BULETINUL INSTITUTULUI POLITEHNIC DIN IAŞI
Publicat de
Universitatea Tehnică „Gheorghe Asachi” din Iaşi
Volumul 63 (67), Numărul 1, 2017
Secţia
MATEMATICĂ. MECANICĂ TEORETICĂ. FIZICĂ

LIQUID CRYSTALLINE PHASE IN BIOLOGICAL PROCESSES

BY

CRISTINA-DELIA NECHIFOR[1,*], MIHAI ANGHELUȚĂ[2], ANA MARIA CIUBARA[3] and DANA ORTANSA DOROHOI[4]

[1]“Gheorghe Asachi” Technical University of Iaşi,
Faculty of Machine Manufacturing and Industrial Management
[2]“Iulius Hațeganu” University of Medicine and Pharmacy, Cluj - Napoca
[3]“Dunărea de Jos” University of Galați,
Faculty of Medicine and Pharmacy
[4]“Alexandru Ioan Cuza” University of Iași,
Faculty of Physics

Received: January 10, 2017
Accepted for publication: February 27, 2017

Abstract. The role of liquid crystals in biological processes is revealed by the model membranes which represent globules containing phospholipids separated by water. The role of lipid concentration, temperature and drugs in the model-membranes fluidity is discussed in this paper. The cholesterol effect on the phospholipid/water systems in controlling the cell membrane fluidity is also evidenced. The influence of Gramicidin S and cholesterol on the model membrane DPPG/water is illustrated by using IR symmetric and asymmetric bands of $–CH_2$ components of the hydrocarbon chains of DPPG.

Keywords: Phospholipids; lyotropic liquid crystals; Cholesterol; membrane fluidity; IR spectra.

[*]Corresponding author; *e-mail*: cristina.nechifor@tuiasi.ro

1. Introduction

The liquid crystalline phases, or mesophases, are distinct states of matter with ordering properties between the high ordered crystals and total disordered liquids (Muscutariu, 1981; Gray, 1962; Brown *et al*., 1971; Brown, 1975). The compounds giving mesophases under suitable conditions are named mesogens.

Liquid crystals can be classified in thermotropic and lyotropic. The main factor influencing the degree of order of thermotropic liquid crystals is temperature. The lyotropic liquid crystals are obtained at a given concentration of the mesogens in thermodynamically bad solvents, by occurrence between hydrophobic and hydrophilic interactions.

Liquids crystals are present now in the highest domains of the Techniques, Informatics, Medicine and so one (Kallard, 1970; Kallard, 1973; Gray and Winsor, 1974; Brown and Volken, 1979). Liquid crystals and the phenomena bonded by them have important implications in biological processes (Gray and Winsor, 1974; Brown and Volken, 1979). The biological membranes whose functions can be explained by their liquid crystallinity with tridimensional order parameter have a crucial role in all cellular phenomena.

The components of the cell membranes include lipids (phospholipids, phosphatidylethanolamines, phosphatidylserines and phosphatidylcholines), proteins, metal-ions, cholesterol and water which mutually interact and are organized in such way that permits the molecules and ions transport between the outer and exterior of the cells. Associated of each lipid class are fatty acids of different chain length and saturation. The lipids which occur in membranes have liquid crystalline properties. They appear to exhibit long range order with certain amount of short range disorder. These properties are relevant for the membranes structure and functionality.

The mosaic structural model (Muscutariu, 1981) of cell membranes in which they are represented as infinite continuous bilayers of lipids and specific proteins, separated by aqueous phases in an instantaneous thermodynamic equilibrium, can be used to explain the mechanisms assuring the membranes functionality, such as: transmission of the nervous impulse; trans - membrane transport; specific effects of hormones and drugs on the biological cells.

The fluidity of the mosaic structure, based on its liquid crystalline properties is also very important for redistribution of the membrane components by translational diffusion through the viscous tridimensional medium.

