Presented at the DLSU Research Congress 2015
De La Salle University, Manila, Philippines
March 2-4, 2015

A Molecular Dynamics Study on the Permeability of Oxygen, Carbon Dioxide and Xenon on *dioleoyl-phosphatidylcholine* (DOPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) Double Lipid Bilayer

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Abstract : Permeation of gases in lipid membranes shows significant contributions in the fields of chemistry, environmental toxicology, nanotechnology and many other fields. However, difficulties still arise in comprehending the interaction of gas molecules in lipid membranes. This study investigated the permeation of three gas molecules namely: xenon, oxygen and carbon dioxide in dioleoyl-phosphatidylcholine (DOPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) double-lipid bilayer using the MARTINI coarse-grained forcefield. The calculations were implemented using the GROningen MAchine for Chemical Simulations (GROMACS). Each system contained 256 lipids and 3000 water molecules equilibrated at 323 K and 1 atm. In this study, the transient-pore mechanism was used to calculate the diffusion coefficients for both gas molecules and lipid membranes. The measured area per lipid for DOPC and POPE were 0.702 nm^2 and 0.621 nm^2 which agree with experimental data for double-lipid membrane. Only oxygen gas molecules were able to permeate the DOPC membrane by passive diffusion. Other gases were hindered by the strong interaction between the molecules and the phosphate heads of the bilayers. The diffusion coefficient of oxygen in DOPC-water system resulted to 1.271 x 10^{-5} cm^2/s. Permeability of gas molecules was then calculated by getting the ratio of the diffusion coefficients between the gas and the lipid membranes. The permeability of oxygen in DOPC double-bilayer is 0.542. This shows that permeation of molecules is directly related to the diffusion coefficient of the gas in the lipid membrane and the area per lipid membrane.

Key words: Permeability; Lipid bilayer; molecular dynamics simulation; diffusion; Palmitoyloleoylphosphatidylethanolamine (POPE); Dioleoylphosphatidylcholine(DOPC)

1. INTRODUCTION

Lipids are biological molecules characterized by limited solubility in water and solubility in non-polar organic solvents. One of the major functions of lipids is to serve as a permeable barrier in biological membranes. The lipid membranes partition the cell into functional and segregated compartments. It separates biotic and abiotic compounds to keep membrane healthy and intact. In addition, these membranes are permeable allowing functional compounds to enter the cell and prohibit the unwanted substance form entering the cell (Moulton G., 2004). As cells progress to their cycle of life, a large quantity of exchange is needed to maintain stability and function. This process may involve transport of biological compounds and excretion of waste components, which are necessary for the normal function (Pardee,A. 1968). The study of permeability of some known particles has been very useful in the fields of medicinal chemistry, environmental toxicology, nanotechnology and many other fields both in biology and in chemistry. Unfortunately, the concept of particles permeating into a lipid bilayer has not completely been understood and so it is important for us to go through deeper details of the permeation process and the interaction of the particles into the membrane.

The permeation of gas describes the process of membrane separation though a semi-permeable non-porous membrane. Transport caused by membrane selectivity and solution-diffusion, which is

dependent to permeation rates of the components through the membrane. Each gaseous component transporting through the membrane has a characteristic permeation rate that is a function of the ability to dissolve and diffuse. [2] The permeability is therefore relative to the properties of the substance being dealt with. eExperimental methods in determining the rate of permeation of particles can be measured by means of osmotic, nuclear magnetic resonance (NMR) and radiotracer experiments (Finkelstein A., 1984).

