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Molecular Dynamics Study of Prednisolone Concentration on Cholesterol Based Lung Surfactant Monolayer

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Abstract. Research on lung surfactant (LS) and drug interaction promote the development of drug adsorption mechanism on the LS. The in-depth understanding of prednisolone interaction with the LS is important for drug design. Previous studies report the stability and surface activity of the LS for various drug molecules such as prednisolone, prednisone, glucocorticoid, budesonide, and beclometasone. However, proper knowledge of prednisolone concentration effect on the model LS monolayer is essential for better understanding the drug delivery in the targeted area, which is still missing in the existing literature. The current study deals with the concentration effects of prednisolone drug molecules adsorption, diffusing, and transfer on the model LS monolayers. Coarse-grained molecular dynamics simulation has been employed to understand the stability of model LS by using the MARTINI force field. The present study has been conducted for two different surface tensions to replicate the inhalation and exhalation process of breathing. Different structural and dynamical characteristics of the system have computed in this study. Structural changes, for instance, phase behavior, area per lipids, diffusion coefficient, mean square displacement, and lipids chain order parameter may be affected by the aggregation of prednisolone. The findings of this study could be helpful in drug designing through the understanding of the prednisolone diffusion and aggregation mechanism in the surfactant monolayer.

INTRODUCTION

Surface-active biomolecules such as lipids, cholesterol, and proteins (known as lipoprotein) form a thin layer on the inner part of alveolus in the lung. This LS layer secreted into the alveolar space from epithelial type II cells. Dipalmitoyl-phosphatidylcholine (DPPC) is the major lipid component of the lipoprotein, where a minimal amount of POPC (Palmitoyloleoylphosphocholine) and cholesterol (CHOL) are also present in the LS, and it carries small amount of surfactant proteins. In the surfactant, the amphiphilic phospholipids (PLs) have hydrophilic (water attraction) and hydrophobic (water repulsion) regions. The primary role of LS is to maintain the physiological values (at inhalation and exhalation condition) of surface tension (ST) at the air-liquid interface of the alveoli and protect the lung from collapsing. These fascinating characteristics (varying the ST with the surface area) of the LS preserve the alveolar stability. The decrease of lung airways is usually caused due to a narrowing or blockage of lung tubes, which becomes unable to supply oxygen and other gases into/out of the lungs. Asthma, chronic obstructive pulmonary disease (COPD), and bronchiectasis are examples of these diseases. Prednisolone is used as a drug to cure lung diseases (allergies and asthma [1-3]) that are known as the overactive immune system. It is a metabolite of prednisone and an active molecule that plays an essential role in glucose metabolism [4]. The standard and long term treatment have harmful side effects, including impaired immune response, weight gain and behavioral disturbances [5, 6]. Neurological development is also affected when steroids are used to treat allergy or asthma patients [7]. These two drugs are permitted as an essential medicine by the World Health Organization (WHO) for the medication of the basic health system [8]. Less harmful contemporary use of prednisone and prednisolone is essential for the medications than oral administration. In this regard, to cure respiratory distress syndrome (RDS) and obstructive lung diseases, LS is

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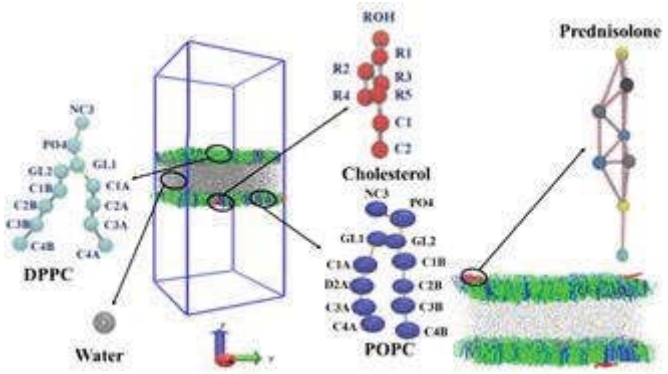
used as a spreading drug agent [9, 10]. The structural, dynamical and chemical properties of LS may help for rapid diffusion, transporting of drugs and dissolution of hydrophobic drugs molecule on the alveolar liquid-air interface and pulmonary epithelium [9]. To get an overall understanding of these properties of the LS, we need to investigate the interaction of drugs with the LS at molecular level. Computational studies using molecular dynamics (MD) simulation play an important role to investigate drug design, drug delivery, and the interaction of the drug with biomolecules. The MD studies have conducted to find out the LS monolayer [11-13] characteristics in the molecular point of view. The understanding of drug molecules interactions with surfactant lipids (headgroups or chains) plays an important role, and this interaction has also influenced surfactant to act as a pulmonary transport vehicle.

