The Effect of Temperature and Pressure on the Self-assembly of Dipalmitoylphosphatidylcholine Using Coarse-Grained Molecular Dynamics

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***Abstract*—The use of coarse-grained molecular dynamics (CG-MD) technique is a promising tool to explore the time and length scales of real physical system beyond what is feasible with atomistic molecular dynamics (MD). Here, structural and dynamics properties of the bilayer comprising 64 molecules of dipalmitoylphosphatidylcholine (DPPC) in water were investigated using coarse-grained molecular dynamics (CG-MD) simulation method. This was done to explore the effect of temperature and pressure on the self-assembly of DPPC. The models prepared were simulated at the temperatures of 298K and 323K under isotropic and semi-isotropic pressures. The aggregation started from the random configurations followed by forming bilayers in the period of 500 ns for all model systems. Area per lipid, thickness, and radial distribution values were different for each model while the bilayer formation pattern was the same.**

***Index Terms*—dipalmitoylphosphatidylcholine, self-assembly, coarse-grained molecular dynamics**

I. Introduction

Lipid molecules exhibit a wide phase behavior in aqueous solution including micelles, rod-like structures and bilayers depending on the concentration, temperature and other physical properties [1] and [2]. Lipids are amphiphilic systems thus they can function as surfactants. Amphiphilic molecules structurally involve two moieties; a hydrophilic head and a hydrophobic tail. Formation of a micelle is a phenomenon where amphiphilic molecules form small aggregates in water [3]. This phenomenon occurs above the critical micelle concentration (CMC). The most important factor in micelle formation is the hydrophobic effect originated from excluding the non-polar moieties from the water to the interior part of the micelle accompanying by an interfacial free energy release at the water-micelle interface [4]-[8].

The use of coarse-grained (CG) models in exploring a variety of structural and dynamics properties of macromolecular systems has been shown valuable results in probing the time and length scales of systems beyond what is feasible with atomistic molecular dynamics (MD) [9] and [10]. CG-MD technique has been used to study a variety of systems, such as micelle formation. For example, the results of CG-MD simulation of sodium dodecyl sulfate (SDS) [11] and polysorbate 80 in aqueous solutions [12] were in a good agreement with experiment and atomistic MD simulation studies. 1 μs CG-MD simulation of the self-assembly of 360 SDS molecules in water revealed the existence of three different aggregates [13]. Kranenburg *et al.* (2004) prepared a coarse grained model to examine the phase diagram of a DMPC bilayer as a function of temperature. They suggested that in the rippled gel phase, the DMPC bilayer consisted of a major gel-like region and a minor fluid like region [14]. Yang and Faller (2012) reported bilayer formation in different pressure condition for a mixture of dioleoylphosphatidylcholine (DOPC) and pegylated DOPC using CG-MD method. From their results, isotropic pressure led to the micelles while semi-isotropic pressure produced fluctuating bilayer [15].

Dipalmitoylphosphatidylcholine (DPPC) has a zwitterionic headed lipid structure, with two hydrocarbon tails and it can act as a surfactant. There are several reports on the dynamics and the structural properties of DPPC as bilayer [16] and as vesicles [17] at high temperatures. Marrink *et al.* (2001) demonstrated an atomistic MD simulation of the aggregation of 64-256 DPPC molecules into bilayers at 323K [16]. A coarse-grained [18] as well as an atomistic simulation [19] of DPPC was carried out to investigate the lecithin ripple phase. The lecithin lipid bilayers showed spontaneous rippled phase when they cooled to 283K. Theoretical studies proved that lipid bilayers could present lamellar phases as a function of temperature [20]. The self-assembly of DPPC in different pressure was studied by Patel and Balaji (2005). They reported that the use of anisotropic pressure coupling in the self-assembly of solution-like configuration of DPPC could lead to the formation of a bilayer or bilayer-like aggregates. In contrast, simulating the same system in isotropic pressure coupling was led to the formation of a cylindrical micelle/lamellar structure with a large water hole [21].

