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TEMPERATURE INFLUENCE ON THE DIPALMITOYLPHOSPHATIDYLCHOLYNE - MODEL MEMBRANES STUDIED BY FTIR

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Lyotropic liquid crystals are realized from certain concentration of amphiphilic molecules, such as phospholipids, in water. The specific interactions in which water is involved are very important for model-membrane stability. Dipalmitoylphosphatidylcholyne (DPPC) model membranes have been studied by FTIR technique at different temperatures in the spectral range {2500 – 4000} cm^{-1}. A Gaussian deconvolution has been performed in order to study the water behaviour from the point of view of hydrogen-bond formation between water molecules and both the hydrophilic heads of phospholipids and the components of buffer solution. This study contributes to the understanding the mechanisms which assure the model membrane stability.

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1. Introduction

Biological membranes [1] are complex and heterogeneous assembly of non-polar and amphiphilic molecules. Biological membranes are crucial cellular components with multiple roles:

- they maintain electrochemical gradients by controlling the diffusion of ions and biomolecules;
- they act as a supporting matrix for embedded enzymes and receptors;
- they engage in stabilizing interactions with skeleton proteins.

In order to explain the biological membrane functions, simplified model-membranes from the main constituents of the biological ones were adopted and studied by various means. So, DPPC model membranes can be achieved in a buffer solution [2, 3] and studied by FTIR technique.

Interactions of DPPC with aqueous dispersions determine the conformational arrangement of DPPC liposomes or bilayers.

The measuring of some spectral parameters like band frequency, width and intensity change provided information regarding the possible structural interactions and conformational rearrangements taking place.

In 1994 Egberts et al [4], carried out preliminary studies of DPPC bilayers, in which they identified various phospholipids-phospholipids and phospholipids-water interactions. They also estimated the rate of water diffusion across the bilayer [4].

DPPC phospholipids bilayers exhibit two principal thermal lamellar phase transitions [5], corresponding to a gel to gel $\left(L_{\beta'} - P_{\beta'}\right)$pretransition and a gel to liquid crystalline $\left(P_{\beta'} - L_{\alpha}\right)$main transition at $T_m = 41.5^0 C$. In the liquid crystalline L_{α} phase, the hydrocarbon chains of the lipid bilayers are conformational disordered, whereas in the gel phase, the hydrocarbon chains are more extended and relatively ordered [6].

Interesting studies about the polarity and degree of hydration that DPPC adopts in solutions can be gain by monitoring thermal alterations in C=O stretching band of DPPC carbonyl group and PO_2^- antisymmetric stretching band of DPPC polar head groups. The decreasing mode of the overall frequency with temperature at

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the two components is an indication of the probability of hydration that DPPC adopt in aqueous solutions as that the ester carbonyl groups are potential recipients of hydrogen bonding interactions with water. The Fourier transforms self-deconvolution and Fourier 2^{nd} derivative revealed two bands: $1741 \pm 1\, cm^{-1}$ and $1727 \pm 2\, cm^{-1}$ attributed to the individual vibrations of the two ester groups (they were attributed to the CO situated to the primary and secondary positions of glycerol backbone)[6,7].

At T_m and upon temperature increasing major structural rearrangements take place in the bilayer as the hydrocarbon chains start to melt. This may lead to an increase in the degree of disruption in the DPPC bilayers causing an increase in the probability of hydrogen bonds to C=O groups.

An appreciated decrease in the FTIR frequency of C=O groups is noticed in the absorption spectrum as these groups admit a concomitant of hydration [2]. The hydration is presumed to increase above the gel to liquid crystalline phase transition which can be assigned as an indicator of increasing interactions between the C=O and solvent and other component moieties [1].

PO_2^- antisymmetric stretching mode can give future indications for hydration or dehydration of DPPC dispersions in different solutions [6]. Hydration will induce a shift of PO_2^- asymmetric stretching band frequency to lower frequencies and de-hydration shifts frequencies to higher values [3, 7].

Based on the experimental data obtained in FTIR spectra corresponding to CO stretching band of DPPC carbonyl group and PO_2^- anti-symmetric stretching band of the DPPC polar head group, we supposed that he hydration of the DPPC head could be also analyzed in the range {2500-4000} cm^{-1}, where the stretching vibrations of the free and bonded water molecules appear. The water clusters and also the presence of the water bonded to the phospholipids head groups could be evidenced in this spectral domain. We made a study in the IR range of the stretching modes of water molecules in order to obtain information about the hydrogen bonds formed between water and phospholipid molecules between water and and also between the water and Na_2HPO_4 from buffer solution molecules.

