

## THEORETICAL STUDY OF SOLVENT EFFECTS AND NMR SHIELDING TENSORS OF DLPC

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Received 18 August 2007; Accepted 21 September 2007

## ABSTRACT

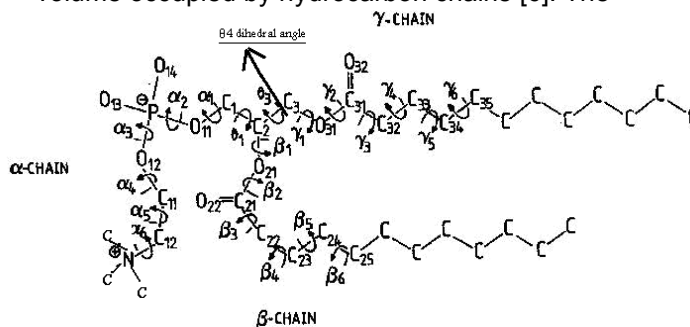
The effect of the polarity of the environment on the conformation of zwitterionic membrane dilauroyl phosphatidylcholine (DLPC) has been investigated with calculation at the Hatree-Fock level using the 6-31G\* basis set with Onsager continuum solvation model. The 'Gauge Including Atomic Orbital' (GIAO) approach is used to investigate *Ab initio* GIAO calculations of NMR chemical shielding tensors carried out within SCF-Hartree-Fock approximation are described. In order to compare the calculated chemical shifts with experimental ones, it is important to use consistent nuclear shielding for NMR reference compounds like TMS. Conformation of DLPC was evaluated with four different solvents with different dielectric constant (Water ( $\epsilon = 78.39$ ), Dimethyl Sulfoxide ( $\epsilon = 46.7$ ), Acetone ( $\epsilon = 20.7$ ) and Heptane ( $\epsilon = 1.92$ ). In concern with conformational energy, Water could be the most suitable solvent for DLPC. Moreover, as the polarity of the medium increase, the conformational stability of this molecule increases faster than that of DLPC in the gas phase. Consequently, the relative energy of DLPC also depends on the polarity of the environment. This subject was considered as well as the most variable in some dihedral angles degree and NMR isotropic shift were in the less dielectric constant ( $\epsilon = 1.92$ ). It could be in polar medium DLPC conformer becomes additionally stabilized by intermolecular ionic and hydrogen bond interactions with polar neighboring molecules. On the basis of this work it can be concluded that the effect of the polarity of the environment clearly are influenced on the isotropic values by geometry variation due to intermolecular motion in molecule.

**Keywords:** Onsager continuum model, DLPC, NMR shielding, isotropic, solvent models, anisotropic

## INTRODUCTION

DLPC (1, 2 - dilauroyl - sn- glycerol - 3-phosphatidylcholine) is one of the biological lipid and is commonly used in biophysical studies. This molecular approach is a prerequisite in the understanding of the functions and organization of the biological membrane [1]. Despite extensive studies have done on the structure, molecular conformation, lateral interaction, and dipole arrangement the head group and how these feature surface affect the properties and topology of the membrane [2]. DLPC is zwitterionic having a negative charge on the phosphate group and a positive charge on the amin. The hydrocarbon chain of this lipid is 12 carbons long (Fig 1) [3]. As the temperature increase, the fatty acid chains in DLPC tend to adopt conformations other than the all-trans straight chain configuration [4], such as the gauche conformation state illustrated in Fig 2, the closeness of the DLPC chains or its "packing" indicates many of the physical properties of the bilayer such as lateral movement of the DLPC chains [5].

