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PARAMETRICAL CONTROL OF THE ELECTRICAL PERMITTIVITY FOR THE L_α PHASE OF THE DIPALMITOLYLPHOSPHATIDYLCHOLINE, DETERMINED BY SIMULATION METHODS

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A simulational study was performed in order to determine the components of electrical permittivity tensor for the hydrated dipalmitolylphosphatidylcholine (DPPC) in the liquid crystal (LC) phase, L_α. The complex behavior of the smectic arranged bilayers in the multi-lamellar structure of this biological lipid was analyzed by structural simulation methods using the HFFS program. Results illustrate a consistent variation of the parallel relative permittivity values (30 to 96) along the multi-layered structure, inside and outsider the bilayers. The simulation method offers us the possibility to vary different physical and geometrical parameters (concentration in solution, buffer ions, temperature) in order to control the electrical permittivity variations at microscopic scale and implicitly the effective permittivity of the biological LC at macroscopic scale. The temperature imposes itself like a very important factor which has to be considered for determining the correct electrical permittivity value, due to the fact that in the temperature range between 21 and 42 ^{0}C more phase transformations from gel to LC occurs in DPPC, each phase presenting different ordering and characteristic parameters. Our simulation method has enabled us a facile control parameters variation at structure level and monitoring of the effects with a satisfactory precision (less 4 % relative error in physical parameters determination).

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1. Introduction

The pulmonary activity is conditioned by the natural lung surfactant, which represent a mixture of lipids and apoproteins secreted by the alveolar cells into the alveoli and respiratory air passages. The surfactant role is to reduce the surface tension of pulmonary liquid, in order to allow it to foam or penetrate solids. Up to 70% weight of the lipid component of the natural surfactant consists of dipalmitoylphosphatidylcholine (DPPC). The efficiency of DPPC is extremely good. Practically the surfactant dispersion is not correlated with the microstructure and the ability to achieve superlow tensions. Consequently, a minimum particle concentration of the surfactant (~ 0.1 wt%) is enough for reproducibly observing superlow tensions [1] .

The applications of the DPPC are multiple: the major constituent of pulmonary surfactant, where modulate the functions of alveolar macrophages; modulates inflammatory functions of monocytic cells; also used for research purposes in studying liposomes, lipid bilayers, model biological membranes and in the formation of reconstituted HDL particles (high-density

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lipoprotein, which enable lipids like cholesterol and triglycerides to be transported within the water-based bloodstream). The understanding of the complex behavior of the structural DPPC in aqueous solutions at different temperatures helps us to manipulate efficiently the phospholipid for a specific application.

Our method of study was the structural simulation, which helped us to reproduce more phase transformations which occurs at structure level under the influence of different parameters like temperature, constituents concentration in solution, buffer ions, pressure, etc. The DPPC in aqueous solution evolves from an "ordered" gel phase to a "disordered" phase of bilayers, presenting even multi-lamellar periodicity, which can be classified like a liquid crystal (LC) phase. Different substances incorporated in the membrane can affect the main phase transition to LC. Some anesthetics (*e.g.* halothane and enflurane) are decreasing the transition temperature and increasing the width of the transition without affecting the enthalpy change. They decrease the degree of cooperative interaction between phospholipid molecules within the bilayer [2], [3]. Other encountered effects: by adding cholesterol to membranes, the average area of membrane becomes smaller and hydrocarbon chains of DPPC have higher order [4]. As a conclusion, the transition temperature is influenced by incorporation of different substances in the membrane and its variation, ΔT_m is function of the additive substance proportion with different control parameters (pH, ionization degree of the additive, etc.) [5].

Various modeling techniques were used until now to explore the dynamic DPPC membrane, such as mean field theory, Monte Carlo simulations, dissipative particle dynamics (DPD) simulations, atomistic and coarse-grained (CG) molecular dynamics simulations. The most successful used was the collective model for slow molecular reorientations in the lipid bilayers, describing the segmental or molecular reorientation in lipid bilayers [6]. We are coming with a new structural simulation strategy with the HFSS program, which reproduces the internal structure at macroscopic level, considering the interactions between microcomponents and with the internal and external fields.