2. Experimental setup

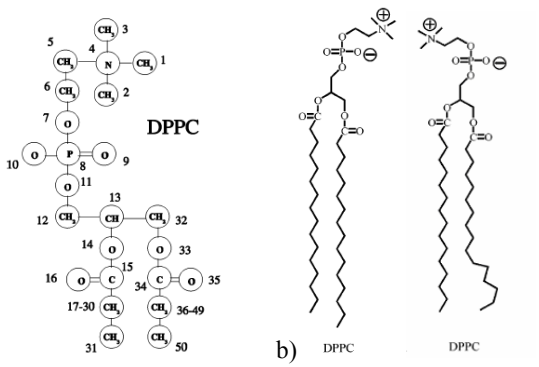


Fig.1 a) DPPCstructure, b) DPPC tayls arranmgement before 41° C and after 41° C

Multilamellar DPPC liposomes were obtained [8,9] as thin filmes in exces Na_2HPO_4 buffer for 20 minutes at $66^0 C$. The liposomes were incubated in a shaking water bath for 1hour at a temperature around $45^0 C$.

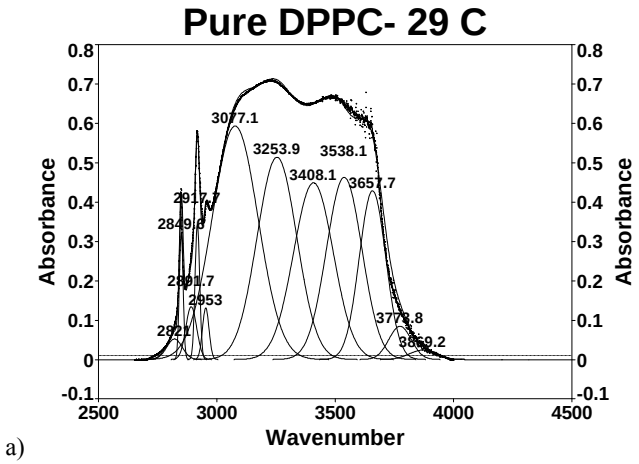
Fourier Transform Infrared (FTIR) spectra were registered using a Bomeme 157 FTIR spectrometer. The interferograms were averaged for 100 scans. The samples were investigated in a large temperature range with increasing temperature from $23^0 C$ to $70^0 C$. The temperature increasing rate was small enough to assure quasistatic processes [9].

An Unicam Digital Temperature Controller unit with a thermocouple located around the edge of the cell window was used for temperature monitoring. Each spectrum was recorded after 15 minutes of temperature stabilization to make sure that the sample is that displayed by digital controlled.

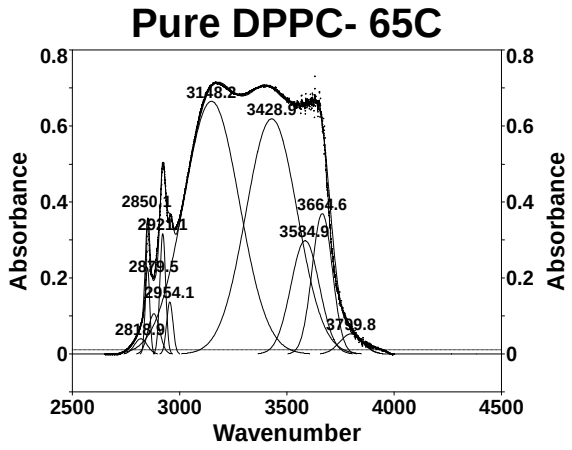
The structural formula of DPPC and also the arrangement of the hydrocarbonic tayls in the gel and liquid crystalline phases of DPPC are given in Figs 1 a) and b), respectively.

3. Results and discussion

The program PeakFit v4.12 [10, 11] was used as a very powerful tool to deconvolute the IR spectra, by using Gaussian (Amplitude) deconvolution. After deconvolution, the program calculates area, amplitude, center and width for every peak.



a)



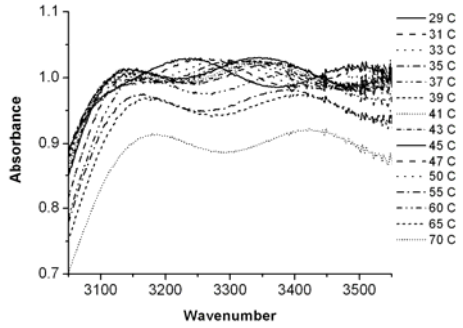
b)