The present study aims to explore the adsorption of prednisolone into PLs monolayers. Herein, the coarse-grained MD simulation is used to model the LS monolayer composed of DPPC, POPC, CHOL (7:3:1) with and without prednisolone. The effects of prednisolone on the LS monolayer components (with and without cholesterol) have been studied to examine the changes in the structural and dynamical properties of the monolayer system including phase behavior, area per lipids (APL), lipids chain order parameter, lateral diffusion coefficient, and mean square displacement. The simulations have performed at two extreme conditions such as inhalation (at ST 0 mN/m) and exhalation (at ST 20 mN/m) to mimic the natural breathing process.

MODEL DESCRIPTION, PARAMETERIZATION, AND SIMULATION

The preliminary lung surfactant (LS) model was constructed by using an INSANE (INSesrt membrANE) script [14]. The composition of phospholipids (PLs) was maintained (DPPC, POPC, CHOL = 7:3:1 molar ratio) according to surfactant monolayer composition [15, 16]. The INSANE script was used to generate a bilayer with a dimension of 17 × 17 nm^2. Two monolayers (parallel to XY plane) were prepared by splitting the bilayer and maintaining at a distance 6 nm between them. The gap between these two monolayers was replaced by a water box with a dimension of 17 nm × 17 nm × 6 nm and filled with ~ 12500 CG water beads keeping a density of ~1000 kg/m^3. Each monolayer contained a total of 484 CG PLs and cholesterol molecules as presented in Fig 1. Polar head groups of the lipids aligned towards the water and the hydrophobic lipids tails oriented towards the air. The whole system (lipids, water) was placed in a periodic box with a box size of 17 nm × 17 nm × 50 nm in such a way that the lipids monolayers were separated by ~40 nm of vacuum in total. For the model validation, the primary structure was made by employing the same process as Estrada-López *et al.* [15] and Hossain *et al.* [16] models. Four different systems were prepared for the simulation with the various compositions (see Table 1 and Fig. 1) of the surfactant and prednisolone drug molecules. According to experimental studies, the higher amount of cholesterol (40 mol%) declines the ability of LS to achieve minimal ST and physiological levels of cholesterol increase the adsorption of surfactant lipids at ST ~0 mN/m [17]. In the present study, we prepared monolayers with the composition of cholesterol (~5 wt/wt) maintaining the physiological levels of cholesterol in the surfactant. Various concentration of prednisolone was used to understand the LS mechanism in the absence and presence of cholesterol in surfactant monolayer. All simulations were achieved with the help of molecular computational package GROMACS version 5.1.4 [18]. The MARTINI force field [19, 20] parameters for DPPC, POPC, cholesterol, and water were used to describe the interaction between molecules. The coarse-grained prednisolone molecule was parameterized as the same procedure of cholesterol model and modified according to Estrada-López *et al.* [15] interaction parameters. Before starting the simulation, the energy of all the systems was minimized by using the steepest descent method to avoid all the unrealistic steric clash among molecules. Targeted energy (1000 KJ/mol) was fixed to get a relaxed system with no overlapping molecule groups. Periodic boundary condition (PBC) was set up in all directions. Coulomb interactions cut-offs were shifted to zero between 0 and 1.2 nm, whereas the Lennard-Jones interactions cut-offs were considered zero between 0.9 and 1.2 nm. Lipids, prednisolone, and water molecules were coupled independently at temperature 310 K using v-rescale thermostat. The relative dielectric constant was considered 15 for this force filed [20]. The simulations were performed on the system with and without presence of prednisolone at STs 0 and 20 mN/m. These STs were considered to maintain the physiological conditions (inhalation and exhalation, respectively) of lung surfactant for all the twelve independent simulations. The equilibration and production run of the simulations were conducted in NVT (constant particle number, volume and temperature for 100 ns) and NPT (constant particle number, pressure and temperature for 500 ns) ensemble by using Berendsen pressure coupling with compressibility 4.5 $\times 10^{-5}$ bar^{-1} in the xy-plane. In order to keep fixed box height along the monolayer normal, the value of compressibility of the system was set zero. All the systems were simulated for 500 ns equilibration and 2 μs production run (with 20 fs time steps) by using leap-frog integrator algorithm. To display and analyse the molecular assemblies, the Visual Molecular Dynamics (VMD [21])

was used with a variety of rendering styles. All the calculations (order parameter, density profiles, area per lipids, diffusion coefficient and mean square displacement) were carried out based on the trajectories for the last 1 μs of production run.