The amphiphilic components (lipids and proteins) composing the biological membranes do not interact between them; the liquid crystalline order is determined by the interactions between the hydrophilic heads of the amphiphilic constituents with water molecules. In aqueous medium they form a stratified structure (double layers separated by water molecules), resulted from the maximization of hydrophilic interactions and the minimization of the

hydrophobic ones. The double layers are stabilized by specific interactions between ionized heads of the phospholipids and water molecules.

The hydrocarbon chains are oriented out of water contact. Long chains fatty acids esters and glycerides are packed in this way due to the favorable dispersion which arises from parallel packed chains. The stability of the biological membranes is determined by the balance between hydrophobic and hydrophilic interactions of amphiphilic molecules and water.

The biological membrane structure corresponds to the minimum of the free energy determined by the structural features of lipids and proteins and by their interactions with aqueous medium.

The structure of the biological membranes is more complicated than that of the phospholipid double layers, used for simplicity as model-membranes. This very simple model membrane is used to establish some properties bonded to the membrane fluidity as function on temperature, pressure and impurities.

The aim of this study is to emphasize the role of external factors in modifying the cell membrane fluidity by making as model the DPPC/water or DPPC derivatives/water systems.

2. External Factors Influencing the Membrane Fluidity

The liquid crystalline nature of biological membranes facilitates modifications in its fluidity under the external factors. Lyotropic liquid crystals are primarily influenced by the lipid concentration in the polar solvent (in biological processes the polar solvent is water). Temperature, pressure and the drug presence can also influence the membrane fluidity and so, their functionality.

The behavior of DPPC (dipalmitoylphosphatidylcholine) in water has been summarized by D. Chapman (Chapman 1974; Chapman *et al.*, 1967) in a diagram alike that from Fig. 1. In the gel phase (Chapman, 1974) water separates the double layers of lipids with their hydrocarbon chains in a hexagonal lattice.

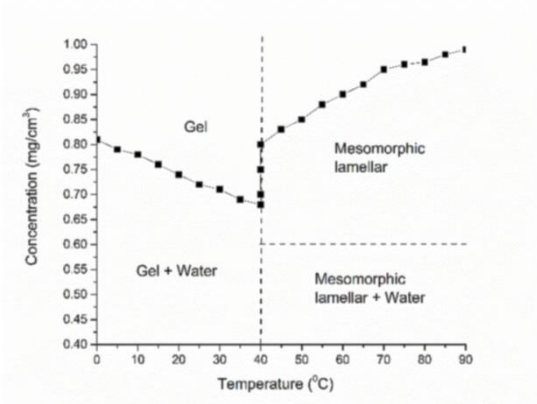

Fig. 1 − Phase diagram of DPPC/water system.

From the phase diagram of DPPC/water system (Fig. 1) it results that after the main phase transition (t > 42ºC), the ability of lipids to disperse in water increases and the system gel + water passes in mesomorphic lamellar phase + water. For lipid concentration higher than 0.6 and t > 42ºC, water is eliminated from the mesomorphic system of DPPC/water. At high temperatures and in the presence of small quantities of water, the cubic phase occurs (C < 0.8 and T < 40ºC).

At small temperatures, water separates the double layers of lipids and their hydrocarbon chains are packed in organized crystalline manner. When water content increases, temperature of endothermic transition decreases. The decrease is not indefinite; for DPPC it ranges up to 42ºC. It was explained that water can produce loosing of the ionic structure of the lipid heads causing reduction of the dispersive forces between the hydrocarbon chains.

Some biological membranes can exhibit the main phase transition many degrees below the biological environmental temperature. So, these membranes can be in a high mobile and fluid condition at biological environmental temperature. These conditions are realized especially in the case of highly unsaturated lipids.

The drug presence can decrease the main phase transition, as it was shown for the DPPC model membrane (Stan *et al*., 2006; Severcan *et al*., 2008).

3. Physico-Chemical Methods in Study of the Liquid Crystalline Phase

Thermotropic mesomorfism of lipid molecules can be evidenced by optical and spectroscopic means.