2. Simulational model

The dipalmitoylphosphatidylcholine (DPPC), with formula $C_{40}H_{80}NO_8P$, can also be written as 1,2 - dipalmitoyl - *sn* - glycero - 3 - phosphocholine. The phospholipid structure consists of a polar headgroup connected by ester linkages to two hydrophobic alkane chains (Fig. 1). Among the physical parameters considered for our simulation can be enumerated: molecular weight of 734.038862 g/mol; molecular polarizability: 82.39 a.u. = $13.584 \cdot 10^{-40}$ C·m^2/V, polar surface area: 121 Å^2, solvent accessible surface area: 1403.77 Å^2; effective dipole moments of DPPC: $1.564 \cdot 10^{-30}$ C·m (DPPC has a negative vertical dipole due to the relative positioning of a negatively charged phosphate group and positively charged trimethylammonium group); OH bond length: r_{OH} = 0.9572 Å; HOH bond angle: θ_{HOH} = 104.52 ^{0} [7], [8], [9]. The coordination numbers of various atoms around the ions were considered.

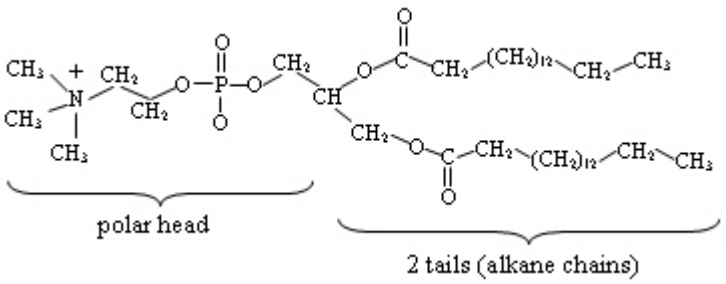

Fig. 1 Molecular structure of the dipalmitoylphosphatidylcholine ($C_{40}H_{80}NO_8P$).

Like phospholipid, the DPPC molecules are amphiphilic and form a self-assembled bilayer membrane in the presence of excess water (about 40 - 100 water molecules per lipid molecule). The molecules arrange themselves in two layers, with their hydrophilic polar groups towards the surrounding aqueous medium, and their lipophilic chains towards the inside of the bilayer (Fig. 2). The structure has a non-polar region between two polar ones.

For simulations, the anisotropic rotational diffusion model describing the segmental or molecular reorientation in lipid bilayers was replaced with a collective model for slow molecular reorientations in lipid bilayers, in agreement with the results presented in literature [6]. Structural changes of the membrane with temperature were watched out, at normal atmospheric pressure.

When temperature increases, the DPPC bilayer membrane undergoes three types of the structural changes: the subtransition from subgel (L_c phase) to lamellar gel (L_β' phase) at 21.2 °C (due to the acyl chain ordering and the head group dehydration), the pretransition to ripple gel (P_β' phase) at 34.3 °C, and finally the main transition to the liquid crystalline (L_α phase) at T_m = 42.0 °C [10], [11]: $L_c \xrightarrow{21.2\ ^0C} L_\beta' \xrightarrow{34.3\ ^0C} P_\beta' \xrightarrow{42\ ^0C} L_\alpha$ (Fig. 2). By our simulation method we have represented the "ordered" phases (L_c, L_β', P_β'), transforming from one to another, as well as the L_α "disordered" phase. We have considered for the main transition: ΔH = 7.4 ± 0.4 kcal/mol; $\Delta G_{(H2O)}$ = 6.57 ± 0.3 kcal/mol; E_a = 50.34 ± 5.46 kcal/mol. The simulational analysis has pointed out an important fact: different phases might coexist and a phase diagram is specific for the complex thermal behavior of the structure. Like extra-domain, under special condition an interdigitated structure appears, a non-bilayer structure, formed in a finite temperature range at high pressures above ca. 1000 atm [10].

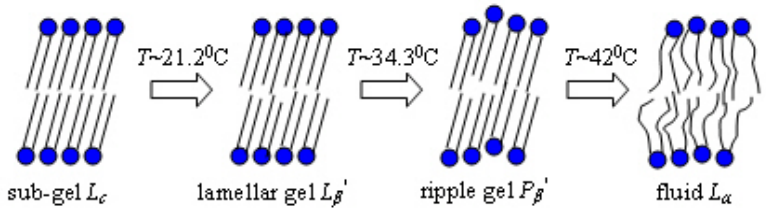

Fig. 2 Thermal induced phase transformations in the DPPC bilayer membrane in the presence of excess water.