(a) (b)

FIGURE 1. Schematic representation of the simulation system with lipids monolayers separated by 6 nm water layer having 40 nm vacuum. The lipids monolayer was constructed by the composition of DPPC, POPC, and CHOL (a) without prednisolone molecules and (b) with prednisolone molecules.

RESULTS AND DISCUSSION

The present study has been conducted to calculate the structural and dynamical properties of the LS monolayer with and without cholesterol in the presence and absence of prednisolone drug molecules. Before going to analyze drug interaction, we first validate our model to conform the actual representation of LS monolayer. For this, we compare the properties area per lipid (see Table 1) and order parameters (see Fig. 2) from different lipid components at different STs. The calculated area per lipid (APL) values from this study are in good agreement with previous data from simulations. The APL is one of the vital structural characteristics of the biological membrane. The APL values of twelve independent simulations illustrate the biophysical characteristics of the lipids monolayer which is shown in Table 1.

TABLE 1. Area per lipid of LS monolayer during the breathing process for the lung surfactant monolayer systems composed of DPPC: POPC, and DPPC: POPC: CHOL, with and without prednisolone (PRED). Uncertainties of results represent by standard deviations for comparison data from previous studies, where values are given as reported in the paper.

System		ST, mN/m	APL Present Study nm^2	APL Estrada-López et al. nm^2 [15]	Hossain et al. nm^2 [16]
DPPC, POPC (I)		0	0.484±0.000891	0.477±0.01	0.475±0.001
		20	0.569±0.001256	0.569±0.03	0.560±0.001
DPPC, POPC, CHOL (II)		0	0.464±0.000821	-	-
		20	0.530±0.001257	-	-
DPPC, POPC + Prednisolone (III)	PRED (10)	0	0.484±0.000794	0.478±0.001	-
	PRED (120)		0.560±0.002699	**Collapsed**	-
	PRED (10)	20	0.571±0.001216	0.573±0.001	-
	PRED (120)		0.598±0.001455	0.618±0.002	-
DPPC, POPC, CHOL+ Prednisolone (IV)	PRED (10)	0	0.482±0.000792	-	-
	PRED (120)		**Collapsed**	-	-
	PRED (10)	20	0.532±0.001243	-	-
	PRED (120)		0.560±0.001397	-	-

The phase behavior of the lung surfactant monolayer depends upon the APL values. The obtained values of the APL at 0 mN/m ST lie in the range ~0.46-0.49 nm^2 (except for system-III). The range of APL values conform that the simulated surfactant monolayer is in the liquid condensed (LC) phase representing the monolayers in exhalation state. On the other hand, the APL at ST 20 mN/m of the LS monolayer reaches at an intermediate phase known as liquid condensed-expanded (LC-LE) phase from liquid expanded (LE) phase. The transition of the phase behavior of the monolayer depends on the breathing conditions that could be confirmed by evaluating the order parameter of the lipid's tail groups. Fig. 2 shows the order parameters for the *sn*-1 chain of the DPPC and *sn*-2 chain of POPC from simulations of DPPC: POPC in the absence of prednisolone (system I) at ST 0 and 20 mN/m. As a comparison, data from previous studies of the same system are shown. Both the *sn*-1 chain of DPPC and the *sn*-2 chain of POPC show much higher order parameters at 0 mN/m ST than that at 20 mN/m.