A critical feature that distinguishes fatty acids from their corresponding two-chained lipid molecules is that they can freely partition into membranes and can "flip-flop" or distribute evenly between the two leaflets of the membrane and also rotation can occur around the C-C bounds allows the chains giving either a trans or gauche configuration (Fig 2). Multiple changes from *Trans* to *gauche* conformation increase the total volume occupied by hydrocarbon chains [6]. The



**Fig 1.** Atom numbering and notation for torsion angles according to Sundaralingam 1997.

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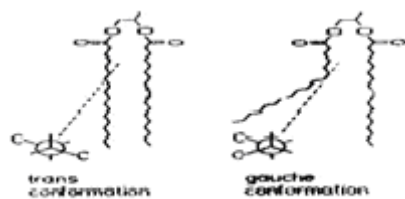


Fig 2. Disposition of phospholipids diacyl chains.

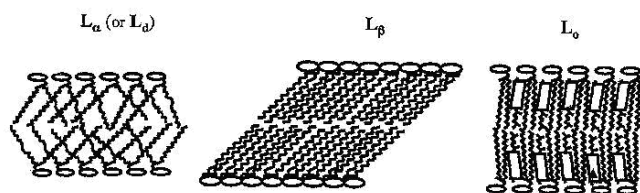


Fig 3.  $L_\alpha$  (also called  $L_d$  for liquid disorder), the normal fluid phase;  $L_\beta$ , the nonphysiological ordered gel phase formed by cooling membranes and the  $L_o$  (liquid ordered) phase in which the lipids are ordered (as on  $L_\beta$ ) (1).

conformational features observed in crystal structures of phosphatidylcholines (PC) in aqueous dispersion and natural membrane system showing that a preferred conformation is predominant also in dynamic systems. The concept lateral segregation in biological membranes developed over the last 30 years as a purely thermodynamic description by physicist to explain the coexistence within a bilayer of more than one lipid phase include  $L_\alpha$ ,  $L_\beta$ ,  $L_o$  (Fig 3). But retain their freer rotational and lateral diffusion (as in  $L_\alpha$ ) and which can occur at physiological temperature[4].

During the past decade, there has been increasing interest in calculating solution free energies via self-consistent reaction field (SCRf) [7]. The development of methods to calculate the free energies of solvation of molecules is a crucial task of computational chemistry [8]. Such methods have been rather successful in predicting solvation free energies [9]; the nuclear magnetic resonance (NMR) shielding tensor [10] is influenced by several factors: molecular structure, temperature, electric gradients and fields and the environment. Its observation thus leads to precious information about phenomena on the molecular level of the DLPC in the some polarity of the environments [11]. The experimental data show that the conformation is largely independent of the hydration state and head group packing patterns [12].

Our goal was to investigate the torsional dependence of a molecule's tensor and the resulting average effect on general shielding properties and also decided to find the relation between chemical shifts and conformations of DLPC in solvents. However, we reported in a preliminary fashion in the solvation free energy prediction of our SCRf methods for DLPC. As the temperature increase, the fatty acid chains tend to

adopt conformations other than all-trans straight chain conformation, such as the gauche conformation state illustrated in Fig 1. As far as environment affects the confirmation of the DLPC dipole [13]. Our approach was to investigate we studied the effects of some polarity of the environment on the minimum energy conformation of DLPC and also on general shielding properties. It is clear that a realistic description of the intermolecular interactions occurring in the hydrated bilayer should include some different effects, such as intermolecular electronic effects (determining the conformation of DLPC in the gas phase), interaction between neighboring head group in the bilayer, and interaction between DLPC and water molecules [14]. Therefore we should consider a model with at least four the dielectric constants. In this work we made use of the *Ab-initio* calculations to determine minimum energy conformations of the dilauroyl pshphatidylchline and have performed calculations according to the continuum solvating model by Onsager [15]. And also the 'Gauge Including Atomic Orbital' (GIAO) method [16] was used, which has recently become a widely used technique leading to gauge-independent results.

## COMPUTATIONAL METHODS

### Geometry Optimization

All the calculations were done with the Gaussian 98 at the Hartree-Fock (HF) level theory. First, the geometry of DLPC was full optimized at the RHF/ 6-31G\*, 6-31G, 3-21G and STO-3G levels of the theory in the gas phase without any constraints and then optimizing all remaining geometrical parameters were bond angles. Geometry optimization was repeated to consider solvent effects on geometry and conformation dependence on the surroundings.

