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The effect of Environmental exposure to some chemical solvents on DPPC as important component of lung surfactant: an *ab initio* study

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ABSTRACT

One of the main components of lung alveoli is surfactant. DPPC (Dipalmitolphosphatidylcholine) is the predominant lipid component in lung surfactant that is responsible for lowering surface tension in alveoli in this article. We used a very approximate model with computational method of *Ab initio* to describe the interactions between DPPC as important component of lung surfactant and some chemical solvents such as Benzene, Toluene, Heptane, Acetone and Ethanol which cause Lung injuries that contribute to respiratory distress such as ARDS. The effect of these solvents on the conformation and disordering DPPC head group has been investigated with calculation at the Hatree-Fock level using the 6-31G basis set with Onsager continuum salvation, GAIO and frequency models. In concern with conformational energy, Water could be the most suitable solvent for DPPC. It could be in polar medium DPPC conformer becomes additionally stabilized by intermolecular ionic and hydrogen bond interactions with polar neighboring molecules. On the other hand, this study shows that Ethanol has the most effect on conformation and lipid disorder DPPC head group of lung surfactant in our model.

Keywords: lung surfactant, DPPC, ARDS, Benzene, Toluene, Heptane, Acetone and Ethanol.

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INTRODUCTION

One of important phenomena in the process of respiration is the role of the fluid coating the walls of the alveoli of the lungs that is called pulmonary surfactant. Lung surfactant is produced by the alveolar type II cells and it is secreted as phospholipid aggregates at the surface of the alveolar membrane. The air-water interface lung surfactant adsorbed from the aggregates form a film [1]. The function of surfactant is reducing the energy required to inflate Lung; it also decreases the likelihood of Lung collapse and increases the pulmonary compliance [2]. Furthermore, it stabilizes alveoli at low lung volumes and prevents the formation of alveolar edema [3]. Between 90% and 95% of lung surfactant is made up of lipids, with the remainder being proteins [4].

The main component that is responsible for considerable lowering of the interfacial tension is dipalmitoyl phosphatidyl choline (DPPC) which accounts for 50-70% of the PC [5].

DPPC has a gel to liquid crystalline transition temperature (T_m) of 41.5 °C [6].

Therefore, at a physiological temperature of 37 °C it is in an ordered gel state. Because DPPC contains two saturated acyl chains, it can be tightly packed in the orientated monolayer on the surfactant film. The position of DPPC in the film is due to its amphiphilic properties that the hydrophilic head group is soluble in water subphase (Figure 1) and two acyl chains have hydrophobic interactions with surfactant protein B. The dynamic/rheological properties of DPPC monolayer at small areas per lipid and physiological temperature do not allow for rapid re-spreading that can follow the alveolar space expansion during inhalation [7].

The SP_B is thought to facilitate the re-spreading of DPPC [8].

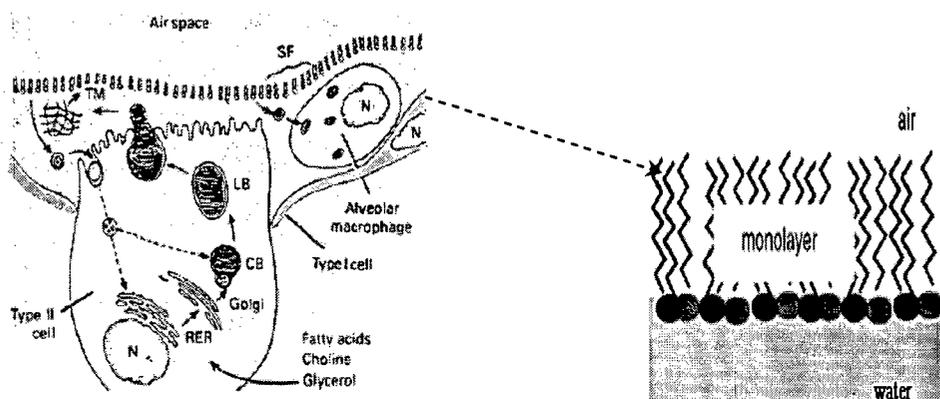


Figure 1. Metabolism of surface active material. ER=endoplasmic reticulum, G=Golgi body, LB =lamellar body, MVB=multivesicular body