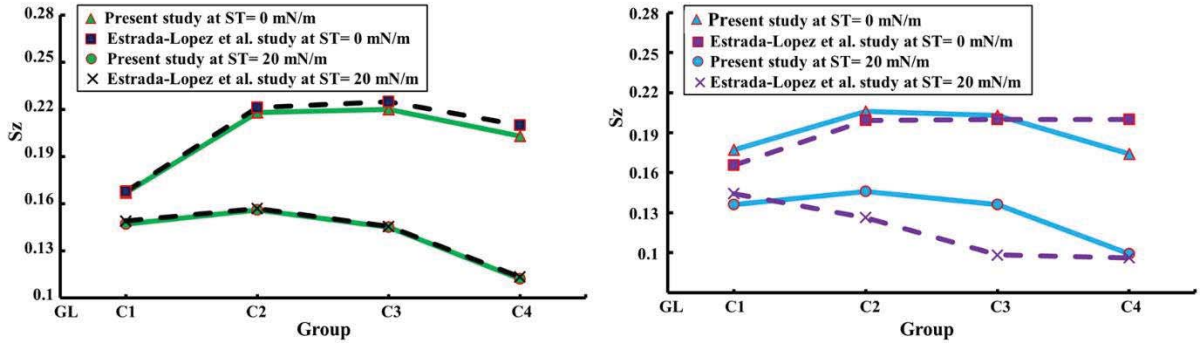


FIGURE 2. Order parameter of lipids in lung surfactant monolayers composed of DPPC: POPC (7:3). For comparison data from a previous study (reference) is shown. (a) DPPC *sn*-1 chain. (b) POPC *sn*-2 chain.

Figure 3 shows the order parameters for the *sn*-1 and *sn*-2 chains for DPPC and POPC from simulations of DPPC: POPC: CHOL in the absence of prednisolone (system II) at ST 0 and 20 mN/m. As expected, the saturated lipid DPPC is more order than unsaturated lipid POPC at both STs. The PLs are found more order at low ST than high-pitched ST without the existence of prednisolone. As the increase of prednisolone concentration, a phase transition suppresses the surface activity of the DPPC, POPC, CHOL monolayer film than DPPC, POPC monolayer films.

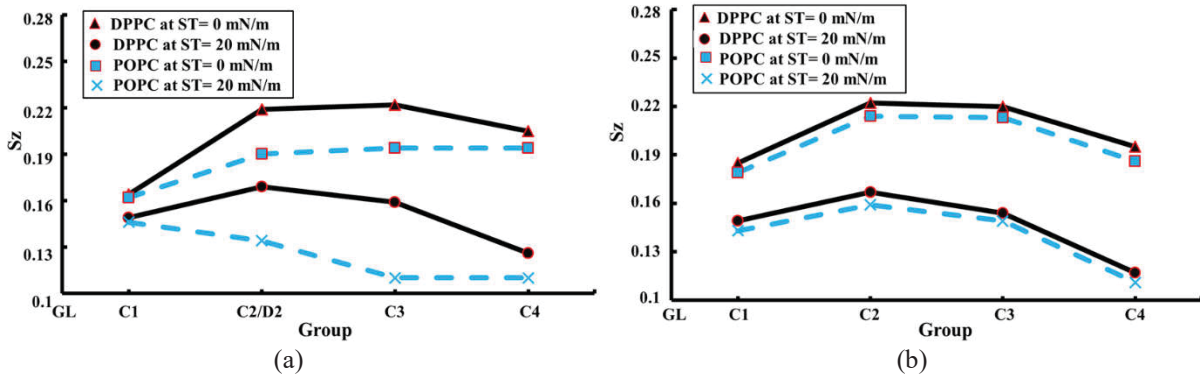


FIGURE 3. Order parameters of lipids tail groups (DPPC, POPC, CHOL model) at STs 0 and 20 mN/m. Order parameter of DPPC, POPC lipid-tail beads (a) *sn-1* (b) *sn-2*.

Density profiles of the DPPC, POPC, CHOL, and water of the system have evaluated at both STs (0 and 20 mN/m) that have illustrated in Fig. 4. From the figure, the density profiles of each LS component at the air-water interface has comparably higher values as expected in LC state at ST of 0 mN/m than the ST of 20 mN/m in both systems. A slight drop in density profiles is observed with the increase of prednisolone concentration (not shown here for brevity). This propensity of density profiles shows the gradual decrease of lung surfactant components (DPPC, POPC, and Cholesterol) due to the increase of drug concentrations from ~0 to ~11 % w/w. The presence of prednisolone contributes to expanding the APLs up to ~0.60 nm^2 and diffuse into the surfactant layer.

