Temperature Dependence of the Phospholipids Bilayers Stability, Studied by FTIR Spectroscopy

FERIDE SEVERCAN[1], CATALIN AGHEORGHIESEI[2] , DANA-ORTANSA DOROHOI[2]*

[1]Faculty of Biology, Department of Biophysics, Middle East Technical University, Ankara, Turkey

[2]Faculty of Physics, Department of Optics and Spectroscopy, Al.I.Cuza University, 11 Carol I Bdv, 700506, Iasi, Romania

The temperature induced modifications in the lipid bilayers in water were studied by using 2D-FTIR Correlation Spectroscopy. The spectral range analysed in this paper corresponds to symmetric and asymmetric stretching vibrations of the –CH_2 groups from the acyl chains of DPPC. Autocorrelation peaks at 2916 cm^{-1} and 2848.5 cm^{-1} and asynchrone modifications in the spectral ranges 2916-3000 cm^{-1} and 2848-3000 cm^{-1} were evidenced. The revealed modifications indicate changes in the conformers of the hydrocarbon chains in the phase transition from the gel to liquid crystalline phases, as well as the variations in the concentration of the two phases with temperature increasing.

Keywords: DPPC model membrane, 2D-FTIR Correlation Spectroscopy

Phatty acids are abundant in biological membranes mainly as components of phospholipids and cholesterol esters. Their presence, free or bound to phospholipids, modulates the lipid membrane behaviour [1].

The phospholipids model membranes [3,4] are usually studied in order to elucidate the thermal changes induced in the biological membranes. Their use eliminates the multitude of factors that influence the biological membrane fluidity, a natural condition for their functionality. The influence of the external electric fields [5,6] of concentration [7,8] or of temperature [9-11] on the lyotropic or thermotropic liquid crystals was intensively studied in the past years by electric [5], DSC [12] or spectral means [10,11,13-15], in order to establish the properties of the model membranes.

The amphiliophylic nature of the phospholipids (fig.1) determines the bi-layers formation at appropriate concentrations of the phospholipids in water. The bi-layers have the hydrophilic heads of the phospholipids in contact with water and the acyl chains (hydrophobic tails) oriented to the middle of the bilayer.

The model membrane stability is due to the maximization of the hydrophobic interactions concomitantly with the minimization of the hydrophilic ones. It is affected by temperature and by the impurities.

The phase transition of the type (1) takes place as temperature increases.

$$\text{Gel phase} \Leftrightarrow \text{Liquid crystalline phase} \qquad (1)$$

By the phase transition the model membrane passes from the highest ordered system to the more disordered one, when temperature increases (fig.2).

Temperature produces important changes in the order degree of the model membrane. At low temperatures the model membranes are structured as lamellar symmetric bilayers, forming a gel phase with a high degree of order, while, at high temperatures, they become disordered and pass in a liquid crystalline phase. The passing from the gel to the liquid crystalline phase is very quick and is characterized by a main phase transition temperature, T_m. At the main phase transition temperature, the bilayers contain equal percentages of ordered and disordered phospholipids. The value of the main phase transition is