Theoretical and experimental studies of biological particles may involve complicated analysis and not cost-efficient methods of calculations. These problems are mainly addressed by the use of computational methods, specifically, computer simulations to predict behaviors, molecular structures and interaction of biological molecules[4]. A study by Steven L. Fiedler and Angela Violi used simulation of nanoparticle permeation through a lipid membrane (Biophys J., 2004). Another is a computer simulation of small molecule permeation across a lipid bilayer (Bemporad et al., 2004). Although the use of these methods would allow us to further study atomistic properties of lipid membranes, some of the main aspects of experimental and computations, such as time and length scale, should also be considered. Due to those limitations, the coarse-grained simulation (CG) was developed in order for us to use a system with reduced degrees of freedom. As a result, a simulation using a coarse-grained (CG) system allows faster computation with fewer resources compared to system that runs in an all-atom model. [3] There are different CG simulations that can now be used for different purposes. One of which is MARTINI, a coarse-grain force field suited for molecular dynamics simulations of biomolecular systems. This simulation has been utilized for studies involving fundamental structures of lipid bilayers and their thermodynamic properties.

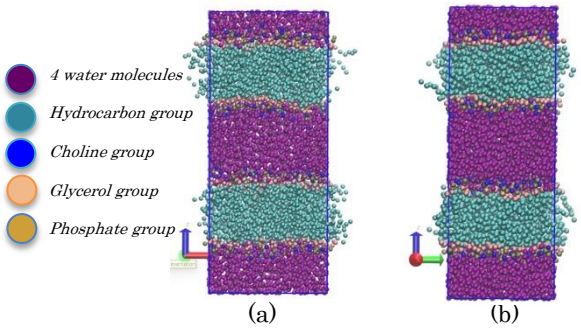
In this study, we have used the MARTINI coarse-grained simulation to probe the permeation of some gases in lipid membranes without the aid of protein. This is in line with the study of gas permeability in lipid membranes, specifically dicapryloylphosphatidylcholine (DCPC), dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC), using LAMMPS coarse-grained molecular dynamics by Huajun Yuan. We have used the same gas molecules namely: xenon, oxygen and carbon dioxide and two lipid membranes such as Palmitoyloleoylphosphatidylethanolamine (POPE) and Dioleoylphosphatidylcholine (DOPC). The gas molecules have low solubility in water which is imperative in our study of permeation. The goal of this investigation is to compare computational results observed from MARTINI coarse-grained simulation from the experimental results. This study is limited to only two lipid membranes due to time constraint and only to investigate permeation of three gases through calculation of diffusion coefficient and not to completely analyze the interaction of the gas molecules and the lipid membranes.

2. METHODOLOGY

The GROningen MAchine for Chemical Simulations (GROMACS) software was used in the implementing the molecular dynamics of the system using the MARTINI forcefield conceptualized by Marrink S. et al. (2007). The gas molecules and the lipid membranes are shown in figures 1 and 2. Visualization Molecular Dynamics (VMD) application was used to visualize the system.

(a) (b) (c)

Figure 2: Eight gas molecules of (a) xenon (b) oxygen and (c) carbon dioxide prepared in MARTINI



(a) (b)

Figure 3: Left image is a sample of a Dioleoylphosphatidylcholine (DOPC) double bilayer. Right image is a sample of Palmitoyloleoylphosphatidylethanolamine (POPE) double bilayer. (axis colors: x(red), y(green), z(blue))

The unit cell of the system was composed of 256 lipids with 3000 water molecules at 323K forming double lipid bilayer as shown in figure 2 with an initial size of 8.5 nm by 8.5 nm by 20.0 nm. Each system equilibration was implemented for 100 ns with a time step of 20 fs for stability at 323 K and

1 atm, initially in canonical ensemble (NVT) using Berendsen thermostat followed by equilibration in the isothermal–isobaric (NPT) ensemble using Parinello-Rahman barostat to optimize the unit cell.

After the equilibrations, the bilayer thickness, area per lipid of DOPC and POPE double-bilayer were measured, and they were compared with the experimental values. After which, eight gas molecules were inserted at the middle aqueous part or hydrophilic portion of the double-bilayer. This is similar to the configuration by Wang Y. et al. (2007). Lastly for the MD production, the system was equilibrated at NVT ensemble.