### Solvent Model

For simulation of a polar environment the Onsager self-consistent reaction field (SCRf) model was used as implemented in Gaussian 98 program [17]. The simplest SCRf model is Onsager reaction field the basic assumption. In this model is that the solute is placed in a spherical cavity of radius  $a_0$  inside the solvent, cavity / dispersion effect are neglected and only the electrostatic effects of solvation, the net charge and dipole moment of the molecule are taken into account. The total energy of solute and solvent, which depends on the dielectricity constant and also the solute dipole moment, induces a dipole moment of opposite direction in the surrounding medium [19].

### The GIAO Method

The GIAO type method was introduced by Ditchfield and relies on the London orbitals. This

technique is invariant with respect to the choice of the gauge for any basis set size. Therefore, the geometries of all the compounds were full optimized at the RHF/ 6-31G\*, 6-31G, 3-21G, STO-3G levels of theory. Then the restricted Hartree-Fock (RHF) approach combined with the 6-31G basis set was employed for full optimization of the relevant geometries, and then the restricted Hartree-Fock (RHF) approach combined with the 6-31G basis set was employed for full optimization of the relevant geometries, and then GAIO was used for computation of corresponding energies and nitrogen NMR shielding.

### NMR chemical shift

The resulting NMR chemical shift of the nucleus, characterized by the chemical shielding tensor  $\sigma$ , and  $\sigma_{iso}$  is measured by taking the average of  $\sigma$  with respect to the orientation to the magnetic field, i.e:

$$\sigma_{iso} = \frac{\sigma_{11} + \sigma_{22} + \sigma_{33}}{3}$$

General information about NMR parameters can be found in [12] the inequalities:

$$|\sigma_{11} - \sigma_{iso}| \geq |\sigma_{33} - \sigma_{iso}| \geq |\sigma_{22} - \sigma_{iso}|.$$

If we define as the chemical shift anisotropy  $\Delta\sigma$  is defined by:

$$\Delta\sigma = |\sigma_{33} - \sigma_{iso}|.$$

The asymmetry parameter  $\eta$  is defined

$$\text{By: } \eta = \frac{\sigma_{22} - \sigma_{33}}{\Delta\sigma}$$

## RESULT AND DISCUSSION

In a pervious study, we investigated the influence of intermolecular rotation around the glycerol C (2)-C (3) bonds as dihedral angle  $\theta_4$  (Fig 1). Molecular dynamic simulations starting with only the *trans* dihedral angle  $\theta_3$  about The C2-C3 bond in both leaflets for more than 80% of the simulation, while  $\theta_4$  spent most of the simulation in the *gauche*<sup>+</sup> state in both leaflets [22] in contrast to the fluid phase simulation results, which indicate almost equal populations of both the *trans*/*gauche*<sup>+</sup> and *gauche*<sup>+</sup>/*gauche*<sup>±</sup> states for the  $\theta_2/\theta_4$  pair. For the attitude of DLPC in the shapes and forms during  $L_\alpha$  phase, several shielding tensors were calculated at a 10° increment of the dihedral angle, Dihedral angles vary from 55° to -145° with an increment of 10 degree. Several shielding tensors were calculated at a 10° increment of the dihedral angle, According to results, this molecule is not able to have  $\theta_4$  less than 55° and also after 175° got negative degree and the energy was also increased and the conformation of hydrocarbon chains were totally disordered and the distance between them was too increased. Therefore those results related to angles from 175° to -145° were omitted from our calculation. The most stable form was about  $\theta_4 = 65^\circ$

**Table 1.** Conformational energy of DLPC obtained by geometry optimization for basis set 6-31G\*, 6-31G, 3-21G, STO-3G levels

primitive	E/Kcal.mol <sup>-1</sup>
6-31G* = 1465	-1407380.314
6-31G = 1204	-1406773.985
3-21G = 834	-1399682.524
STO-3G = 834	-1390166.993

because the less energy was calculated at this degree. We used the calculated shifts  $\sigma_{iso}$  for the 12 conformers  $i=1\dots 12$  with  $\theta_4=55^\circ, 65^\circ,$  and  $75^\circ\dots 175^\circ$ . The calculated isotropic values compared with the minimum energy conformation.