Optics of the thin films of lipids shows that: they are birefringent when are viewed between crossed polarizers; they loss birefringence by heating below the phase transition temperature and they completely loss birefringence at the melting point.

For example, dimystiroylphosphatidylethanolamine (DMOPEA) (Chapman, 1974) at room temperature is birefringent and after heating near its first transition temperature (120ºC) it was emphasized small decrease of its birefringence. After this temperature, near the melting point (200ºC), the birefringence is definitely lost.

When the lipid temperature is near the temperature of the main phase transition, the IR spectrum (Chapman *et al*., 1967), undergoes remarkable changes and loss all the fine structure and the details from the lower temperature. For temperatures higher than the main phase transition temperature, the IR spectrum is then similar with that obtained with phospholipids dissolved in a solvent such as chloroform.

The differential thermal analysis shows that a marked endothermic transition (with heat absorption) occurs near 120ºC. After this temperature the system DMOPEA/water becomes fluid (Chapman and Collin, 1965).

The phase transition is primarily concerned with the hydrocarbon chains of the phospholipids (evidenced by X-ray, IR and PMR spectra). The trends in the transition temperatures also confirm that the phase transition is primarily associated with the melting of the hydrocarbon chains of the phospholipids directly correlated with the dispersion forces between the hydrocarbon chains.

4. Results and Discussions

DSC and DTA are also used in establishing the membranes fluidity.

The transition temperature for different phospholipids classes differ even though they contain exactly the same fatty acid residues (Fig. 2) (Chapman and Collin, 1965).

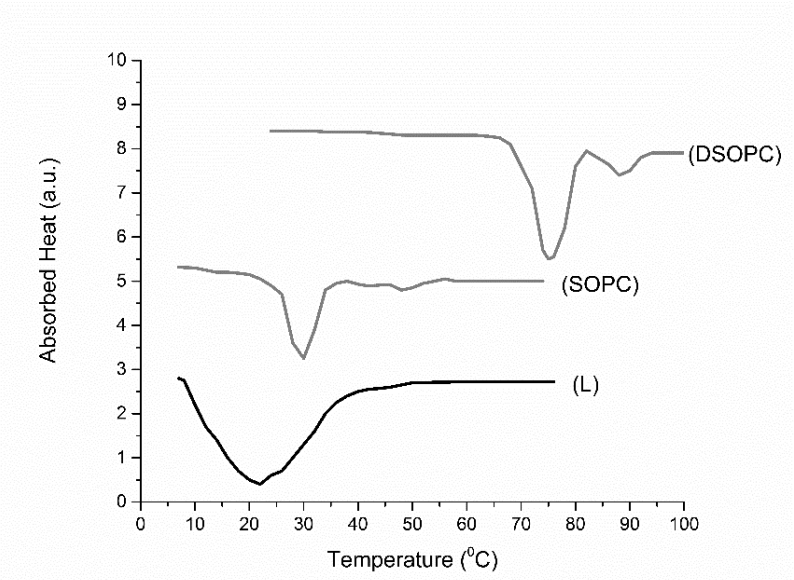

Fig. 2 – Differential thermal analysis (DTA) heating curves for some phospholipids: (L) – egg yolk lecithin; (SOPC) 1- stearoyl-2-oyl-DL-phosphatidylcholine; (DSOPC) 1,2- Distearoyl-DL-phosphatidylcholine.

Phospholipids having exactly the same fatty acid residues are characterized by different transition temperature, as it results from Fig. 2 for SOPC and DSOPC. The egg yolk lecithin (L) is a mixture of phospholipids, but it has only a main point transition, at a temperature smaller than its components.

The high saturated derivatives exhibit transition temperatures much higher than the room temperature, while the natural phospholipids from erythrocytes or mitochondrial membranes contain large numbers of unsaturated cis-double bonds, and therefore, in dry conditions, have endothermic transition temperature either near or below the room temperatures.