Fig. 2. The deconvoluted spectra for 29 and 65° C

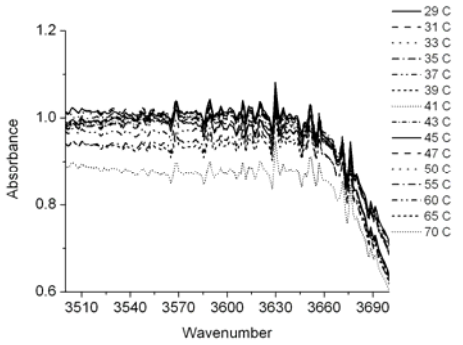
The first IR band in the range {3000-3800} cm^{-1} Figs. 2a) and b) is characteristic of buffer solutions, the bands with wavenumbers bigger than 3600 cm^{-1} could be attributed to free water molecules and the FTIR band with the wavenumbers smaller than 3600 cm^{-1} are characteristic to band water molecules, between them or with phospholipids head groups. Fig. 2a) and b) give the deconvolution of the TIR spectra recorded at two different temperatures.

The hydrogen bond number was estimated from the IR band area and the half-bandwidth of the IR band was considered as order indicator.

In order to estimate the possibilities of hydrogen bond formation between the water molecules and phospholipid heads and only between the water molecules, a spectral range {2500-4000} cm^{-1} for which a few studies were published until now, was considered. In this range appear the symmetric and asymmetric stretching bands of $-CH_2$ and $-CH_3$ groups {2817-2955} cm^{-1} and also the stretching and bending vibrations of free or bound water molecules {3100-3800} cm^{-1} [12,13].



a)

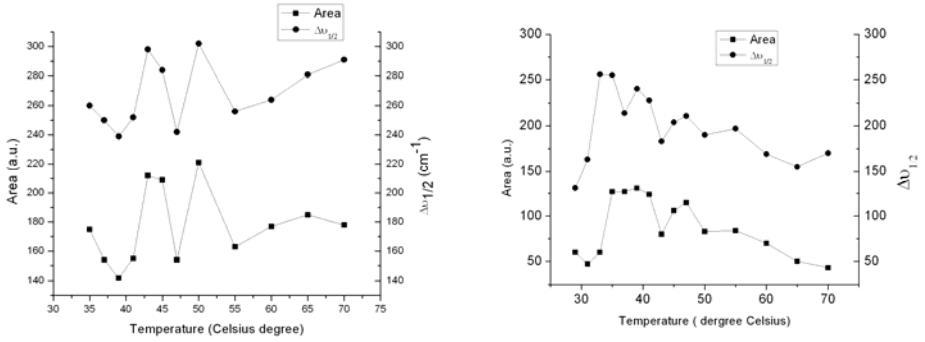


b)

Fig. 3. a) FTIR spectra of free and bonded water molecules; b) FTIR rotational structure of OH vibrations for free water molecules.

The deconvoluted spectra at $29^0 C$ and at $65^0 C$ are given in Fig.2, in which the spectral ranges listed above can be seen. The spectral range $\{2817\text{-}2955\}\, cm^{-1}$ is widely studied in literature [5, 8, 9]. For these reasons we analysed the range $\{3000\text{-}3800\}\, cm^{-1}$ in which different types of bound and free water molecules are expected to absorb. The free water molecules give the high wavenumber bands with wavenumbers higher than 3600 cm^{-1} [6]. At smaller wavenumbers the water dimers or polymers [12-14] appear.

From Fig.3 it results that for temperatures higher than the main transition temperature [2], the band width increases by temperature, indicating an increase in the thermal motion of water molecules in the separating liposomes water layer. Concomitantly the area of the same band, corresponding to the bound water molecules, decreases showing a decrease in the number of the bound water molecules.



a b

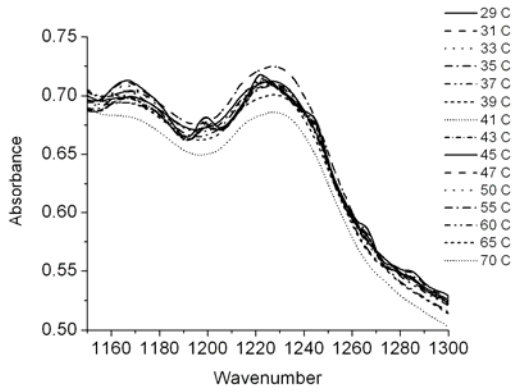
Fig. 4: Area and bandwidth for a) 3300 cm^{-1}; b) 3600 cm^{-1}.