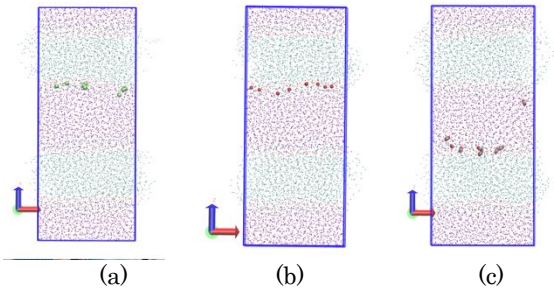


Figure 4: (a) Eight xenon gas in DOPC double bilayer. (b) Eight oxygen gas in DOPC double bilayer. (c) Eight carbon dioxide gas in DOPC double bilayer. (sizes are for visualization purposes only)

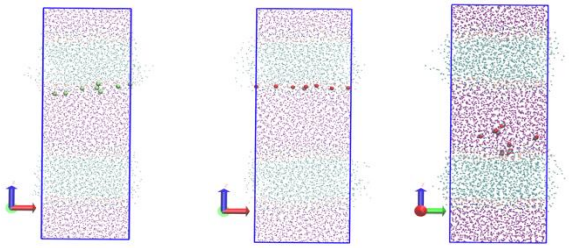


Figure 5: (a) Eight xenon gas in POPE double bilayer. (b) Eight oxygen gas in POPE double bilayer. (c) Eight carbon dioxide gas in POPE double bilayer. (Sizes are for visualization purposes only)

2.1 Permeability of Gases

There are a number of ways to compute for the permeability of gases. Other studies used the solubility-diffusion model proposed by Marrink and Berendsen. This model shows that the permeability coefficient can be calculated using the equation:

$$\frac{1}{P} = \int_{z_1}^{z_2} R(Z)\mathrm{d}Z = \int_{z_1}^{z_2} \frac{\exp\{\Delta G(Z)/k_B T\}}{D_z(Z)}\mathrm{d}Z. \qquad \text{(Eq. 1)}$$

where:

Z_1 and Z_2 = bulk aqueous phase coordinates
$\Delta G(Z)$ = free energy,
k_B = Boltzmann constant
T = temperature
$D_z(Z)$ = Diffusion coefficient of small molecules permeating across a membrane. (Marrink and Brendsen, 1994)

On the other hand, a simpler way to analyze the permeation process through the transient-pore mechanism was proposed by Jansen and Blume. This model suggests that water molecules transport mainly occurs across transient pores. We chose three gases with small amount of solubility in water and positioned the gas molecules in the hydrophilic portion of the bilayer so that the only movement it would produce is to migrate into the hydrophobic part of the bilayer. We have studied one gas in a lipid bilayer at a time. We started with placing eight molecules of xenon gas in DOPC bilayer. The total energy of the system was minimized and the system was equilibrated at 323K at 1 atm. Then, the density profiles were plotted and the diffusion coefficient of the molecules in the membrane was measured. We then calculated the ratio of the diffusion coefficients perpendicular component (along z) to the parallel component (along x and y) of the membrane.

$$P = \frac{D_{\perp}}{D_{//}}. \qquad \text{(Eq. 2)}$$

where:

P = permeability
$D_{\perp}$ = diffusion coefficient along the z-axis
$D_{//}$ = diffusion coefficient along x and y-axis

If the ratio is equal to zero, the membrane is absolutely impermeable (Murad S., 1998). On the other hand if the ratio is one, the gas molecules can freely permeate through the membrane by passive diffusion.

3. RESULTS AND DISCUSSION

After equilibration, the thickness of the double lipid bilayer was measured using the difference of the peaks of the phosphate heads shown in figure 6. The lipid bilayer thickness of DOPC and POPE were 4.3166 nm and 4.4865 nm, respectively. The final volume of the unit cell of POPE after equilibration was 6.30954 nm x 6.31069 nm x 18.69382 nm and 7.12089 nm x 6.31281 nm x 16.60229 nm for DOPC.