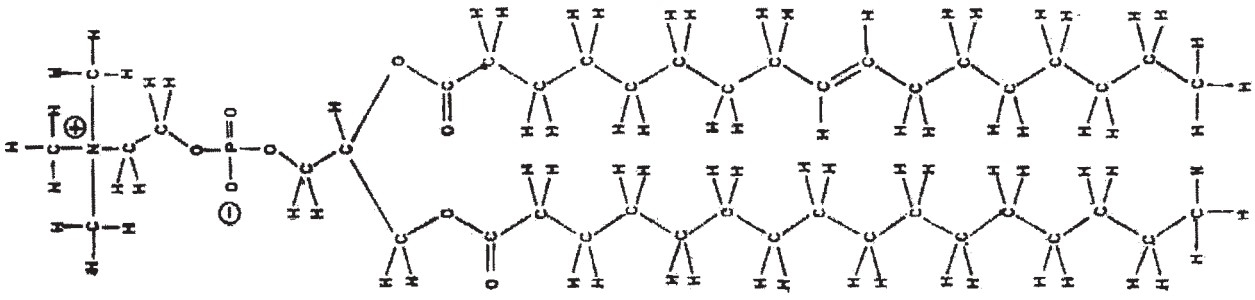

Fig. 1. Structural characteristics of DPPC

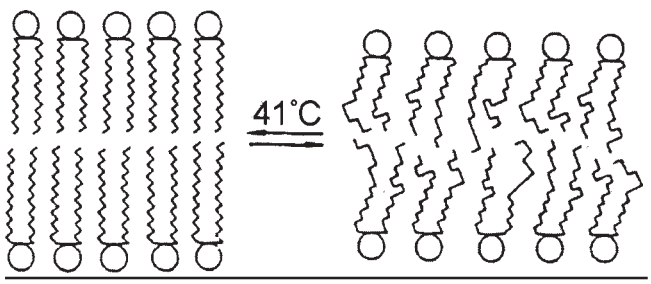

Fig. 2. Changes induces by temperature in the order degree of the long acyl chains of DPPC

* *Tel.: 0232201363*

dependent on the chemical nature of the model membrane constituents and also on their concentration in the model membrane.

The disordered hydrophobic acyl chains, in the liquid crystalline phase, induce a curvature stress in the bilayers that can determine pore formation. Substance transfer through the bilayer becomes possible in these conditions. Some antibiotics were used in order to test the phospholipids model membranes fluidity [4, 13]. These studies are important for understanding of the biological membranes functions.

FTIR spectra indicate the degree of order in the phospholipids bilayers by the shifts of the stretching symmetric and asymmetric vibration bands of the CH_2 groups belonging to the acyl chains. Important changes in the IR spectra, such as spectral shifts, or bandwidth modifications, are induced by the phase transition from the gel to the liquid crystalline phase when temperature increase.

2-D correlation spectroscopy is an important instrument for understanding the process taking place into phospholipids bilayers under external perturbations, such as temperature. This method allows the identification of the simultaneous or successive processes induced by the external perturbation, permitting us to propose a scenary for these processes at molecular structural level.

Experimental part

DPPC was purchased from Sigma Chemical Co. St. Louis Mo and used without purification. Multilamellar vesicles were obtained from DPPC dried films and phosphate buffer solution, using the procedure proposed by Severcan et all [4,13-15].

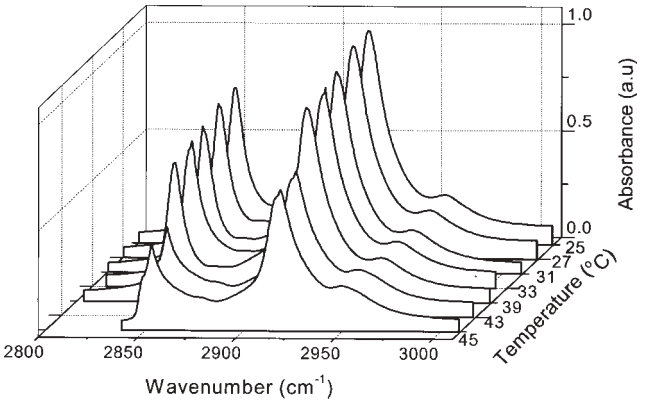

Fig. 3. FT-IR spectra at some temperature

FTIR spectra of the DPPC vesicles were registered with a FT-IR BOMEM MB 157 spectrometer in $CaCl_2$ cells. A Unicam Specac Temperature Controller was used for temperature modifying. The FT-IR spectra were averaged from 100 scans. The water vaporous influence was eliminated by subtracting the FTIR spectra of buffered solution from the model membrane spectrum, at each studied temperature (fig. 3). The temperature was modified in the limits 25-61^{0}C.

Results and Discussions

Having a high degree of order, the gel phase of the model membrane offers few possibilities for the vibrational and rotational motions of the acyl chains than the liquid crystalline phase, where the low degree of order enlarges the dynamics of these motions. The IR bands corresponding to the symmetrical or antisymmetrical modes of the acyl chains of DPPC are shifted to the high frequencies when the model membranes pass from the gel to the liquid crystalline phase.