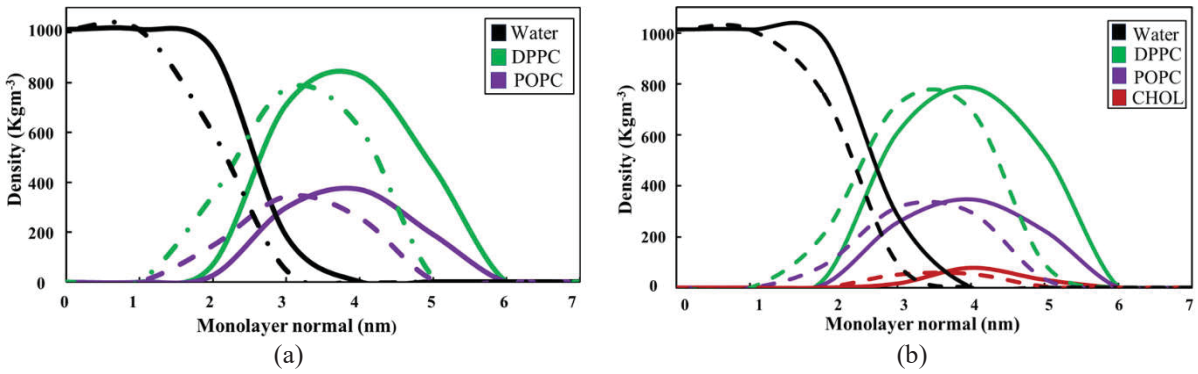


FIGURE 4. Density profiles of DPPC, POPC, CHOL, and Water at surface tension 0 (solid line) and 20 (dotted line) mN/m for (a) DPPC, POPC and (b) DPPC, POPC, CHOL monolayer systems.

The surfactant molecule's dynamical properties, such as mean square displacement (MSD) have approximated by the least square fitting to the straight line ($MSD(t) = D \times t + c$) through the MSD curves from representative time, t = 1200 ns to t = 2000 ns in Fig. 5. Lateral diffusion coefficient has calculated at different STs to estimate the rate of diffusion, and the values of the diffusion coefficient have presented in Table 2. According to Table 2, the diffusion coefficient of LS monolayer components at ST 20 mN/m is ~2.30 to ~2.60 times higher than those at ST 0 mN/m.

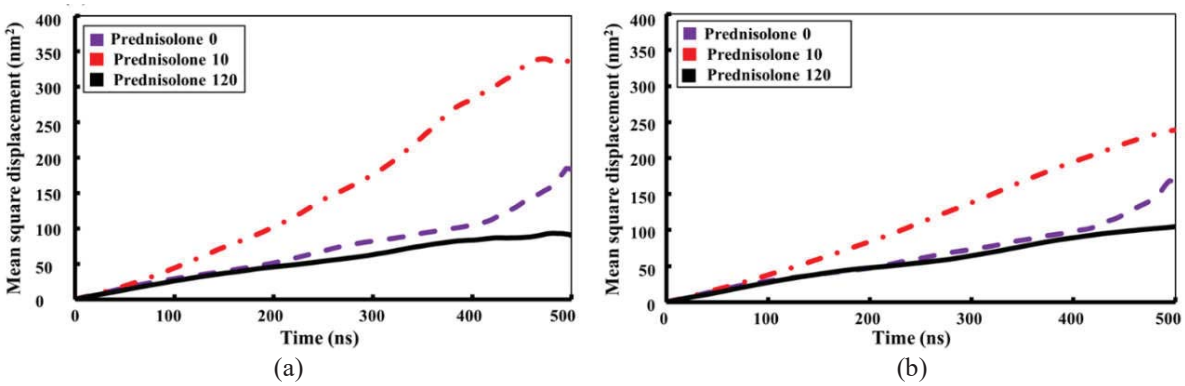


FIGURE 5. Mean square displacement (MSD) at ST= 20 mN/m. (a) phospholipids (DPPC and POPC) and (b) cholesterols with different prednisolone concentrations.

In general, the lateral diffusion coefficient fluctuates around two orders of magnitude, depending on the phase behaviour of the lipid's monolayer. The calculated diffusion values satisfy the existing data of lung surfactant monolayer [21]. The maximum diffusion coefficient of prednisolone is found $(9.98\pm0.16)\times10^{-7}$ cm^2s^{-1} at liquid condensed-expanded (LC-LE) phase with a low concentration (~1 % w/w) of drug molecules whereas the diffusion coefficient of prednisolone at liquid condensed (LC) phase is observed $(4.37\pm0.03)\times10^{-7}$ cm^2s^{-1}. The diffusion of drug molecules is significantly affected by the monolayer ST. The increase of ST contributes to enhancing rate of diffusion throughout the lung surfactant phospholipids monolayer. The higher concentration of prednisolone shows an adverse effect (slowing down of diffusion rate) on the monolayer, and sometimes, it causes the monolayer collapse (see Fig. 6) due to the deposition of the prednisolone at ST 0 mN/m. The aggregation of prednisolone molecules plays a crucial role in forming buckle shaped structures of the lung surfactant layer. The opposite scenario is observed when ST is an increasing trend that is presented in Fig. 6d. As it is seen from Fig. 6d, the prednisolone molecules on the surfactant layer are equally distributed, and the drug molecules are transferred to the DPPC, POPC, CHOL mixed monolayer. The MSDs of both PLs (Fig. 5a) and cholesterol (Fig. 5b) illustrate that the maximum deviation of the position of PLs and cholesterol molecules have retrieved at prednisolone concentration (~1 % w/w) concerning the reference position over time. The spatial extent of arbitrarily dispersed lung surfactant biomolecules is spreading owing to diffusion.

