Field Scanning Optical Microscope (NSOM), which allows for observing nanoscale modifications. The setup contains mainly an Ar⁺ ($\lambda = 514.5$ nm) laser, a local probe with a "point" (necessary for working in near-field), the sample, a detector and a computer for the feedback loop and the image processing. The feedback gives the possibility of obtaining topographic images in parallel with luminescence images in near field. Thus, a correlation between the topography and the fluorescence images, also a local field manipulation of a single molecule could be done. For optical alignment a He-Ne de 6328 Å and a moving mirror were used.

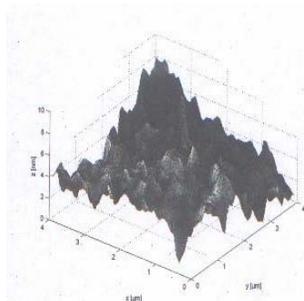


Fig. 10. Topographic levelling image in 3D of the sample.



Fig. 11. Superposition of the topographic level lines on the fluorescence image of the sample.

Details could be found in [8, 9, 10]. Nondestructive investigation of thickness, hydration, roughness, fluctuations and composition of lipids as well as their interactions with proteins were investigated by specular neutron reflectivity, (performed at Institute Laue-Langevin, Grenoble, France, with cold neutrons of 4.52 Å and $\sim 10^6$ neutron/cm²s. Thanks to the programs WETDOC 1.0 and AFIT (<http://www.ill.fr/Computing>) the reflectivity curves were fitted [11].

Deposition of the lipid BL on solid substrate, as single BL and double BL and studied by neutrons reflectivity methods is reproducible at a pressure of 30 mN/m. They are stable over days and are flat and ordered.

3. Teoretical considerations and discussion of the results

In literature, in general, the membrane potential problem is correlated with an electrodynamic system composed of two phases in contact. An electric voltage appears as a result of electric charges transfer between the two phases [12]. The membrane is considered as a structure that obstructs the transfer of substances, allowing some species of particles to pass from one phase to another, at different levels of restriction. We have made several studies upon the membrane potential for BLM composed by AR and AA and mixtures with Ch. These studies show, as it was earlier suggested [13], that substantial frontier potentials appeared at the interface membrane - watery medium.

Our experimental data can be explained with a simple model of three capacitors connected in series; we have studied the modification of the potential and of the membrane capacitance when Ch concentration in the membrane was modified. The electric interactions in membranes can be caused either by an excedentary positive and negative electric charges repartition at the membrane level or by the chemical intermolecular forces. The internal potential in membrane, - the Galvour potential Φ - can be considered to be the algebraic sum between the potential corresponding to the inhomogeneous charge distribution inside the membrane (the so-called Volta potential Ψ) and the one characterizing the dipolar superficial layer (the so-called superficial potential X). The intermolecular potential is neglected. The presence of a chemical potential gradient between adjacent phases of a membrane contributes to the membrane electric potential. It can appear also as a consequence of an existing asymmetry in the membrane structure, in absence of a chemical potential gradient.

Let's consider the BLM extended from $x = 0$ to $x = d$. The membrane is situated between two symmetrical watery phases that contain the ions of the natural salts existing in water. The chemical and electrostatic potentials, especially the pre-existent dipolar one and the image - charge potential, combine so that deep potential gaps for the ions in watery solution occur, close to the solution-membrane interfaces (Fig. 12a).

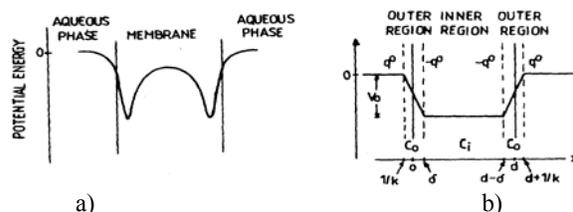


Fig. 12. Schematic presentation of the membrane between the two watery compartments.

The addition of cholesterol in the lipid membrane produces an electrostatic potential variation between the watery phase and the interior of the membrane (Fig. 12b).

Four electric charge planes and three dielectric regions appear and one can write the external and internal superficial capacitances C_0 and C_i , respectively, with the dielectric internal constant ϵ_r^{int} and ϵ_r^{ext}

$$C_0 = \epsilon_r^{ext} \epsilon_0 / (1/k + \delta) \quad (1)$$

and

$$C_i = \epsilon_r^{int} \epsilon_0 / (d - 2\delta) \quad (2)$$

Let's call V_0' , V_0'' as the external electrostatic membrane potentials and V_i as the internal potential and suppose that the addition of cholesterol in membrane determines the modification of the electric charge with Q . The membrane potential V can be simply written:

$$V = Q (2 / C_0 + 1 / C_i) \quad (3)$$

or

$$V = [Q + b (q^{int} - q_0)] / C \quad (4)$$

where:

$$1/C = 2/C_0 + 1/C_i \quad (5)$$

is the membrane capacitance, $b = C/C_i$, q^{int} - the internal space charge in membrane, and q^0 - the internal space charge at the equilibrium.

One can see that the total membrane potential modifies when Q and b change; at a constant membrane potential V , a growth in the capacitance is determined by the charge induced by cholesterol.