2D-FTIR Correlation

Model-membrane proprieties were investigated using two dimensional correlation spectroscopy methods developed by I. Noda and Ozaki [16]. The scheme of the generalized correlation spectroscopy has been used in our investigations.

The calculus was made in the matrix form using Hilbert transform. The synchronous matrix is:

$$\Phi = \frac{1}{n-1} MM^T \tag{2}$$

and the asynchronous matrix is given by:

$$\Psi = \frac{1}{n-1} M(HM^T) \tag{3}$$

where: M is the matrix of the dynamic spectra; M^T is transposed matrix of M; n is number of spectra in series and H is Hilbert-Noda transform matrix (i.e. $H_{mn}=0$ if $m=n$ and $H_{mn}=1/\pi(n-m)$ otherwise):

$$H_{mn} = \begin{cases} 0, & m = n \\ \dfrac{1}{\pi(n-m)} & m \neq n \end{cases} \tag{4}$$

The dynamic spectra corresponding to the temperature perturbation in the range $25 \leq T \leq 61^oC$ were obtained. In order to obtain the 2D correlation spectra we used the average spectrum (fig.4) and the dynamic spectrum (fig.5). A program written in Matlab was developed in order to obtain mean spectrum (fig. 4) and dynamic spectra (fig. 5).

The mean spectrum was obtained by averaging the FTIR spectra recorded at different temperatures. The dynamic spectrum was obtained by subtracting the mean spectrum from each FTIR spectrum recorded at a given temperature.

From these maps analysis we can see that almost all the correlations between different IR bands are synchronous (maximum value of the correlation coefficient in synchronous map is 0.2722 compare to the 0.02247 in asynchronous map).

Synchronous spectrum

In synchronous spectrum two strongly auto-correlated peaks are evidenced at 2916 and 2848.5 cm^{-1} (peaks from the main diagonal in fig. 8). This type of autocorrelation peaks indicates that the 2916 and 2848.5 cm^{-1} are IR bands whose intensity strongly modifies by temperature modification, compared with another bands. Such synchronous modification suggests the existence of a