The phase captivating the most interest is the LC phase, which presents an interesting organization and dynamics. Like mechanism, water can penetrate deep inside the bilayer almost up to the starting point of the aliphatic chains [12]. The water molecules penetrate with the help of the NMe(3) group belonging to the headgroup and can form hydrogen bonds with carbonyl oxygen present inside the bilayer [12], [13]. The effect is the disordering of the tails in the L_α phase (above T_m), with unhindered lipid tails in random motion. With temperature increasing, the level of interdigitation between lipid leaflets increases, but the bilayer structure is still kept.

Finally an arrangement which remember us of a smectic liquid crystal was experimentally found [14] and reproduced here by our simulation method, with a relative constant distance between double leaflets (Fig. 3). The buffers and additives, like Na_2HPO_4, or NaCl, Ca ions [9], [14], etc., can control this distance in order to obtain LC structures with different macroscopic parameters.

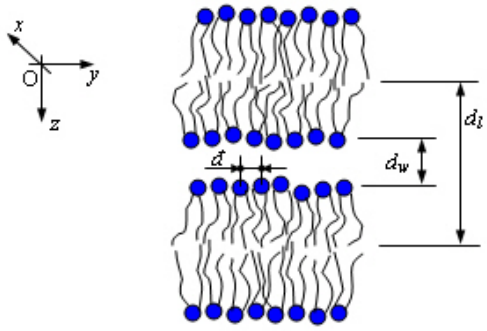

Fig. 3 The lamellar liquid crystal structure of the DPPC with excess water (plus additives).

The electrical permittivity of the lipid membrane in excess of water can be expressed in tensorial form, considering the formula for the electric displacement inside the liquid crystal structure:

$$\vec{D} = \begin{pmatrix} \varepsilon_{rII} & 0 & 0 \\ 0 & \varepsilon_{r\perp} & 0 \\ 0 & 0 & \varepsilon_{rII} \end{pmatrix} \cdot \vec{E}, \qquad \text{with } \varepsilon_{r\text{II}} = \varepsilon_{r\text{II}}' - \text{j}\varepsilon_{r\text{II}}'',\ \varepsilon_{r\perp} = \varepsilon_{r\perp}' - j\varepsilon_{r\perp}'' \tag{1}$$

The $\varepsilon_{r\text{II}}$ and $\varepsilon_{r\perp}$ are the parallel, respectively transverse permittivities, calculated for the field propagation direction parallel, respectively transverse on the long molecular axis $\vec{n}$. The permittivity tensor components were calculated on the basis of a physical algorithm written for the LC samples exposed to the microwave field of a horn antenna. The simulational set up was structured with help of the HFFS program.

The physical algorithm implies calculation of the energy density variation when the testing field propagates through the material sample. The Poynting's theorem in integral form was used:

$$\frac{\partial}{\partial t} w \cdot dV + \oint_{\partial V} \vec{S}_{Poynting} \cdot d\vec{A} = -\int_V \vec{J} \cdot \vec{E} \cdot dV \tag{2}$$

where $\vec{S}_{Poynting} = \vec{E} \times \vec{H}$ is the Poynting vector, w represents the energy density, $w = w_e + w_m$; $\vec{J}$ is the current density and ∂V is the surface which encloses the sample volume V.

The dw_e variation is linked by the electric susceptibility tensor components:

$$dw_e = \vec{E} \cdot d\vec{P} = \vec{E} \cdot d\left(\varepsilon_0 \cdot \overline{\overline{\chi_e}} \cdot \vec{E}\right) = \varepsilon_0 \cdot \left(\vec{E} \cdot \overline{\overline{\chi_e}} \cdot \vec{dE}\right) \tag{3}$$

For the susceptibility determination, the dw_e variation was calculated using the data given by the HFSS.

3. Results for the LC permittivity

A complex simulational study of the DPPC bilayer-water system was performed in HFSS 13.0. The system evolution over the temperature range of 18 - 52 ^{0}C has been analyzed and the transformation of phases has been monitored in detail at structure level. The contribution of constituents, sub-structures and ionic groups, their modification and evolution over the phase transformations were carefully observed.

The considered bilayer-water system was a stable one, with a molecular ratio water/DPPC of 40:1, at atmosferic presure. System evolves through phases to a multi-lamellar system when temperature is increased. For describing the force field for DPPC and water, intramolecular parameters for bonds, angles, proper and improper dihedral were taken from literature [15], [9]. A

testing cell has included 10 bilayers with 128 x 2 lipids per bilayer (128 per leaflet). The projected membrane area $A = L_x \times L_y$ corresponded to an area per lipid of 64.3 Å^2; the lipid volumes were of ca. $(12 \pm 1) \cdot 10^2$ Å^3/lipid, close to the experimeltal reported values [16].