Deficiency and dysfunction of lung surfactant contribute to the pathophysiology of several severe lung disorders such as respiratory distress syndrome (RDS) and acute respiratory distress syndrome (ARDS) [9]. Several factors appear to increase the risk of ARDS including pneumonia, septic shock, trauma, air pollution, chemical inhalation, cigarette smoking and alcohol consumption [10].

In contrast, there is less information about some other chemical effect on DPPC. In this article we discuss the effects of these harmful chemical solvents on surfactant, DPPC with computational simulation methods.

We indicate the effect of some chemical inhalation of solvents that could be finding in air pollution, cigarette smoking and alcohol consumption on alternation of surfactant

DPPC that lead to ARDS by using *Ab initio* method.

2. Computational Methods and Theory

2.1. Ab initio calculations:

The GIAO type methods are invariant with respect to the choice of the gauge for any basis set size and also the previous studies [11]. For a large molecules and ions, the free energies of solvation obtained from HF, MP2, and DFT methods are very similar. As far as the calculations are concerned, we have chosen the Hartree-Fock approach using the 6-31G basis set.

The *ab initio* calculations were carried out using the Gaussian 03 (Revision 3.2) software package [12]. The restricted the Hartree-Fock (RHF) approach combined with the 6-31G basis set was employed for full optimizations of the relevant geometries, and then for computations of the corresponding energies and NMR shielding; the latter were evaluated using the coupled Hartree-Fock (CHF) approach and the gauge-included atomic orbital (GIAO) method. The isotropic part σ_{iso} of σ is measured by taking the average of σ with respect to the orientation to the magnetic field, i.e., $\sigma_{iso} = \left(\frac{\sigma_{11} + \sigma_{22} + \sigma_{33}}{3} \right)$

2.2 Solvent Model

For simulation of a polar environment the Onsager Self-Consistent Reaction Field (SCRf) model was used as implemented in Gaussian 03 program in this model the solvent as a polarizable medium with a continuous dielectric constant, ϵ , with the solute forming a cavity in the bulk solvent. General information about SCRf parameters can be found in [21 main].

2.3. Thermo chemistry

Geometries in constant pressure 1 atmosphere and temperature 310K have been optimized at 6-31G basis set and vibrational frequencies obtained at the same levels. Gibbs energies have been calculated as the energy differences of solvent for head group of DPPC by Gaussian 03.

Three equations will be used to derive the final expression used to calculate the different components of the thermodynamic quantities printed out by Gaussian [12].

The partition function from any component can be used to determine the entropy contribution S from that component, using the relation [McQuarrie, § 7-6, Eq. 1]:

$n = N/NA$, and substitute $N_A k_B = R$.

$$S = R \ln(q(V, T)e) + RT \left(\frac{\partial \ln q}{\partial T} \right)_v$$

The internal thermal energy E can also be obtained from the partition function [McQuarrie, §3-8, Eq. 2]:

$$E = NK_B T^2 \left(\frac{\partial \ln q}{\partial T} \right)_v$$

And ultimately, the energy can be used to obtain the heat capacity [McQuarrie, §3.4, Eq. 3]:

$$C_p(T) = \frac{K(T) \Delta H_{vH} \Delta H_{cal}}{(1 + k(T)) RT^2}$$

RESULTS AND DISCUSSION

The recent studies of the lung surfactant structure and orientation of DPPC molecules at the air-water interface and the interface between water and an immiscible organic liquid is presented, with particular emphasis on studies that DPPC are deposited, the result is a monolayer, a one molecule thick film. Most DPPC forming monolayer consists of a polar head group and a non-polar tail group.