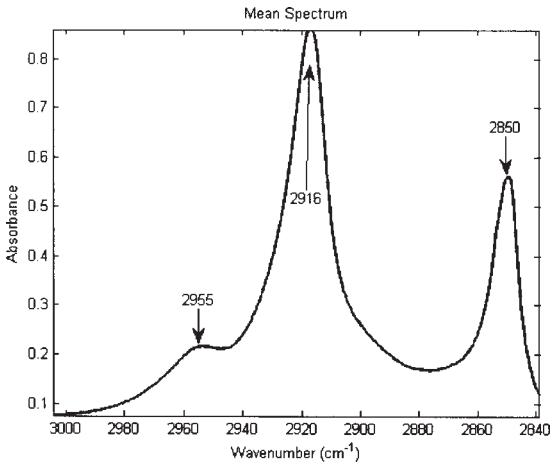

Fig. 4. Mean spectrum from figure 3

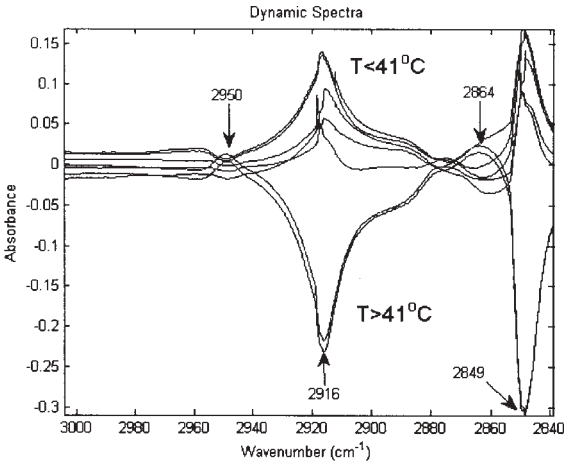

Fig. 5. Dynamic spectra from figure 3 using mean spectrum as in figure 4

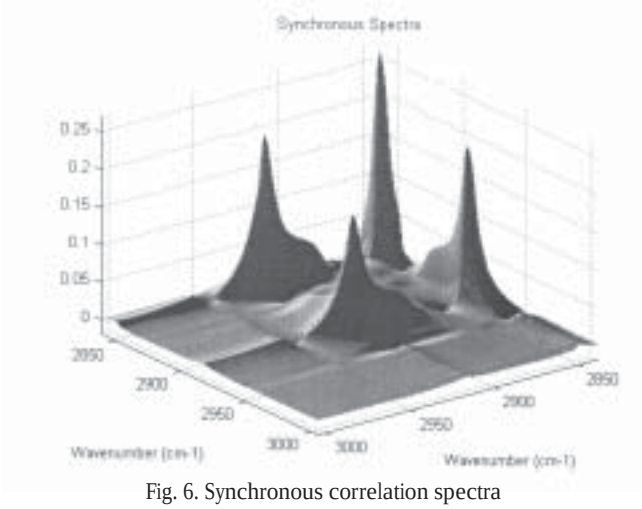

Fig. 6. Synchronous correlation spectra

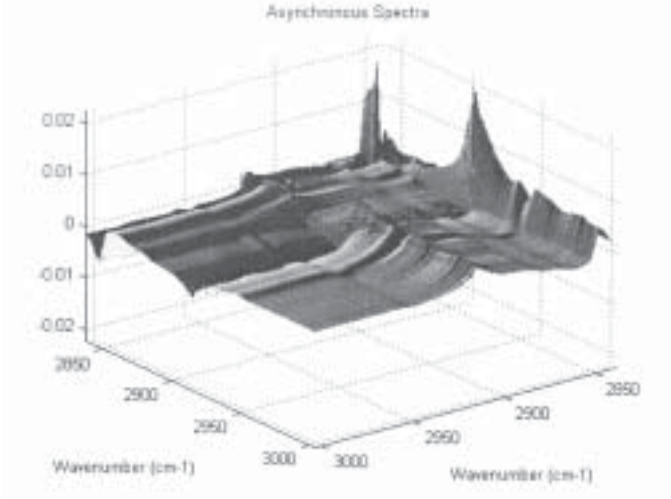

Fig. 7. Asynchronous correlation spectra

coupling between the variations of 2916 and 2848.5 cm^{-1} band intensities. This fact is due, evidently, to the simultaneous changes in the symmetric and asymmetric stretching vibrations of CH_2 from the acyl chains induced by temperature, in conditions of the phase transition of the type (1).

The off diagonal peaks indicates that there are positive correlations for 2916cm^{-1} and 2864cm^{-1} pair bands and negative for 2864cm^{-1} and 2848.5cm^{-1}. This is clear due to the temperature changes in the membranes and transition between gel phase and crystal liquid phase.

Asynchronous spectrum

Compared with the synchronous spectrum, the asynchronous spectrum evidences the successive correlations in the dynamic spectra (variations of the intensities in the dynamical spectra with different velocities, when temperature varies.

Using the two dimensional correlation spectroscopy technique we can obtain information about simultaneous or coincidental changes (using temperature as perturbation parameter) of two separate intensities variations measured at two different wavenumbers from synchronous spectra and out of phase changes from asynchronous spectra respectively.

From figures 6 and 7 it results a very good correlation between the symmetric and antisymmetric vibrations in the CH_2 groups of the acyl chains, showing simultaneous changes of these bands with the temperature modification.

Figures 8 and 9 represent the same synchronous and asynchronous spectra from 6 and 7 but using more commonly contour plots.