Formation of the self-assembled bilayer membrane due to the amphiphilic nature of the DPPC was simulationally reproduced in the presence of excess water, until the water content was kept under a physical threshold. Upon temperature increasing starting from below the first phase transformation, the ordered, stretched, tilted at 33 ^{0}, alkane chains begin to distance and bent, respectively the polar heads loose a little of their regular planar order, while the water molecules are penetrating deeper the leaflets. Hydrogen bonds water-water, or phospholipid head-water become consistent, $-PO_2^-$ and $-CO$ groups being able to make hydrogen bonds with water molecules [9]. At the main phase transition, major structural rearrangements take place in the bilayer as the hydrocarbon chains start "to melt".

Determinations of the $\varepsilon_{r\text{II}}$, $\varepsilon_{r\perp}$ permittivities were performed by simulation for the hydrated DPPC membrane in the L_α phase. A lamellar periodicity in pure water $d_l \approx 65$ Å was considered. We had to take into account that the multilamellar dispersions of DPPC in the LC state (describing the segmental or molecular reorientation in the lipid bilayers) is a function of temperature and frequency [6]. The operational temperature of represented graphs was of 49 ^{0}C, above the T_m, and the results were obtained for an exposure field with frequencies in GHz range: 0.915 - 2.45 - 5.8 GHz (ISM frequencies), where applications for bio-MEMS works. Graphs in Figs. 4 and 5 were given for f_o = 2.45 GHz and the frequency dispersion of the permittivity curves was illustrated in Fig. 6.

Values of the parallel permittivity, calculated for an applied electric field $\vec{E}$ propagating parallel with the molecular dipoles, evolves along the propagation direction from maxima specific for the polar head regions to low values in the hydrocarbon core and then to minima in the bulk aqueous between bilayers.

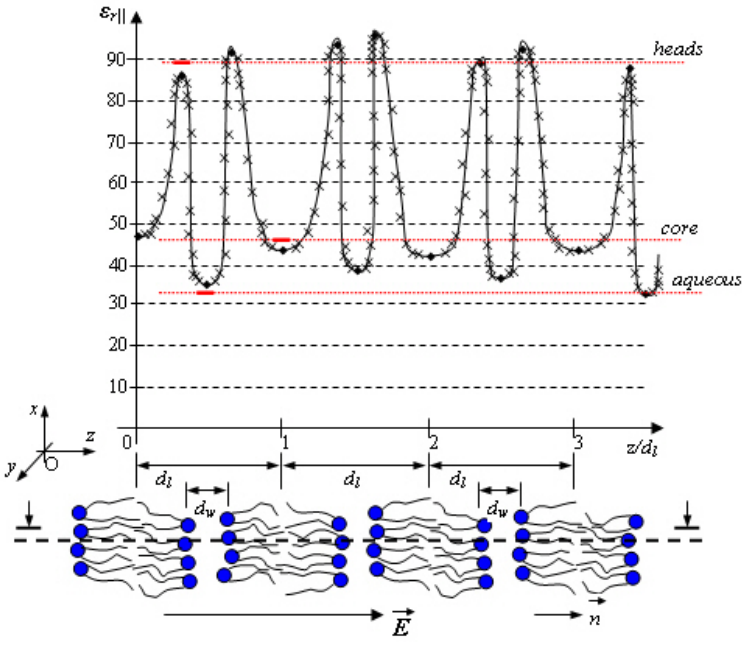

Fig. 4 Variation of the parallel relative permittivity of the liquid crystal L_α phase of the DPPC in water solution, molecular ratio 1:40, represented in the direction transverse on bilayers (f_o = 2.45 GHz, T = 49 ^{0}C). The permittivity values reported in literature in the headgroup region, in the hydrocarbon core of the lipid bilayer, respectively in the bulk aqueous were represented with red dot lines.

Due to the excess of hydration, the alkane chains have enough spacing and freedom for moving, in order to allow to the molecular dipoles parallel with the long molecular axes to align themselves after the field direction. One dipolar end is relatively stable and blocked in the polar head region, where the parallel permittivity is high, the other end being free to move in the rarefied hydrated core (with low permittivity) and is oriented by the field.