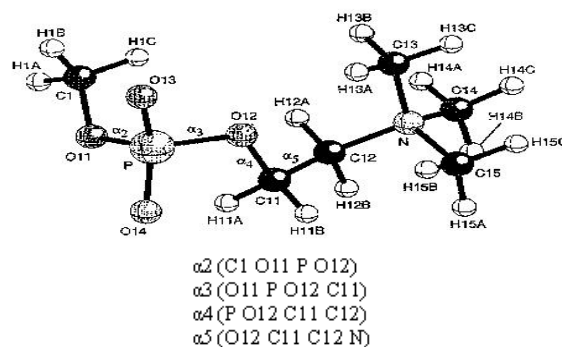
Assuming that molecule is rigid, because of the low summarization rates of dihedral angle reflect the reduce mobility of the glycerol backbone relative to the rest of the molecule.

### Geometry Optimization of DLPC in the Gas Phase

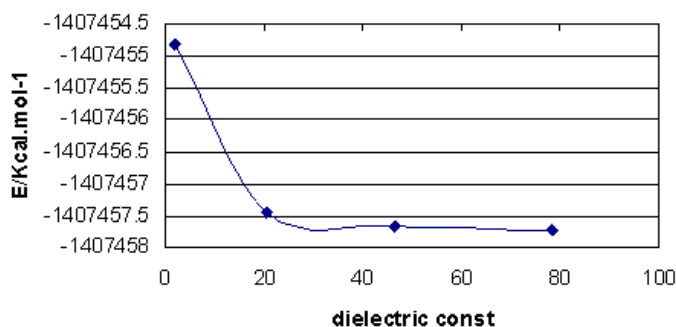
All computational calculation was done that reported by Sundaralingam [20] as initial geometry. In our work it has been shown that minimum energy of DLPC which obtained by geometry optimization (GAIO/6-31G\*, 6-31G, 3-21G, STO-3G levels of Theory, Table 1) is in the internal dihedral angle of rotation  $\theta_4 = 65^\circ$ . Then this conformation was chosen as an initial form of our study.

The preference for the extended DLPC conformation is supported by high-resolution NMR spectra by Hauser and co-workers [21], which show that the PC head group has a distinct preferred conformation with  $\alpha_4$  in the range 150-160° and  $\alpha_5 \pm$  *gauche*, both in solution and in lipid aggregates [12,14]. In our treatment, we assume that this is also the case for the sites on the chains backbone.

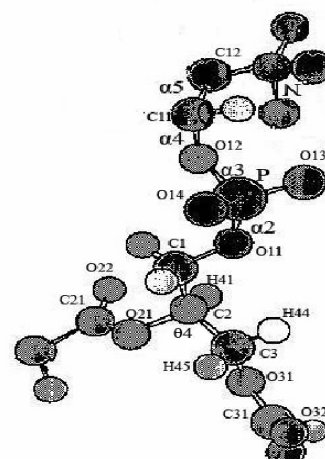
In the case of our model, for the P-O-C-C  $\alpha_4$  is 123.995°, C-O-P-O  $\alpha_2 = -87.753^\circ$ , O-P-O-C  $\alpha_3 = 162.407^\circ$  and O-C-C-N  $\alpha_5 = -47.733^\circ$  and  $\theta_4 = 65^\circ$  in gas phase.