The self-assembly of DPPC at room temperature with different ratios is very important for palm kernel oil-

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based nano-emulsions' formulation. Theoretical studies have been shown that nano-emulsions have a great potential to be applied in transdermal drug delivery [22]-[24]. Lecithin is an important part of nano-emulsion formulation because it is a surfactant with Generally Recognized as Safe (GRAS). Lecithin shows a tendency to form lamellar and other liquid-crystal phases, thus, the self-assembly of DPPC which is one of the lecithin compounds at different temperatures and pressure will be very helpful for understanding the basics of nano-emulsion preparation. The main reason is that the tendency of lecithin in forming the lamellar and liquid crystal phases will influence the type of co-surfactant selection [25]. Here, we report coarse-grained molecular dynamics (CG-MD) models of 64 molecules of DPPC at room temperature and a higher temperature of 323K under isotropic and semi-isotropic pressure condition to investigate the self-assembly of DPPC in water.

II. Methodology

A. Model Preparation

CG-MD model system were prepared using MARTINI model version 2.0[9]. The MARTINI force field employs 4:1 mapping scheme for non-ring structures, where on average 4 heavy atoms are represented by 1 interaction center, each has an effective size of 0.47 nm and a mass of 72 amu. MARTINI model for coarse-grained DPPC was consisted of 12 beads where choline (NC3) group was represented by type Q0 (blue), phosphate group (PO4) was represented by type Qa (yellow), glycerol group (GL1, GL2) and hydrocarbon tail were represented by type Na (magenta) and C1 (cyan) respectively (see Fig. 1).

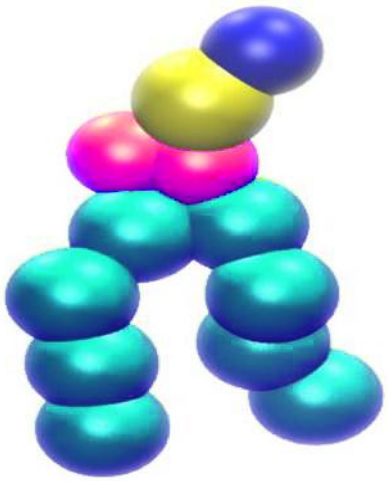


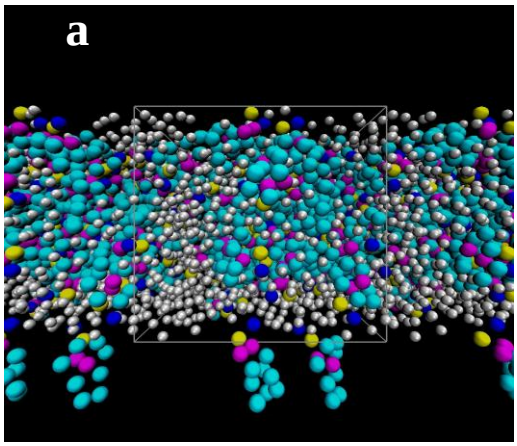
Figure 1. The DPPC molecular structure after coarse-graining.