At the first termothropic transition temperature, the molecular motion of the chains increases until the first transition point; lateral expansion of the crystal lattice is forced to take place. This expansion allows even greater chain mobility, possible involving cooperative motion of the chains with rotation about C-C bonds.

Near the endothermic transition temperature, a given phospholipid can be in a highly mobile condition, with its hydrocarbon chains flexing and twisting. The more unsaturated the chains, the lower is the main phase transition temperature at which this phenomenon occurs.

In natural membranes the interactions with other molecules can produce inhibition of the chain motion. Due to less perfect packing arrangements, at environmental temperatures, even greater mobility of the chains of the lipid and indeed of the whole lipid molecules could be expected.

When phospholipids are examined in the presence of increasing amounts of water, the various physical-chemical techniques (IR, DSC, NMR, Raman) show that as the amount of water increases, the marked endothermic transition temperature for a given phospholipid falls (Fig. 3).

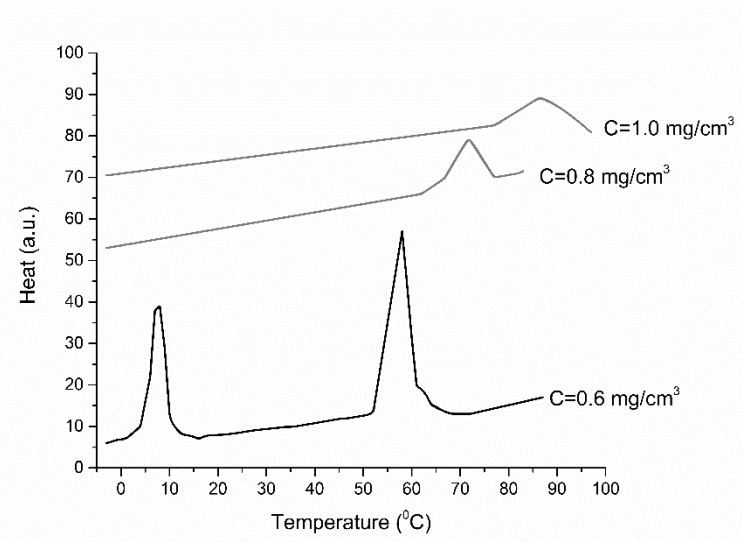

Fig. 3 – DSC heating curves of 1,2 Stearoylphosphatidylcholine (SOPC) in water at different phospholipid concentrations: C = 0.6; 0.8 and 1.0 mg/cm^3.

In the presence of increasing amounts of water, the main phase transition temperature of phospholipids decreases due to water addition to the head of amphiphilic molecules, which causes the reduction of the dispersive forces between the phospholipid chains.

The transition temperature does not fall indefinitely; it reaches a limiting value independent of the water content.

The decrease in the main transition temperature is limited by the energy required to counteract the dispersion forces between hydrocarbon chains necessary to determine the chain melting. The values of the main phase transition temperature are determined by the saturation degree of phospholipids; it is lower for the unsaturated phospholipids. At the biological environmental temperature, the unsaturated phospholipids composing the biological membranes are in highly mobile and fluid conditions.

The natural phospholipids extracted from biological membranes usually exhibit phase transition crystalline – liquid crystalline phases at many degrees below the biological environmental temperature, at which one expects the phospholipids containing unsaturated chains to be in a highly mobile and fluid conditions.

5. The Role of Cholesterol in Biological Membranes

The ability of lipids to disperse in water increases markedly above the main phase transition temperature (Gray and Winsor, 1974; Brown and Volken, 1979).

The drugs can decrease the main phase transition temperature of phospholipids. There are important studies to sustain this affirmation. For example, Gramicidin S (Severcan *et al*., 2008, Stan *et al*., 2006) added to a model membrane of DPPG in water decreases the main phase transition temperature with about 1.5ºC (see Figs. 4, 5 and Table 1).