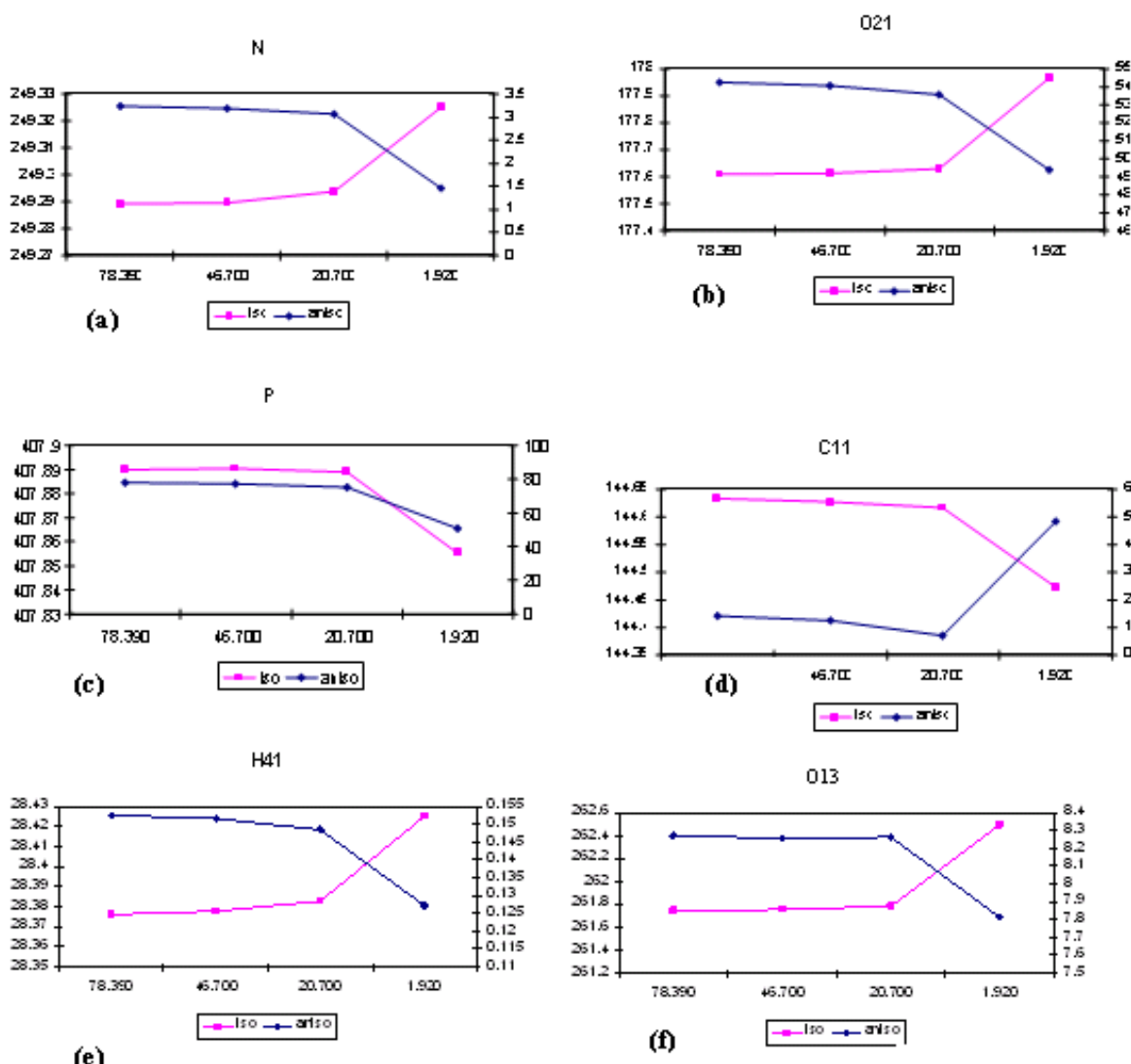
**Fig 4.** Atom labeling and dihedral angle notation for the DLPC head group (13).