The polar head groups are hydrophilic, and will thus "dissolve" in the aqueous layer. The tail groups are hydrophobic and remain on the surface of the subphase. Therefore, in our model DPPC head group was contacted with solvents. As far as, the most aqueous layer around head group DPPC region in lung surfactant is water, all of the solvent obtained results were compared with the result of water as a blank solution. All computational calculation was done that reported by Sundaralingam [14] as initial geometry. The obtained theoretical values compared with experimental values which they were

available. Initial geometry was given from calculations compared with NMR determined structures. In the case of our model, for the P-O-C-C α_4 is 178.355° , C-O-P-O $\alpha_2 = 157.354^\circ$, O-P-O-C $\alpha_3 = 72.410^\circ$, O-C-C-N $\alpha_5 = 52.412^\circ$ and $\theta_4 = 52.036^\circ$ in gas phase.

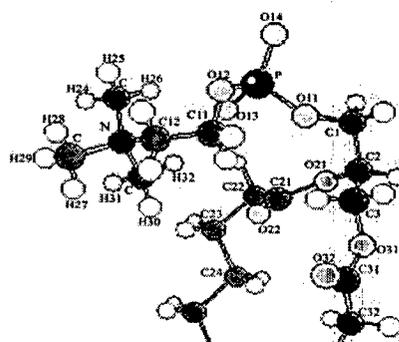


Figure 2: Atom numbering and notation for torsion angles of head group DPPC region according to Sundaralingam 1997

α_2 (C1 O11 P O12)
 α_3 (O11 P O12 C11)
 α_4 (P O12 C11 C12)
 α_5 (O12 C11 C12 N)
 θ_4 (O21 C2 C3 O31)

3.1. Solvent effects on geometry

Structural molecular properties obtained with HF/6-31G on basis set was optimized in solvents. For each DPPC conformer in solvents was calculated as the difference between the most stable and the most labile conformer. The values are given in Figure 2; Critical dihedral Angles of the DPPC Head Group in each the optimized geometries of solvents at the same level are shown in Table 1.

According to obtained results of Table 1, the relative energies of DPPC headgroup also depend on the polarity of the environment. In all of the solvents, headgroup conformer in Water ($\epsilon = 78$) has the lower energy in compare with the others. Therefore, in concern with conformational energy, Water could be the most suitable solvent. It could be in polar medium DPPC conformer becomes additionally stabilized by intermolecular ionic and hydrogen bond interactions with polar

neighboring molecules. Moreover, as the polarity of the medium increase, the conformational stability of this molecule increases faster than that stability of DPPC in nonpolar solvents. But the geometry of DPPC head group conformer is largely unaffected by changes of the dielectric constant. Table 1: shows that the only α_4 of the dihedral angles is slightly unaffected by the Polarity increase, changing from 174.673° ($\epsilon = 78.39$) to 175.199° ($\epsilon = 1.92$), while the remaining geometry parameters are affected. Dihedral angle α_2 changes from 152.751° ($\epsilon = 78$) to 153.582° ($\epsilon = 1.92$), Dihedral angle α_5 turns in the opposite direction from 42.206° ($\epsilon = 78$) to 54.687° ($\epsilon = 1.92$). These results are confirmed by NMR which also indicates that the only significant PC group in aqueous solution is somewhat increased α_5 torsion in PC: [15]. The dihedral α_3 and α_4 of this model are little or not affected by polarity changes. In concern with water phase the most variation in dihedral angles was considered in Ethanol.