The interaction of cholesterol with phospholipids is of a great biological importance. Cholesterol occurs in many membranes, particularly in myelin sheaths, or in red blood cell membranes. Cholesterol is solubilized by phospholipids; it has vehicular possibilities. The effect of cholesterol is to disrupt the ordered array of the hydrocarbon chains of the lipids in the gel phase. The presence of cholesterol in mixtures phospholipids/water determines a relative "lipid fluidization".

When cholesterol is added to a model-membrane made by DPPG in water, it decreases the main transition temperature of phospholipid with about 2.5^0C (see Figs. 4, 5 and Table 1). Cholesterol has condensing effect on phospholipids, diminishing the area occupied by the fatty acids.

In Figs. 4 and 5 are given the IR symmetric and asymmetric bands of $-CH_2$ components of the hydrocarbon chains of DPPG as function of temperature for DPPG/water membranes and also for the DPPG/water membranes containing Gramicidin S (GS) and cholesterol (Ch).

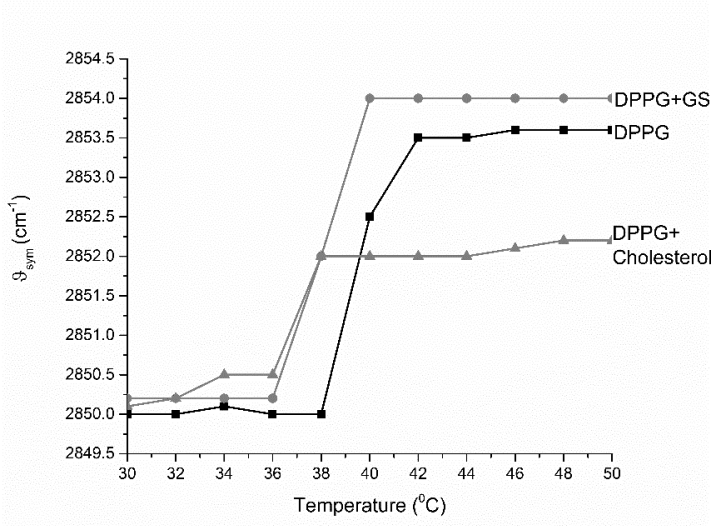

Fig. 4 – Symmetric IR bands of -CH_2 components of the hydrocarbon chains of DPPG in model membranes containing Gramicidin S and Cholesterol.

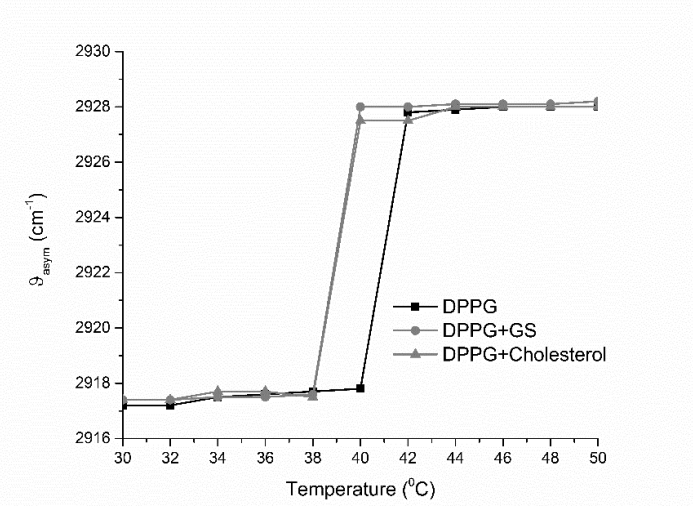

Fig. 5 – Asymmetric IR bands of -CH_2 components of the hydrocarbon chains of DPPGin model membranes containing Gramicidin S and Cholesterol.

The cholesterol presence causes the hydrocarbon chains of different phospholipid molecules to be in an "intermediate fluid" condition. When cholesterol and lecithin are present in equimolar ratios, all the hydrocarbon chains are in a fluid condition. The mixture becomes dispersible in water over a much wider temperature range than occurs with the individual phospholipids (Stewart, 1974).