**Fig 5.** Conformational energy of DLPC obtained by geometry optimization on basis set 6-31G\* level in water ( $\epsilon = 78.39$ ), dimethyl sulfoxide ( $\epsilon = 46.7$ ), acetone ( $\epsilon = 20.7$ ) and heptane ( $\epsilon = 1.92$ )



**Fig 6.** DLPC conformer in a  $\epsilon =78.39$  (Polar environment / water)



**Fig 7.** The isotropic values oiso (left-hand scale) and anisotropies (right-hand scale) for carbon (d), (b) Hydrogen (e), Isotropic value constants for oxygen, (c, f) gives the isotropic value and anisotropy for phosphorus and for nitrogen (a) in DLPC as a function of the dihedral angle  $\theta_4$  characterizing the rotation.

**Table 2.** Dihedral angles of the DLPC head groups optimized at four  $\epsilon_s$  using the onsager salvation model at the HF/6-31G\* level of theory<sup>a</sup>

dielectric const	$\alpha_2$	$\alpha_3$	$\alpha_4$	$\alpha_5$	$\Theta_4$
<b>A</b>					
78.39	-74.22	179.051	128.95	-66.622	69.128
46.7	-74.286	179.051	128.922	-66.61	69.118
20.7	-74.564	178.902	128.826	-66.577	69.077
1.92	-77.537	177.117	127.852	-66.112	68.418
<b>B</b>					
	-87.753	162.407	123.995	-47.733	69.126

<sup>a</sup>Dihedral angles in degrees; DLPC in solvent (A), DLPC in gas phase (B)

### Geometry Optimization of DLPC with Dependence on Dielectric Constant

Structural molecular properties obtaining with HF, 6-31G\* on basis set was optimized in solvent. When DLPC is used as starting geometry for minimization at Water ( $\epsilon = 78.39$ ), Dimethyl Sulfoxide ( $\epsilon = 46.7$ ), Acetone ( $\epsilon = 20.7$ ) and Heptane ( $\epsilon = 1.92$ ) the only significant difference concerns dihedral angle  $\alpha_5$ , which differs by approximately  $18^\circ$  for the  $\epsilon = 78.39$  in compared with DLPC in gas phase (Table 2). This molecule in gas phase has the most resemblance with molecule in Heptane phase ( $\epsilon = 1.92$ ) in concern with conformational energy (Fig 5).

The geometry of DLPC conformer is largely unaffected by changes of the dielectric constant. Table 2, shows that the changes is rather similar to the constrained gas-phase conformer, at  $\epsilon = 1.92$ . Only  $\alpha_4$  of the dihedral angles is slightly unaffected by the Polarity increase, changing from  $128.95^\circ$  ( $\epsilon = 78.39$ ) to  $127.852^\circ$  ( $\epsilon = 1.92$ ), while the remaining geometry parameters are affected.

Dihedral angle  $\alpha_2$  changes from  $-74.22^\circ$  ( $\epsilon = 78$ ) to  $-77.537^\circ$  ( $\epsilon = 78$ ), while  $\alpha_5$  turns in the opposite direction from  $-66.622^\circ$  ( $\epsilon = 78$ ) to  $-66.112^\circ$  ( $\epsilon = 1.92$ ) (Table 2). The dihedral  $\alpha_3$  and  $\alpha_4$  of this model are little or not affected by polarity changes. As can be seen in Table 2,  $\theta_4$  angle, it has the less variation in compared with  $\alpha_5$ ,  $\alpha_4$ ,  $\alpha_3$  and  $\alpha_2$  and  $\alpha_1$  angles. Therefore head group of DLPC has the most conformational changes when the molecular treated with different dielectric constants. The relative energies of DLPC also depend on the polarity of the environment. In all of the four solvents DLPC conformer has the lower energy in compare with gas-form; however, at ( $\epsilon = 78$ ) is the less energy (Fig 5 & 6).