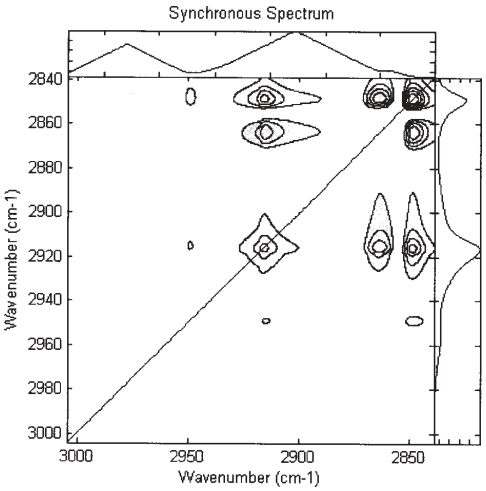

Fig. 8. Contours map for synchronous spectrum

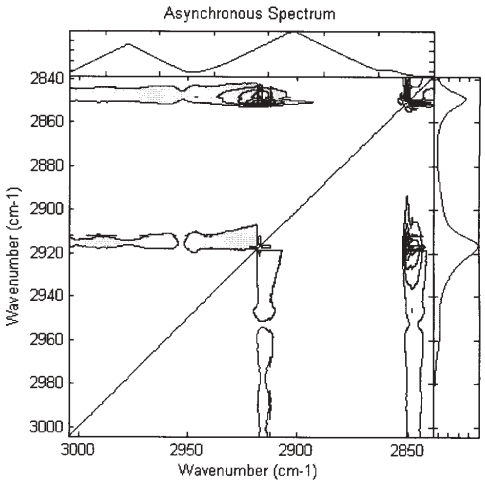

Fig. 9. Contours map for asynchronous spectrum

From figure 9 it results that the asynchronous changes appear in the spectral range 2916-3000 cm^{-1}, but there are represented by very narrow width bands. This fact could be explained by the conformational changes in the chains at the main phase transition of the type (1) and also by the variation of the two phase's concentration at temperature variation.

There are two strong autocorrelation peaks at about 2916cm^{-1} and 2848.5cm^{-1} (peaks from main diagonal in fig. 8)

Conclusions

2D correlation FTIR spectroscopy permits to evidence the correlations between the vibrations of different structural groups and the modifications induced by temperature (as a perturbation factor). The dynamic spectra give the temperature of the main phase transition, their peaks being positive for $T<T_m$ and negative for $T>T_m$.

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References

1. JAIN M.K., Introduction to Biological Membranes, John Wiley and Sons, 1988
2. EPAND R.M., Biochim. Biophys. Acta, **1376**, 1998, p. 353
3. BRANDEN C., TOOZE J., Introduction to Protein Structure, Gareand Publishing, Inc. New York, London, 1991
4. SEVERCAN F., KAZANCI N., BAYCAL U., SUZER S., Bioscience Reports, **15**, p.205, 2000
5. SLAVNICU E., GHELMEZ M., SLAVNICU D., STERIAN A.R., Rev. Chim. Bucuresti, **56**, 10, 2005, p. 1024
6. DUMITRA ª CU I., DUMITRA ª CU L., DOROHOI D.O., J.O.A.M., **8(3)**, 2006, p.1028
7. HONCIUC M., PĂUN V.P., Rev. Chim. (Bucure°ti), **54**, nr. 1, 2003, p. 74
8.OPRESCU B., TOPALÃ C., TĂTARU L., ANGHEL S., Rev.Chim. (Bucure°ti), **54**, nr. 7, 2003, p. 591
9. TOPALÃ C., OPRESCU B., GIOSANU D., Rev. Chim. (Bucure°ti), **54**, nr. 3, 2003, p. 221
10. STAN C., CRISTESCU C.P., SEVERCAN F., DOROHOI D.O., Rev. Roum. Chim. **49**, nr. 9, 2004, p. 777
11 STAN C, CRISTESCU C.P., SEVERCAN F., DOROHOI D.O., Mol. Cryst.& Liquid Cryst. **457**, 2006, p. 27
12 TOPALÃ C., MELTZER V., Rev. Chim. Bucure°ti, **55**, nr. 1, 2004, p. 31
13. AKINOGLU B.G., GHEITH M., SEVERCAN F., J. Mol. Struct., **565-566**, 2001, p.281
14. SEVERCAN F., DURMUS H.O., EKER F., HARIS P.I., AKINOGLU B.G., Talanta, **53**, 2000, p.205
15. SEVERCAN F., TOKMAK S., AGHEORGHIESEI C., DOROHOI D.O., An. Univ. Al. I. Cuza, Ia°i, s. Chimie, t.**X**, 2002, p.259
16. NODA ISAO, OZAKI YUKIHIRO, Two-Dimensional Correlation spectroscopy, John Wiley and Sons, 2004

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