B. Simulation Parameters

The model systems prepared were composed of 64 DPPC molecules in 772 coarse grained water beads (3088 real water molecules). The MD production simulation was performed for 500 ns in a NPT ensemble by using Gromacs 4.0 software package [26]. Periodic boundary condition was applied with a nonbonded cut off of r_{cut} = 1.2 nm. Bonded interactions were defined by harmonic potentials, whilst nonbonded interactions were defined by Lennard-Jones potential. The Lennard–Jones potential was shifted from r_{shift} = 0.9 nm to r_{cut} and the Coulombic potential was shifted from zero to r_{cut}. The DPPC and water particles were independently coupled to a 298K and 323K bath with a relaxation time of τ_p = 1.0 ps. The pressure was maintained isotropically and semi-isotropically at 1 bar using the Berendsen thermstat [27] with a coupling time of τ_p = 5.0 ps and an isothermal compressibility of 3.0×10^{-4} bar^{-1}. The equations of motion were integrated using the leap frog algorithm [28] with a timestep of 30 fs. Four set of simulations were run; 64 DPPC and 772 coarse-grained waters in an isotropic condition at 298K and 323K, and 64 DPPC and 772 coarse-grained waters in semi-isotropic condition at 298K and 323K. The box sizes for all models were chosen as $10.0\times10.0\times10.0$ nm^3. The CG potentials are much smoother than the atomic potential, thus by coarse-graining the particles, the effective sampling time will be four times faster than the actual simulation time. This has been confirmed by the comparison of diffusion constant for atomic and CG water molecules [9].

III. Result and Discussions

The structures of the bilayer obtained under isotropic pressure are shown in Fig. 2(a) to Fig. 2(c) at 298K and Fig. 3(a) to Fig. 3(c) at 323K. Fig. 4(a) to Fig. 4(c) and Fig. 5(a) to Fig. 5(c) illustrate the simulation results for semi-isotropic pressure at 298K and 323K, respectively. The self-assembly process was visualized by using VMD [29]. The tail groups are represented by the cyan beads, GLY groups with magenta beads while NC3 and PO4 groups are blue and yellow. Similar bilayer formation pattern was observed in all model systems. The bilayer formation was started from a random configuration. As shown in Fig. 2 (c) at 9.4 ns, the bilayer was completely structured with the head groups faced towards the water molecules and the tails inside the bilayer. This lamellar shape was retained until the end of simulation. The same trend was detected at 323K under similar pressure condition while the bilayer was completely formed at 8.0 ns (Fig. 3 (c)). For systems under semi-isotropic pressure, a complete bilayer formation was spotted at 8.3 ns for system at 298K (Fig. 4(c)) whilst 7.6 ns was the time for system at 323K (Fig. 5 (c)).



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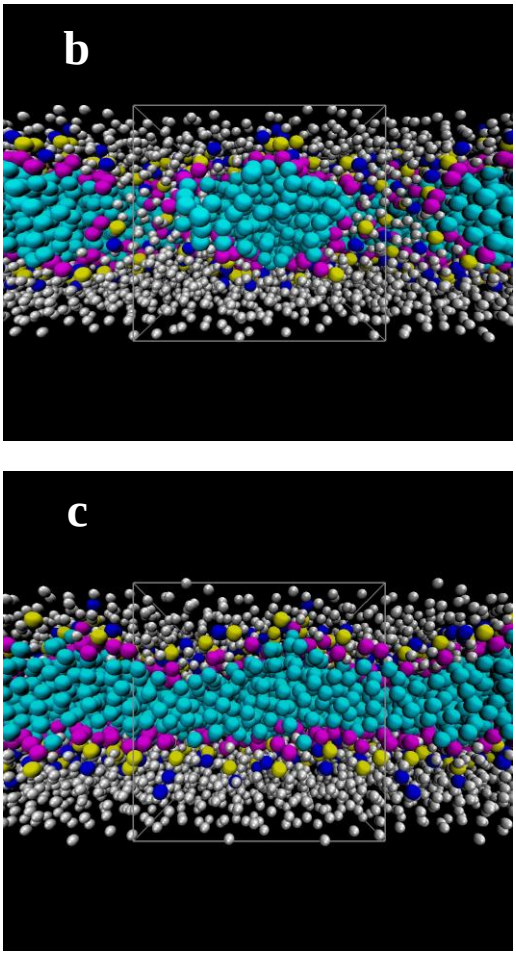

Figure 2. The snapshot pictures for the self-assembly of system of 64 DPPC in isotropic condition at 298 K at (a) 0 ns, (b) 1 ns, and (c) 9.4 ns.