### Calculation of NMR parameters in Solvent Model

The chemical shielding tensors calculated with the GAUSSIAN 98 Program is quantum mechanical entities. The computation of absolute shielding constants carried

out for NMR reference molecules. We calculated shielding tensor for different dielectric constants ( $\epsilon = 78, 46.7, 20.7, 1.92$ ). At each dielectric constant, the nuclear shielding was calculated each geometrical structures (Table 3). The changes in  $\rho_{iso}$  and  $\Delta\sigma$  were increased from Acetone ( $\epsilon = 20.7$ ) to Heptane ( $\epsilon = 1.92$ ) as well as changes in dihedral angles ( $\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5, \theta_4$ ) and conformational energies which were studied (Table 2, Fig 5).

### CONCLUSION

*Ab initio* calculations show that without influence from a polar environment, i.e. in the gas phase, the positive-charged ammonium or choline group of the PC head group interacts intermolecular with one of the unspecified phosphate oxygen, this conformation of the PC head group in a polar environment is in line with NMR data by Akutsu and Kyogoku [22], which also indicate that the only significant PC group in aqueous solution is a somewhat increased  $\alpha_5$  torsion in PC [13]. Recent molecular dynamics simulations of PC membrane domains [5] have demonstrated that intermolecular ionic interactions take place between neighboring lipid head groups and are important stabilizing factors in dynamic bilayers. The in strict stability of the minimum energy conformation of DLPC and its favorable stabilization in aggregated lipid phases thus can explain the predominance of this conformer in crystal structures, [23] aqueous dispersions, and biomembranes. In this investigation, conformation of DLPC was evaluated with four different solvents with different dielectric constant, Dimethyl Sulfoxide, Acetone and Heptane. In concern with conformational energy, Water could be the most suitable solvent for DLPC. Moreover, as the polarity of the medium increase, the conformational stability of this molecule increases faster than that of DLPC in the gas phase. It is known that the head group, and probably the glycerol backbone, in phosphatidylcholin bilayer membranes in excess water are strongly hydrated. Then it is possible that they could stabilize certain geometry of the glycerol moiety. Of course, the  $^2\text{H}$  NMR spectra of phosphatidylcholin- $2\text{H}_2\text{O}$  systems indicate that the exchange rates between these head group sites and the bulk water environment would need to be rapid on the NMR time scale [24]. Consequently, the relative energy of DLPC also depends on the polarity of the environment. This subject was considered as well as the most variable in some dihedral angles degrees and NMR isotropic shift was in the less dielectric constant. It could be in polar medium DLPC conformer becomes additionally stabilized by intermolecular ionic and hydrogen bond interactions with polar neighboring molecules.

**Table 3.** 6-31g calculations of the  $\sigma_{iso}$  in ppm, of the nuclear magnetic shielding tensor  $\sigma$  for some atoms around  $\theta_4$  and head group.