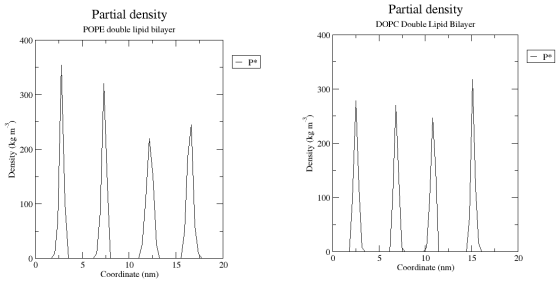


Figure 6: Density profiles of the phosphate head of POPE double lipid bilayer (on the left) and DOPC double lipid bilayer (on the right).

3.1 Area per Lipid

The area per lipid of each double-bilayer was calculated. The area per lipid is one very important aspect not just because it describes the asymmetric distribution of lipids but it also correlates to the permeability of the lipid bilayer. The greater the area of your lipid, the more difficult it is for the molecules would pass through. We have computed the area by multiplying the Box-X and Box-Y components and divide it by half the number of lipids. The area per lipid computed for DOPC is 0.702 +/- 0.002 nm^2 and for POPE is 0.621 +/- 0.001 nm^2. The results were somehow close, with less than 10% error, to the accepted value of the area per lipid which is 0.65 +/- 0.05 nm^2. (Marrink S et.al, 2007)

3.2 Density Profile

The partial density profile of the different lipid membrane systems for 100 ns simulation were plotted as shown in figure 7.

The density profile of the lipid membranes, both DOPC and POPE, contain peaks as compared to bulk water which shows uniform density profile. The same behaviors were also observed in the gas density profiles, which show peaks near the interface of gas and lipid membrane.

We can see from the graphs that only oxygen gas permeated in the DOPC membrane (figure 7). Other gas molecules on DOPC and POPE are stuck in the interface of water and were not able to penetrate the membrane.

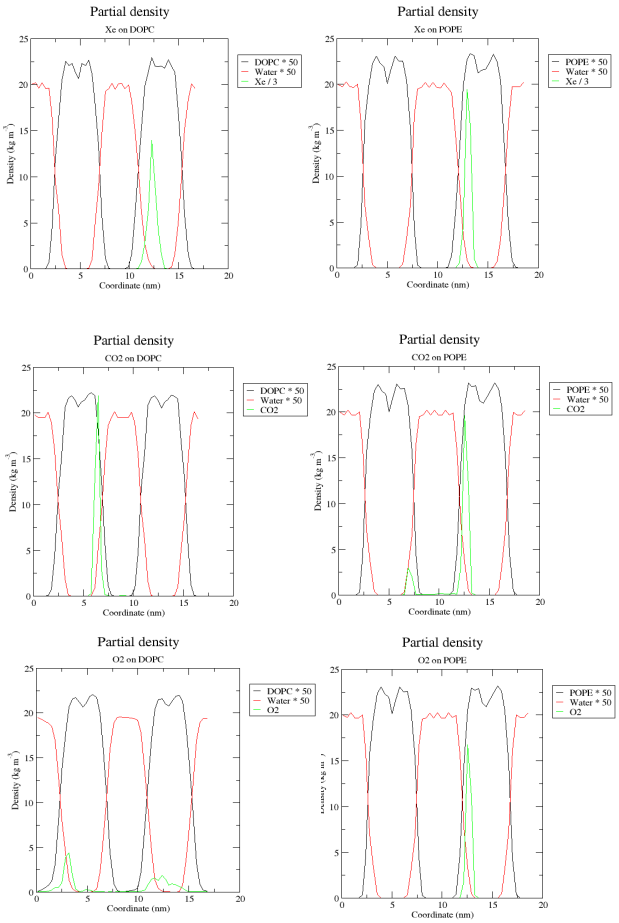


Figure 7: Density profiles of xenon, oxygen and carbon dioxide in DOPC (first column) and POPE (second column) double-bilayer