The hydrophobic effect is the driving force for the aggregation of lipid molecules into bilayer [30]. From our results, all systems showed the bilayer shape at the end of simulation. However, it seems that the system in semi-isotropic condition at 323K showed a faster bilayer formation compared to other systems while the system in isotropic condition at 298K took longest time. This may be due to the fact that lipid assembly can be strongly affected by the restriction of the constant box size ratio in isotropic condition. Therefore, it can be suggested that the increase of temperature in semi-isotropic pressure would lead to the fastest bilayer formation.

Area per lipid is usually used to study the structural behaviour of a bilayer system [20]. In order to obtain the area per lipid, the area of the simulation box can be divided by the number of lipids in the model prepared. From our results, the average value of the area per lipid in isotropic condition at 298K was 0.48 nm^2 while the same system at 323K showed the value of 0.49 nm^2. The area per lipid for system under semi-isotropic pressure at 298K was 1.00 nm^2 whilst for system at 323K the area per lipid was 1.02 nm^2. The experimental values of area per lipid for DPPC in anisotropic pressure at 273K, 293K and 323K are 0.46 ± 0.01 nm^2, 0.48 ± 0.01 nm^2, and 0.64 ± 0.01 nm^2, respectively [20] and [31]. Our simulation results showed a good consistency with previous studies. The increasing of temperature increased the area per lipid because DPPC was at its more ordered gel state at 298K, thus the area per lipid will be smaller than 323K. Both temperature under isotropic condition produced similar values compared to previous experimental results at 293K. However coupling the pressure in semi-isotropic condition produced the higher value of area per lipid compared to those in isotropic condition. This might suggest that the increment of temperature and changing the pressure condition could affect the observed area per lipid. Table I reports a summary of our results.