Table 1
Transition Temperatures of DPPG Model Membranes with Gramicidin S and Cholesterol

Sample	Transition temperature CH_2Symmetric IR band [ºC]	Transition temperature CH_2 asymmetric IR band [ºC]
DPPG	40	41
DPPG+Gramicidin S	38.5	39
DPPG+Cholesterol	37.5	38

The effects of cholesterol in membranes is to control the fluidity of the hydrocarbon chains of the phospholipids providing a coherent structure stable over a wide temperature range and permitting some latitude of the fatty acid content of the component lipids.

6. Conclusions

The main components of the cell membranes are phospholipids and proteins. Their liquid crystalline behavior in water is a determinant factor for the cell membranes functionality.

The liquid crystalline phase of the phospholipids is very important for biological processes due to the fact that assures the permeability of the cell membranes for the ion and molecule transport inside and outside of the cells.

The cell membranes fluidity is influenced by temperature, by some drugs and also by cholesterol.

REFERENCES

Brown G.H., Doane J.N., Neff V.D., *Structure and Physical Properties of Liquid Crystals*, Butterworth, London, 1971.

Brown G.H., *Advances in Liquid Crystals*, Acad. Press, New York, Vol. I 1975; Vol. II 1976; Vol. II 1978; Vol. IV 1979; Vol. V, 1980.

Brown G.H., Volken J.J., *Liquid Crystals and Biological Structures*, Acad. Press, New York, 1979.

Chapman D., Collin D.T., *Nature*, London, **206**, 189-191 (1965).

Chapman D., Williams R.M., Ladbrooke B.D., *Chem. Phys. Lipids*, **1**, 445-475 (1967).

Chapman D., *Significance of Liquid Crystals in Biology*, in: Liquid Crystals and Plastic Crystals, Physico-Chemical Properties and Methods of Investigation, Eds. G.W. Gray and P.A. Winsor, Ellis Horwood Limited-Chichester Halsted Press - a Division of John Willey and Sons Inc., New York, London, Sidney, Toronto, Vol. 2, 1974.

Gray G.W., *Molecular structures and the properties of liquid crystals*, Acad. Press, New York, 1962.

Gray G.W., Winsor P.A., *Liquid Crystals and Plastic Crystals*, Vol. I and II, Halsted Press, New York, 1974.
Kallard T., *Liquid Crystals and their Applications*, Optosonic Press, New York, 1970.
Kallard T., *Liquid Crystal Devices*, Optosonic Press, New York, 1973.
Muscutariu I., *Cristale lichide și aplicații*, Edit. Tehnica, București, 1981.
Severcan F., Agheorghiesei C., Dorohoi D.O., *Rev. de Chim. București*, **59**, *3*, 356-359 (2008).
Stan C., Cristescu R., Severcan F., Dorohoi D.O., *Mol. Cryst. Liq. Cryst*, **457,** 27-41 (2006).
Stewart G.T., *The Role of Liquid Crystals in Life Processes*, Cpt.6.2 in Liquid Crystals and Plastic Crystals, Vol. II, (Editors. G.W. Gray, P.A. Winsor), Halsted Press, New York, 1974.

FAZA CRISTALINĂ LICHIDĂ ÎN PROCESELE BIOLOGICE

(Rezumat)

Rolul cristalelor lichide în procesele biologice este pus în evidență de modelele membranelor reprezentate sub formă de globule care conțin fosfolipide separate de apă. În această lucrare este discutat rolul concentrației lipidelor, temperaturii și a unor medicamente în studiul fluidității membranelor. Un aspect important pus în evidență este efectul colesterolului asupra fluidității sistemelor formate din fosfolipide și apă. Influența Gramicidinei S și a colesterolului asupra membranelor DPPG/apă este ilustrată prin utilizarea benzilor de vibrație simetrică și antisimetrică în IR ale -CH2 ce sunt componente ale lanțurilor de hidrocarburi din DPPG.