Changes in intensities and wavenumber for FTIR bands in figure 3a illustrate the continuous modification of bonded water molecules number and also of the bond types. The temperature can cause broke of some hydrogen bonds and water molecules participation to water-water or phospholipids-water new hydrogen bonds. This kind of behaviour is also demonstrated by figure 4, where the area and bandwidths temperature dependences are similar with those obtained for a complex liquid [15] in which weak hydrogen bonds act.

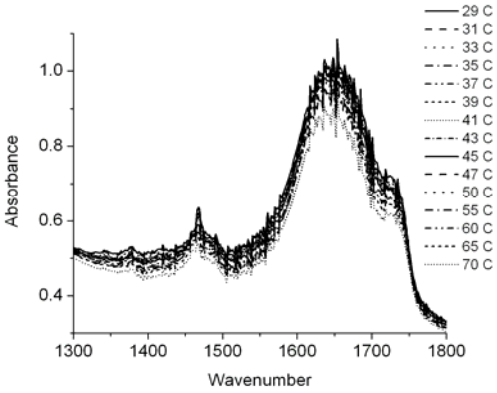
The hydrogen bond formation can be evidenced in the spectral ranges {1160-1300} cm^{-1} corresponding to stretching modes of $-PO_2^-$ (figure 5 a) and in the range {1300-1800} cm^{-1} corresponding to stretching mode of CO group (fig. 5b).

In fig 5a) are illustrated the modifications induced by temperature in the $-PO_2^-$ stretching vibration range. The frequency decreasing in this range demonstrates the hydrogen bond formation between the phosphate groups and water molecules.

In the CO stretching vibrations range, two distinct bands appear for C = O band and free in hydrogen complexes with water molecules.



a)



b)

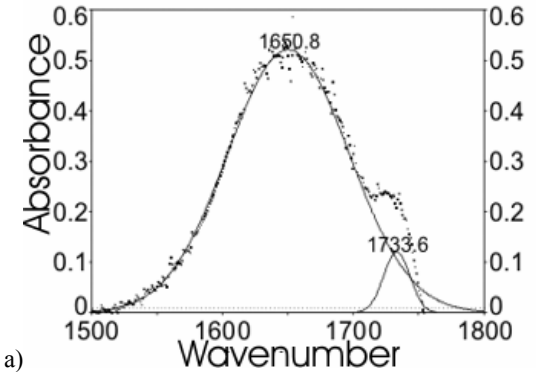
Fig. 5. a) PO2- stretching vibration bands; b) CO stretching vibration bands

Having in view the great number of water molecules 30:1- 100:1 in phospholipid solutions in which the liposomes or bilayers can spontaneously appear [10], large possibilities to participate to hydrogen bonds (water-water, or phospholipid head-water) are opened to water molecules.

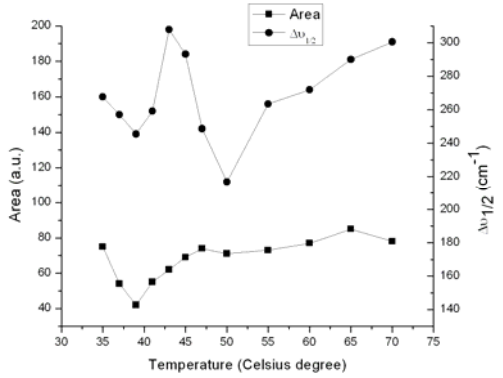
Cluster formation of various types is possible in the water layers separating the head groups of the DPPC bilayers.

The structure of DPPC offers various possibilities in achieving hydrogen bonds with water molecules. So, the $-PO_2^-$ and $-CO$ groups are able to make hydrogen bonds with water molecules.

From deconvoluted spectra Fig 6a) it results an increase of CO groups which participate to H bonds with temperature increasing. The decrease of the CO stretching mode frequency from 1733 cm^{-1} to 1650 cm^{-1} proves the hydration of CO groups from the phospholipids polar heads. In figure 6b the dependence of the area on temperature is illustrated.



a)



b)

Fig. 6. CO stretching mode: a) deconvolued spectra; b) Area and bandwidth for 1650 cm^{-1}

4. Conclusions

Pulmonary surfactant has unusual surface that reduce the mechanical work of breathing and prevent lung collapse. During respiration, the surface layer is compressed and expanded. L-dipalmitoyl phosphatidylcholine (DPPC) is the most abundant phospholipid component of pulmonary surfactant.

The de-convoluted IR spectrum in {2500-4000} cm^{-1} can offer information about the specific interactions at which the water molecules are involved in the water layer separating the liposomes or the phospholipid bilayers. This study is important to read-in the mechanism of pulmonary membrane disruption in lung diseases, influenced by temperature and drugs.

Further work is the study of antibiotic influence on the DPPC model membrane fluidity.

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