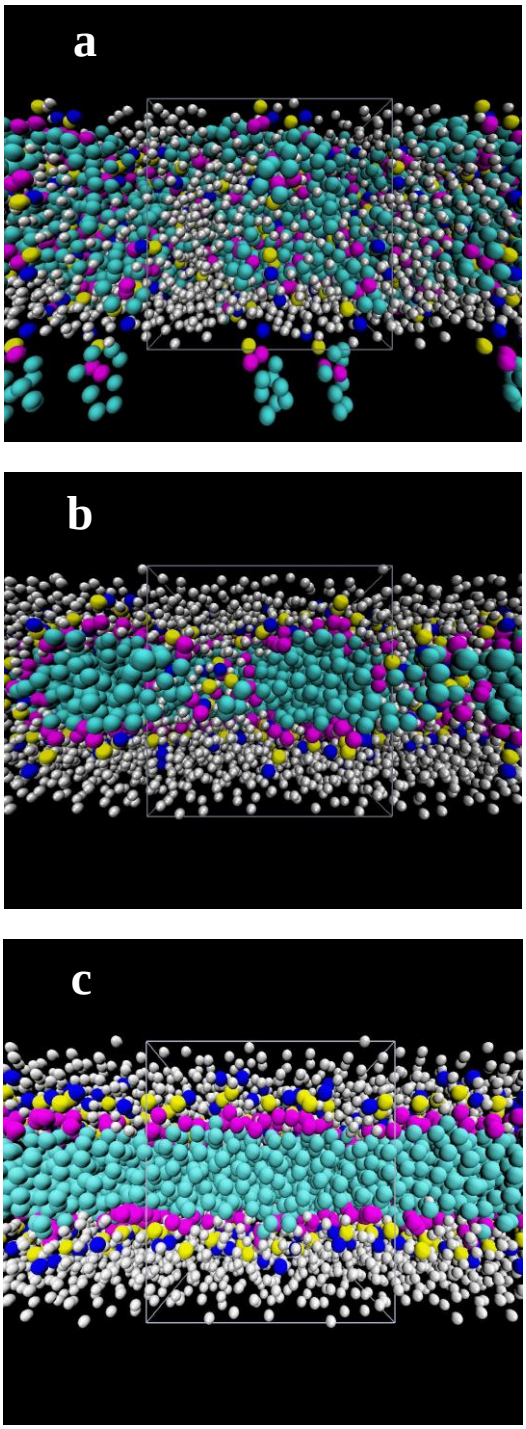

Figure 3. The snapshot pictures for the self-assembly of system of 64 DPPC in isotropic condition at 323 K at (a) 0 ns, (b) 1 ns, and (c) 8.0 ns.

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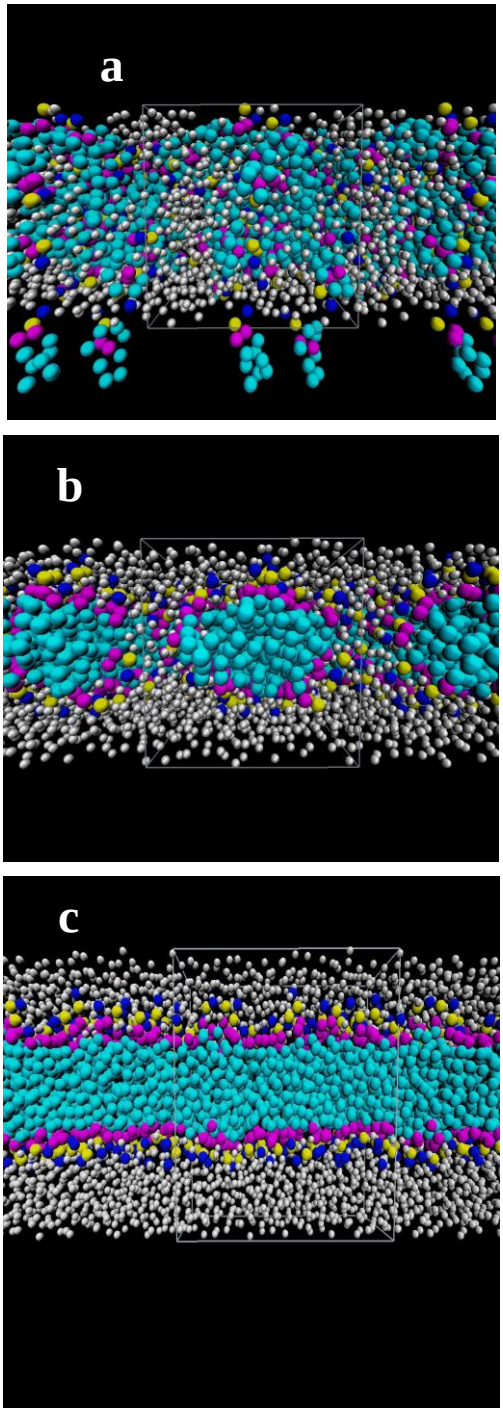

Figure 4. The snapshot pictures for the self-assembly of system of 64 DPPC in semiisotropic condition at 298 K at (a) 0 ns, (b) 1 ns, and (c) 8.3 ns.

TABLE I. A SUMMARY OF BOX SIZE AND THE NUMBER OF LIPID PER LEAFLET OF 500NS SIMULATION TIME.

Pressure condition	Temperature (K)	Box-X/Y (nm)	No. of DPPC/leaflet (top/bottom)
Isotropic	298	5.59±0.002	33/31
Isotropic	323	5.63±0.002	33/31
Semiisotropic	298	8.03±0.200	32/32
Semiisotropic	323	8.07±0.200	33/31