$\epsilon$	$\sigma_{iso}$	$\Delta\sigma$	$\eta$	$\delta^*$	$\sigma_{iso}$	$\Delta\sigma$	$\eta$	$\delta^*$
O22				O21				
78.4	-89.5472	174.2446	-253.022	422.3382	177.6081	54.2344	172.3928	155.1829
46.7	-89.5594	173.8385	-252.743	422.3504	177.6142	54.0683	172.0301	155.1768
20.7	-89.5765	173.0612	-251.660	422.3675	177.6305	53.5641	170.7072	155.1605
1.92	-89.8013	168.158	-239.871	422.5923	177.966	49.3565	159.205	154.825
O31				O11				
78.4	190.2703	65.0152	178.9838	142.5207	290.0172	20.8066	247.2331	42.7738
46.7	190.2442	64.8103	178.4143	142.5468	290.0362	20.8445	247.4511	42.7548
20.7	190.1575	64.131	176.4162	142.6335	290.095	21.0815	248.2245	42.696
1.92	189.2691	52.9688	152.5892	143.5219	290.778	23.209	256.2217	42.013
O32				O13				
78.4	-100.678	25.8497	146.2114	433.4698	261.7422	8.2712	198.67	71.0488
46.7	-100.724	24.9596	145.8362	433.5152	261.7575	8.2592	198.6019	71.0335
20.7	-100.88	21.8065	144.8547	433.671	261.7843	8.2648	198.7157	71.0067
1.92	-102.456	24.4163	99.16913	435.2477	262.4902	7.815	198.293	70.3008
O12				O14				
78.4	288.2809	36.6023	269.5826	44.5101	254.041	14.6352	214.184	78.75
46.7	288.2755	36.8945	269.3618	44.5155	253.9842	14.615	214.1778	78.8068
20.7	288.2716	38.3248	268.662	44.5194	253.797	14.5941	214.3549	78.994
1.92	288.0975	51.6026	261.5894	44.6935	251.3601	13.5152	214.9291	81.4309
C2				C11				
78.4	143.5185	31.7967	168.1568	64.6917	144.6326	1.3986	54.68653	63.5776
46.7	143.5148	31.7929	168.2005	64.6954	144.6275	1.2659	44.41415	63.5827
20.7	143.5063	31.7947	168.3629	64.7039	144.6159	0.7156	-38.6567	63.5943
1.92	143.5136	31.8642	169.6896	64.6966	144.4724	4.8401	124.3822	63.7378
C1				C3				
78.4	155.1533	22.4492	182.4208	53.0569	148.172	16.3729	126.7869	60.0382
46.7	155.1484	22.4461	182.5045	53.0618	148.1733	16.3448	126.785	60.0369
20.7	155.1215	22.4239	182.7869	53.0887	148.1725	16.2372	126.7727	60.0377
1.92	154.7904	22.2778	185.0018	53.4198	148.1709	15.0312	126.5224	60.0393
H41				C56				
78.4	28.3762	0.1525	-147.671	5.3013	159.753	9.4604	123.3683	48.4572
46.7	28.3779	0.1517	-148.593	5.2996	159.7532	9.4987	123.5722	48.457
20.7	28.3827	0.1484	-152.493	5.2948	159.7518	9.6433	124.3052	48.4584
1.92	28.425	0.1273	-181.965	5.2525	159.7506	11.4043	131.8874	48.4596
N				P				
78.4	249.2891	3.2252	174.9025	-525.500	407.8899	77.74	393.7916	-31.7353
46.7	249.2894	3.1897	174.0933	-525.500	407.8901	77.2475	393.944	-31.7355
20.7	249.2936	3.0585	170.9431	-525.504	407.8889	74.9591	394.7345	-31.7343
1.92	249.3251	1.4471	83.15632	-525.536	407.8552	50.6154	399.7335	-31.7006
H45				H44				
78.4	29.2214	1.7141	5.452503	4.4561	29.268	8.0232	28.14166	4.4095
46.7	29.2242	1.718	5.50053	4.4533	29.2666	8.0231	28.15054	4.4109
20.7	29.2339	1.7351	5.706815	4.4436	29.2609	8.0201	28.18107	4.4166
1.92	29.3563	1.9038	7.681784	4.3212	29.1812	8.0181	28.4268	4.4963
C21				C31				
78.4	29.5541	23.6491	-66.9903	178.6561	29.5967	44.5488	96.72105	178.6135
46.7	29.5489	23.6629	-66.8425	178.6613	29.5994	44.6173	96.66367	178.6108
20.7	29.533	23.6423	-66.2955	178.6772	29.6061	44.856	96.46416	178.6041
1.92	29.3605	23.3594	-60.5903	178.8497	29.7042	48.2742	92.94022	178.506

Standard; TMS: Isotropic carbon shielding tensor=208.2102, \*  
 Isotropic hydrogen shielding tensor=33.6775,  
 Isotropic Nitrogen shielding tensor=-276.2121,  
 Isotropic Phosphorus shielding tensor=376.1546 at GIAO method.

This work shows that isotropic values in different dielectric constants are influenced by geometry variation due to intermolecular motion in our model. Therefore, the effects of different polarity environment depend on the range of the chemical shift variation, which it's better to check experimentally results with nuclei with sensitive chemical shift (Fig 7)

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