Table 1. Dihedral Angles of the DPPC Head Groups Optimized at six dielectric constants Using the Onsager Salvation Model at the HF/6-31G* level of Theory.

dielectric const	α_2	α_3	α_4	α_5	θ_3	$\Delta E/ \text{kcal.mol}^{-1}$
A						
78.39	148.690	59.737	172.737	61.523	54.465	0.0
24.55	-173.237	-49.060	-129.858	70.384	57.694	-0.2
20.7	149.102	60.837	172.889	60.653	54.299	-0.7
2.379	152.751	67.549	174.673	42.206	53.033	-6.1
2.247	152.967	67.841	174.807	55.257	52.98	-6.4
1.92	153.582	68.642	175.199	54.687	52.718	-7.1

3.3. Geometry and frequencies

As standard *ab initio* methods, geometries of the DPPC head group in each solvent have been optimized at 6-31G basis set and vibrational frequencies obtained at the same levels. Gibbs free energy of the reaction was estimated at 310 K and constant pressure 1 atmosphere for head group of DPPC by GAUSSIAN 03 (Table 2).

Entropy has been evaluated by standard statistical thermodynamic methods.

$$\Delta S = \frac{\Delta H_{cal}}{T_m}$$

The chemical shielding tensors calculated with the GAUSSIAN 03 program.

At each dielectric constant, the nuclear shielding was calculated each geometrical structures (Table 3). These dates were compared with respect to the value of ΔG , the most stability of DPPC head group is observed in Water. This can be related to role of electrostatic and hydrogen bond interactions polar neighboring water molecules with head group complex. This result was identified as well as the obtained results of table 1. Comparison of enthalpy and entropy values represents the essential role of entropy in stabilizing of DPPC head group. In conclusion, Ethanol is the less favorite solvent for DPPC because it has the less entropy and the more Gibbs free energy.

Table 2. Enthalpies (ΔH^1 , kcal mol⁻¹), Entropies (ΔS^1 , cal mol⁻¹K⁻¹) and Gibbs free energies (ΔG^1 , kcal mol⁻¹) calculated at 314K and pressure constant from theoretical data.

ϵ	ΔH^1	ΔG^1	ΔS^1	T ΔS^*	$\Delta G-\Delta H^{**}$
78.39	-941520.4515	-941576.8005	0.181774	57.077036	56.349
24.55	-941520.4515	-941576.7955	0.181759	57.072326	56.34398
20.7	-941520.4509	-941576.7986	0.181769	57.075466	56.347745
2.379	-941520.4509	-941576.7992	0.18177	57.07578	56.348373
2.228	-941520.4509	-941576.7992	0.181771	57.076094	56.348373
1.92	-941520.4509	-941576.798	0.181768	57.075152	56.347118

**Gibbs free energy the reaction has been evaluated by using expression; $\Delta G(T) = \Delta H(T) - T\Delta S(T)$.

By comparing the results for entropy dates were given from standard statistical thermodynamic method; T $\Delta S(T)$ and the thermodynamic relation: $\Delta G = \Delta H - T\Delta S$ to our data, $\Delta G - \Delta H$ the calculated values may be observed to lie about 0.8 Kcal.mol⁻¹. (See Table 2).

3.4. Calculation of NMR parameters in Solvent 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.

the result obtained from ¹³C NMR of egg yolk phosphatidylcholine (Table 4) [16]. These dates were identical with the result obtained from ¹³C NMR of phosphatidylcholines and it was confirming that the most variation chemical shift anisotropy occurs in Choline group of head region

Table 3. 6-31G calculations of the $\langle\sigma_{iso}\rangle$ in ppm, of the nuclear magnetic shielding tensor σ for some carbon atoms.