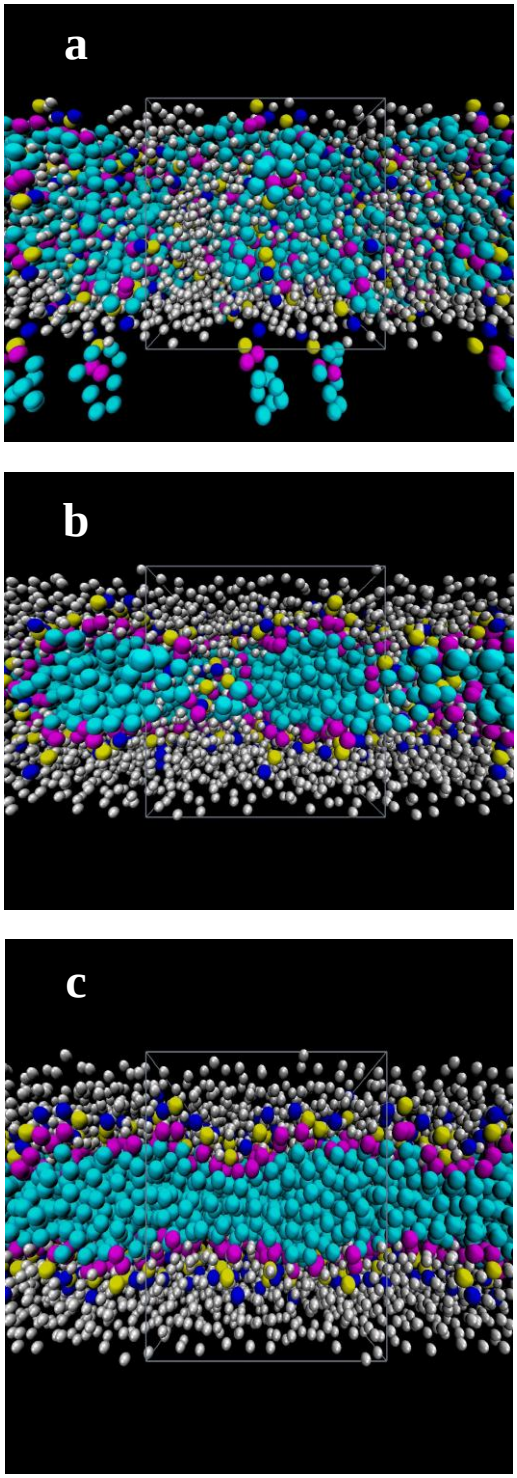

Figure 5. The snapshot pictures for the self-assembly of system of 64 DPPC in semiisotropic condition at 323 K at (a) 0 ns, (b) 1 ns, and (c) 7.6 ns.

The thickness of bilayer formed can be demonstrated by plotting the density profile based on the calculation of the distance between two peaks obtained. The activity of membrane proteins as well as the insertion and orientation of foreign particles in the membrane are critically dependent on the bilayer thickness [32]. Fig. 6 shows the density peaks of head group in our model systems. All systems were plotted along the bilayer normal direction (y-direction). The average value of

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bilayer thickness for systems under isotropic pressure at 298K was 2.91 nm while at 323K, the bilayer thickness slightly increased to 3.04 nm.

Higher values of bilayer thickness were detected under semi-isotropic condition where at 298K, the bilayer thickness was 3.86 nm and at 323K, the bilayer thickness was 3.73 nm. The experimental value at 293K has been reported as 4.42 nm and 3.83 nm at 323K [20]. Our model systems under semi-isotropic condition showed a good consistency with experimental data as the bilayer thickness was decreased with increasing the temperature. However, isotropic condition showed reverse trends which might be due to the presence of high surface tension at this condition [21].