The simulational method has pointed out the high non-uniformity of the parallel permittivity values along the multi-lamellar structure when we follow the direction transverse on the bilayers (*Oz*). Each molecular subgroup presents a strong individuality (polar heads, alkane chains) and the transition to a region to another is sharp. The permittivity peaks corresponding to the headgroup region evidence good insulator properties in that area, which behaves like a barrier of conductive properties of the aqueous membrane. This can be an interesting effect for nanotechnology and applications. The wide permittivity valleys obtained for the hydrocarbon core of the lipid bilayer evidences that the dielectric properties are not so good inside the bilayers. The bulk aqueous permittivity in the zones between the bilayers decreases even lower than the core valleys, the neutral zone being easy to be polarized but suffering the interactional influence of the neighboring bilayers.

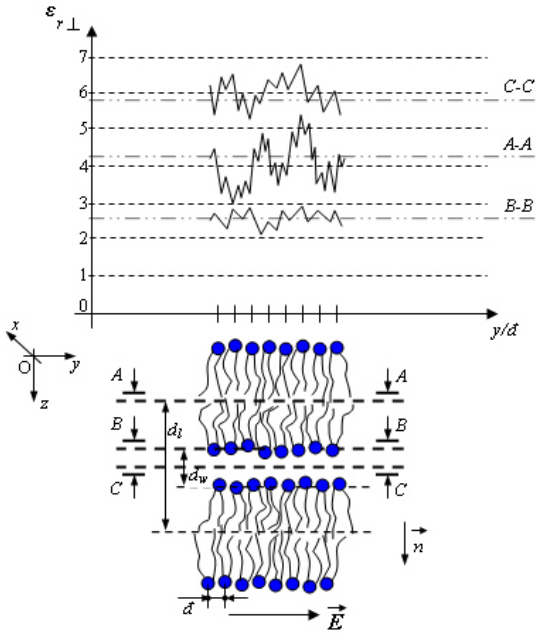

Fig. 5 Variation of the transverse relative permittivity of the liquid crystal L_α phase of the DPPC in water solution, molecular ratio 1:40, represented in the direction parallel with bilayers (f_o = 2.45 GHz, T = 49 ^{0}C). Three xOy section planes were considered: at headgroup level (B-B), in the hydrocarbon core inside the bilayer (A-A), respectively in the bulk aqueous (C-C).

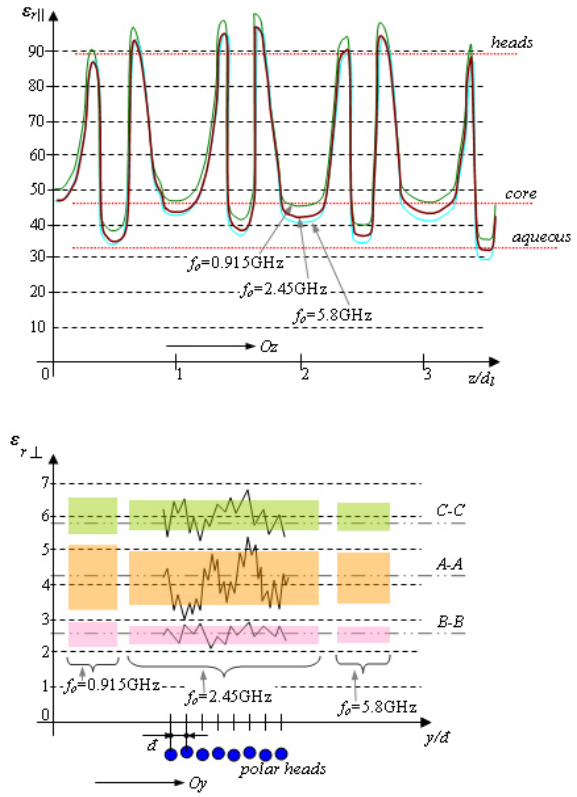

Fig. 6 Frequency dispersion of the permittivity curves for the liquid crystal L_α phase of the DPPC in water solution, molecular ratio 1:40, represented at three ISM operating frequencies in microwave range: 0.915; 2.45 and 5.8 GHz . In the graph below was represented the evolution of the transverse permittivity fluctuation bands at the considered ISM frequencies.