By analyzing the experimental results, we get the following conclusions, in agreement with the theoretical considerations:

- a) The specific capacity of lipid membranes decreases when the potential applied across the membrane increases, similarly to the case of the nonlinear dielectrics, and the amount of cholesterol within the membrane decreases (Fig. 1);
- b) The membrane potential V decreases when the temperature and the Ch amount increase (Fig. 2);
- c) The spatial electric charge increases, and the membrane potential decreases as the amount of cholesterol increases (Fig. 2 and 3);
- e) An increase of the amount of cholesterol in the membrane over 75% leads to an abrupt decrease of the spatial charge.

The behavior of the sandwich-cell SC samples can be explained by the existence of an electric space charge, causing the development of an internal electric field of opposite sign to the external electric one.

The change in the electric state following the electric field action is the occurrence of the electrochemical potential and the change in the number of charges, which takes part in conduction.

The density of the electric current through the sample, when an electric field:

$$\vec{E} = -\nabla\Psi \quad (6)$$

is applied, will be:

$$\vec{j} = -q' n \mu_q \nabla\Psi \quad (7)$$

where μ_q is the mobility of the charge carriers which take part in conduction.

One can consider that during electric conduction, the charge q' which takes part in conduction changes its energy from an energy corresponding to a level (E_j), characteristic of the bound state, to an energy corresponding to a level (E_i), which corresponds to a free or quasi-free state.

The removal of the electrons from their double bonds and their motion under the influence of the external electric field as well as the occurrence of, or increase in electrochemical potential gradient, diminish or even annihilate - as in the case of the AA which has four double bonds C(20:4)- the system's capacity of returning to its initial state.

The most interesting seem to be the $I=I(t)$ plots, t being the time, which allows for the determination of the relaxation time of charge carriers, according to¹⁰, and of the space charge within the sample, by using a dielectric with two layers model [14]. From these curves the internal resistance and the space charge can be determined with a method presented in [13]. The obtained values are represented in Fig. 13. All the measurements took into consideration the Ch percentage and the discussion of the results has been done with respect with the hypothesis that Ch acts similarly with an external electric field, applied on the sample [5].

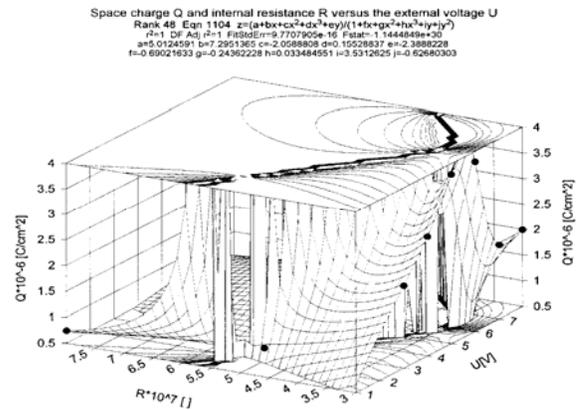


Fig. 13. Space charge Q and internal resistance R versus the external voltage U (Rank 48 Eqn 1104).

One can state the following:

- $I=I(U)$ dependences are parabolic, showing a typical dielectric feature, a hysteric behavior characteristic to ferroelectrics;

- The relaxation time and the space charge are function of the Ch amount in mixture. One can observe an exponential decreasing of the current in time, in AA and mixtures, when the voltage is kept constant. At different voltages, the current intensity I increase and the space charge, too, but slower than I ;

- In general SFA exhibit a slowly increase of the space charge, and the UFA –a more rapid one;

- After a while, a saturation value is reached, therefore the charges are re-distributed in the system and I decrease.

All the results seem to show for the FA and mixtures a ferroelectric feature, valuable for applications in some devices, since they can be biased by an electric field.

The laser interaction with FA in the LC state underline the nonlinear behavior of the FA and mixtures and the Ch influence on the electric conduction and the molecular arrangement in the LC systems, as it was stated in [15]. The “lens-like” effect and the ring pattern appeared in far field under a He-Ne laser beam are due to the self-phase modulation effect in the sample. The presence of Ch in mixtures modifies these effects: the optical nonlinear effects change their feature, increasing in SFA. These changes are in agreement with the microscopical aspects.

Spectroscopic studies of individual molecules movements within the membrane were developed at the Laboratory of Physical Spectrometry (LSP)-University J. Fourier, Grenoble, France, by using a SNOM with a special new design [16]. This microscope offers simultaneously an optical resolution of 50nm and topographic information at nanoscale. This gave the possibility to study the molecular traffic in the membrane and the interaction of the lipids and proteins.

The calculations connected with the experimental setup showed that, in the used configuration, the progressive electromagnetic waves cannot evaluate, but the only part with $kx > \omega t$, where k is the wave vector, and ω –the circular frequency of the wave, for giving information on some structures smaller than $\lambda/2$. In consequence, one can see separately two objects with such dimensions just in near field.

4. Conclusions

In this work, we analyzed thin systems (membranes and ensembles of membranes), under the form of some simple models of biological membrane, realized in the laboratory, in correlation with other previous results obtained by us or presented in the literature. The study underlined the role of the fatty acids and of the cholesterol percentage in membrane, also the possibility to connect this amount with the nonlinear electric and optic behavior of the membrane. The experimental measurements and observations in electric and laser field, respectively, are in accordance with the theoretical assumptions. Studies in near field with the scanning optical microscope showed the intimate (nanoscale) structure of the samples, and give the possibility to act on a single molecule from the membrane.

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