3.3 Diffusion Coefficient and Permeability.

Some studies used direct measurement of the diffusion coefficient through an experiment, which is more complicated. A comparison of our data to other experimental results is a good standard for our fitting procedure. We rescaled our simulation results for effective diffusion coefficient of oxygen in DOPC at 323 K, by the usual factor of four [12], considering the time scale of CG simulations and obtained an effective oxygen diffusion coefficient of D = 1.271 x 10^{-5} cm^2/s. This is in close agreement with the other calculated diffusion coefficients of oxygen in DPPC at 323K by Wang et al (2007) with D = 1.54 x 10^{-5} cm^2/s and by Marrink et al (2004) with D=1.47 x 10^{-5} cm^2/s. Other studies calculated 2.52 and 2.78 x 10^{-5} cm^2/s at 308.1K and 313.2K. (Han., P. et. al, 1996). Another study by Ferrell and Himmelblau shows 3.33 x 10^{-5} cm^2/s at 313.15K (Ferrell, R.T. et al., 1967) and 3.49

x 10^{-5} cm^2/s at 302.75K (Krieger, I.M. et al., 1967). Therefore, the results shown in Tables 1 and 2, for oxygen diffusion coefficient is in fairly good agreement with the experimental values.

To compute for the permeability of gas, we have used the transient pore mechanism which relates the diffusion coefficient to the gases permeability. (Jansen M., 1995). We have used equation 2 to compute for the permeability of the gases.

Table 1. Gas Permeability on DOPC

DOPC membrane		
Gas	**D ($10^{-5}cm^2/s$)**	**P**
Xe	0.036	0.051
O_2	5.084	0.542
CO_2	0.058	0.001

Table 2. Gas Permeability on POPE

POPE membrane		
Gas	**D ($10^{-5}cm^2/s$)**	**P**
Xe	0.261	0.246
O_2	3.385	0.337
CO_2	0.647	0.115

Oxygen has the highest diffusion coefficient in the two bilayers and thus shows higher probability of permeation as compared to the other two gases. Based from the data, oxygen permeated faster in DOPC as compared to when it was placed in the POPE bilayer. The intermolecular force of attraction of oxygen gas and the lipid head groups in POPE is greater as compared to the head groups of DOPC bilayer. (Yuan, H. at al., 2009) On the other side, xenon and carbon dioxide showed lower diffusion coefficients and slower permeation in DOPC. Thus the attraction of the gas molecules and the lipid heads are quite significant which somehow restricted the gases from permeating the membrane.

If the ratio of the perpendicular component or the diffusion along the z –axis and the parallel component or the diffusion along x-y plane is 0 then the membrane is absolutely impermeable and 1 if it is permeable. (Jansen M., 1995). Our results showed that only oxygen gas in DOPC bilayer was able to penetrate the membrane. The rest of the values only show a very small probability, which is almost negligible, of permeating through the lipid membrane through passive diffusion.

4. CONCLUSIONS AND RECOMMENDATIONS

Using a molecular dynamics, the area per lipid and thickness of DOPC and POPE lipid membranes were calculated. Moreover, the permeation of oxygen, carbon dioxide and xenon gas molecules through DOPC and POPE lipid membranes were determined using MARTINI coarse-grain force-field. The results from this study have shown satisfactory agreement with data obtained from experiments specifically on the permeation of O_2 on DOPC.

Our study has shown that some molecules can cross lipid membranes by passive diffusion even without the aid of proteins. In addition, the ability of the molecules to permeate a membrane is related to its solubility in the aqueous phase as well as the force of attraction between the gas molecule and the lipid heads which is denoted by the diffusion coefficients of the gases.

For further studies, it is recommended to check the permeability of gas molecules at different temperature and pressure. Moreover, it would also be expedient to explore on enhancing the permeability of the gases with the use of ions or other possible agents.

5. ACKNOWLEDGMENTS

This study will not be possible without the support of the Department of Physics at De La Salle University through the Computational Materials Design Research Group.

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