When we analyze the transverse permittivity of the liquid crystal L_α phase of the DPPC in water solution, we have to consider that the structure is symmetrical in respect with the *Ox*, respectively *Oy* directions. The structure is relative uniform in the parallel *xOy* planes, the electrical permittivity presenting random fluctuations around a mean value in each plane. Fluctuation magnitude depends on the plane position inside the multi-lamellar structure, as it is illustrated in the Fig. 5.

Fluctuations are due to the medium discontinuities when one passes from a polar head/tail to another. Transverse permittivity fluctuations are stronger in the middle of the hydrocarbon core inside the bilayer, where the alkane chains "disorder" is more advanced, their magnitude and their mean value decreasing when the *A-A* plane become closer to a headgroup plane. The most stable values for the transverse permittivity have been found in the headgroup planes *B-B*, which represent a sub-structure with closely-packed atomic groups. The positional instability of the tails, the variety of their relative positions, deep penetrated by water molecules, determine large fluctuations of the electric permittivity in the *xOy* pane at the hydrocarbon core level inside the bilayer. The bulk aqueous between the bilayers does not present the characteristics of a pure one, but behaves itself like an interstitial medium influenced by near-field interactions with the surrounding bilayers.

4. Discussion

The electrical permittivity determinations for L_α phase of the DPPC in aqueous solution can be performed by simulation methods if we consider a valid domain of variation for the internal and external parameters which influence the structure. Some threshold values of these domains of viability can be determined also by simulation.

The amphiphilic nature of the DPPC molecules allows them to form a self-assembled bilayer membrane in the presence of excess water. The self-assembly processes in water is conditioned by a threshold concentration of water in the lung surfactant with DPPC, higher values meaning that the bilayers can disappear. Our simulation method indicated us that the water molecules have to be in a report of 30:1 - 100:1 with the DPPC molecules, in phospholipid solutions in which the liposomes or bilayers can spontaneously appear. The results were confirmed by experimental results [9]. We have found that over 120:1, the water in excess starts to break the bilayer and the bilayers defragmentation occurs. In the same time, a threshold water content of about 82:1 was found to be critical for the liquid crystal order with relative constant lamellar periodicity.

The errors of the method were estimated in the case of the electrical permittivity measurements, using the experimental results reported by literature as reference. A relative error less than equal to 4 % has been obtained for all calculated values.

The DPPC membrane behavior approved itself to be a dynamic one, its interaction with different additives being not entirely elucidated. As future work, the extremes of ordering and motional anisotropy will make the object of our study, using the instruments of study perfected here.

5. Conclusions

The major constituent of pulmonary surfactant, dipalmitolylphosphatidylcholine in aqueous solution, was studied in L_α phase by structural simulation methods. The liquid crystal phase, existing over 42 ^{0}C and with a molecular report water/DPPC of ca. 30:1 - 100:1, presents a relative periodic multi-lamellar structure which remember us a smectic liquid crystal but not a typical one.

The structure was reproduced and studied by simulation with the 3D HFSS simulator by Ansoft. In a dynamic study we have reconstructed the structure organization over the phase transformations when temperature increases (18 - 52 ^{0}C), in order to establish the influence of different internal and external parameters and to consider the interactions between the microcomponents and with the applied fields.

The parallel and transverse electrical permittivities have been calculated using the simulational data, for a testing field in microwave range (typical 2.45 GHz ISM frequency). A parallel permittivity has been obtained, evolving in the direction transverse on the bilayers from maxima specific for the polar head regions to low values in the hydrocarbon core and then to minima in the bulk aqueous between bilayers. Good insulator properties in the headgroup region were confirmed by the permittivity peaks, the area behaving like a barrier of conductive properties of the aqueous membrane. At nanoscale the effect becomes of interest for applications. The transverse permittivity calculated after the direction parallel with the bilayers presents random fluctuations around a mean value in each plane, stronger in the middle of the hydrocarbon core inside the bilayer, where the alkane chains "disorder" is more advanced.

If we consider the control parameters for the stable L_α phase of the amphiphilic lipid in water (concentration in solution, buffer ions, temperature, etc.), the threshold / critical values can be determined by simulation. For example, a threshold water content of about 82:1 was found to be critical for the liquid crystal order with relative constant lamellar periodicity.

The flexibility of method which allows us to vary simultaneous different parameters and a relative error less than 4 % reported to the experimental results recommend the simulational method like a dynamic analyze tool of such a complex structure in real time.

Acknowledgements

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