ϵ	$\langle\sigma_{iso}\rangle$	$\Delta\sigma$	$\langle\sigma_{iso}\rangle$	$\Delta\sigma$	$\langle\sigma_{iso}\rangle$	$\Delta\sigma$	$\langle\sigma_{iso}\rangle$	$\Delta\sigma$
	C3		C5		C6		C15	
78.39	15.491	32.619	146.5344	7.3153	155.6538	0.4501	13.5071	84.6973
24.55	155.384	32.8455	146.623	7.7358	155.714	0.2243	13.4479	85.2017
20.7	155.356	32.9036	146.6436	7.8482	155.7297	0.1608	13.4319	85.3284
2.379	154.433	34.4469	147.241	11.7006	156.195	2.3709	12.7721	89.0559
2.228	154.3908	34.5009	147.2612	11.9069	156.2116	2.513	12.7356	89.203
1.92	154.2693	34.6213	147.3219	12.4976	156.2573	2.9351	12.6335	89.6752
	C12		C13		C18		C20	
78.39	148.6915	13.9064	132.5199	29.2304	148.1804	5.1853	19.5317	64.5142
24.55	148.705	13.8533	132.521	29.2391	148.175	5.2138	19.5438	63.8668
20.7	148.7084	13.8382	132.5217	29.2417	148.1737	5.2209	19.5472	63.6925
2.379	148.905	13.2843	132.52	29.3993	148.12	5.4195	19.6016	57.4437
2.228	148.9171	13.2447	132.5183	29.4133	148.1166	5.4231	19.6011	57.1436
1.92	148.9524	13.1322	132.5207	29.4436	148.101	5.4485	19.5928	56.2226

**Table 4: Reduced Chemical Shift Anisotropy ($\Delta\sigma$)
For egg yolk phosphatidyl-choline [¹²⁴].**

Type of carbon	Chemical shift anisotropy
Choline	<i>ppm</i>
C(3)*	1.6
C(5)*	4.2
C(6)	-8.6
	Interfacial region
C13 (g)	11.0
C12 (e)	11.3
C18 (g)	11.0
Sn2-CO (C15)	-7
Sn2-CO (C20)	-27.0

Conclusion

The common inhaled solvents cause ARDS are Benzene, Toluene, Heptane, Acetone and Ethanol which found in air pollution cigarette smoking and alcohol consumption. Therefore, we used a very approximate model to describe the interactions between DPPC as important component of lung surfactant and these solvents. The model case of DPPC was chosen because of it's the most common biological lipids in lung surfactant. And there is the less study of the effects of pollutants on DPPC. In this investigation, the molecular modeling suite was used, Gaussian 03, to investigate the

effect of a series of pollutants, some of which have the role of in ARDS on DPPC.

In concern with the obtained results, the most variation of conformation and lipid disorder were found in Ethanol solvent. In general, the effect of harmful solutions within the airways is dependent on the solubility of the reactive chemicals in aqueous media. In our investigation, Ethanol in compare with the other solutions is the most highly water-soluble substance which will also be absorbed by the upper respiratory tract, thus occurring in very low concentrations in the bronchiolar

and alveolar region. Ethanol molecules that penetrate the lower respiratory tract are mostly the vapor forms of the pollutant, or are adsorbed to other components, such as small particles or aerosols. When these vapors are breathed in, they can produce the lung causing breathing problems. Therefore, it could be more effect on dysfunction of DPPC in lung surfactant. Alcohols show experimentally a destabilizing effect on model membranes [18]. It has been observed that upon addition of alcohols, the lipid bilayer becomes thinner and the area per molecule increases. The calculated increase in area/molecule was confirmed by flow experiments where the bilayers of aspirated vesicles were seen to laterally expand upon exposure to aqueous streams containing ethanol. The reduction in the interfacial tension of a bilayer by ethanol is much less than would be predicted by simply making a direct comparison to the behavior of an alkane such as heptane.

In summary, this work shows that Ethanol has the most effect on DPPC head group of lung surfactant in our model. DPPC forming monolayer, consist of a polar head group and a non-polar tail group. The tail groups are hydrophobic and remain on the surface of the subphase.

As the monolayer is compressed, the hydrophobic tails will alter their positions. The different configurations of the molecules on a surface can be distinguished by the behavior of the surface pressure during the monolayer's compression. The various configurations of hydrocarbon chains effects on head group DPPC region. Therefore, the behavior of hydrophobic tails should be considered. Furthermore, it should be checked obtained results with experimentally results.

Finally, we should like to point out the potential of this analysis in further studies of molecular modeling in the lung surfactant. the effects of these solvents in a hydration model of monolayer membrane of lung surfactant, with SP proteins should be considered .

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