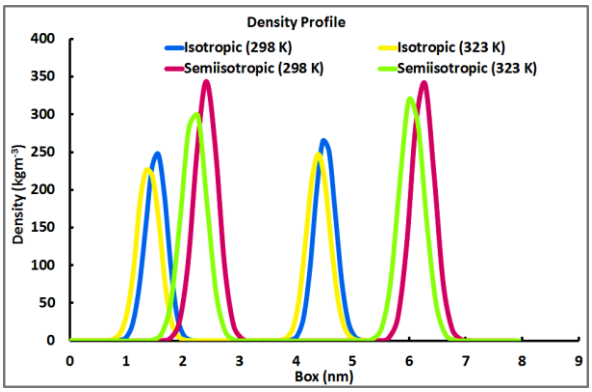

Figure 6. Density profile of all simulated model systems.

Radial distribution functions (RDFs) determines the particle density distribution at the distance *r* around a given particle. Fig. 7 and fig. 8 illustrate the RDF results of water interacting with NC3 group and PO4 group for 500 ns of simulation, respectively. The phosphorus group showed similar fluctuations with nitrogen however the fluctuation was higher for NC3 compare to PO4. This can be resulted in from the higher mobility of the NC3 group, which is able to penetrate the water region more readily than the PO4 group [33]. Headgroups of DPPC can be more hydrated in the isotropic pressure coupling condition than in the semi-isotropic one. Thus, the difference in the pressure coupling may change not only the area per lipid and bilayer thickness but also it may affect the degree of hydration in the lipid system.

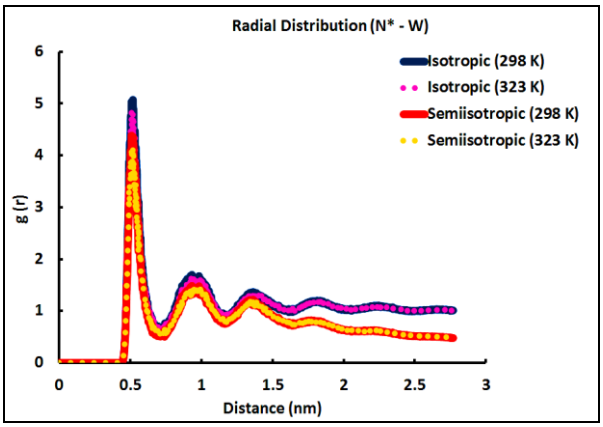

Figure 7. The radial distribution functions correspond to water interacting with lipid NC3 group for the simulation of all systems.

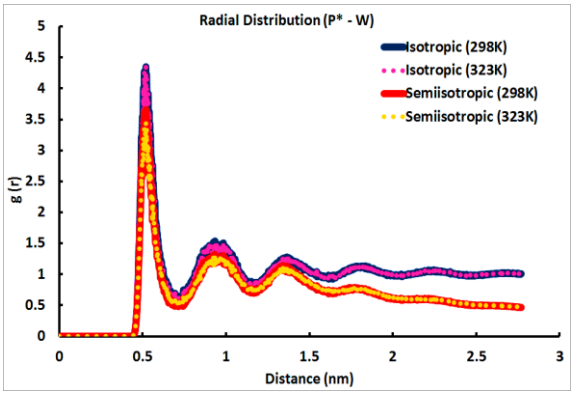

Figure 8. The radial distribution functions correspond to water interacting with lipid PO4 group for the simulation of all systems.

IV. Conclusion

The self-assembly of 64 molecules of DPPC at different temperatures of 298K and 323K and pressure coupling conditions were explored using CG-MD simulation technique. From our results, lowering the temperature from 323K to 298K using isotropic and semi-isotropic pressure coupling did not change the bilayer formation pattern. However, it affected several bilayer properties such as area per lipid, bilayer thickness, and radial distribution. Currently, the bottleneck in the formulation of the nano-emulsions of palm-oil based esters is finding more solubilized and stable micelles with less toxicity and irritancy, and high permittivity through the skin barrier for transdermal drug delivery applications. With respect to the importance of nano-emulsions of palm-oil based nano-emulsions as potential chemical penetration enhancers (CPEs), theoretical studies such as the current one may extend the breadth of knowledge on how a DPPC molecule can act as a surfactant during formulation.

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