TABLE 2. Lateral diffusion coefficient of DPPC, POPC, CHOL, and Prednisolone calculated at ST, 0 and 20 mN/m using Einstein equation.

	ST (mN/m)	Diffusion coefficient $10^{-7}cm^2s^{-1}$ at 10 molecules of Prednisolone	Diffusion coefficient $10^{-7}cm^2s^{-1}$ at 120 molecules of Prednisolone
DPPC	0	3.50±0.04	**Collapsed**
	20	8.86±0.35	8.26±0.39
POPC	0	3.67±0.05	**Collapsed**
	20	9.03±0.33	8.33±0.40
CHOL	0	3.47±0.01	**Collapsed**
	20	8.36±0.11	7.81±0.17
Prednisolone	0	4.37±0.03	**Collapsed**
	20	9.98±0.16	7.56±0.23

The rate of this diffusion is decreasing with the increasing of the prednisolone concentration, and it reaches (0 nm^2 to ~50 nm^2) that is below the MSD curve with no prednisolone molecules. These phenomena of the lateral diffusion of biomolecules depend on the amount of the free surface area in the monolayer, and the faster diffusion occurs due to the large free surface area.

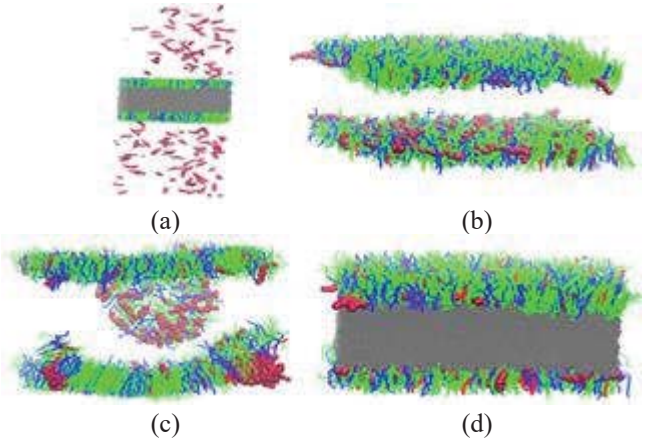


FIGURE 6. (a) Initial configuration of the system with 120 molecules of prednisolone in the vacuum having water slab in the middle of the surfactant monolayer. (b) Equilibrated configuration of the system after 100 ns at ST 0 mN/m. (c) System collapse due to prednisolone aggregation at ST 0 mN/m. after 140 ns production run (d) Prednisolone diffusion (non-aggregation state) at ST 20 mN/m after 1 μs.

CONCLUSION

In the present computational study, we used CG-MD simulations to reports the concentration effect of prednisolone drug molecules adsorption, diffusing, and transfer on the model cholesterol-containing LS monolayers. The preliminary results illustrate the structural and dynamical properties of the monolayers, and we have found that the structural changes may be affected by the aggregation of the prednisolone drug. Calculation of some of these properties show that the adsorption of drug molecules has greatly affected by drug concentration. The literal diffusion coefficient and order parameter is calculated for validating the system parameters. The estimated order parameter of DPPC and POPC shows excellent agreement with the published data. The lateral diffusion coefficients of prednisolone molecules have found as (4.37±0.03) $\times 10^{-7}$ cm^2s^{-1} and 9.98±0.16 $\times 10^{-7}$ cm^2s^{-1} at ST 0 mN/m and 20 mN/m respectively, which are larger than that of theoretical and computational phospholipids mixed monolayer. The hydrophobic nature of the prednisolone contributes to forming the clustering of the drug molecules which occurs at the high fraction (~11 % w/w) in LC phase and it may cause the instability of the LS at ST 0 mN/m. On the other hand, the administration of

prednisolone drug by inhalation at low concentration (both breathing condition) or high concentration (at exhalation) may diffuse quickly and adsorb by the lung surfactant monolayer. The spreading of the drug throughout the lung surfactant may be diffuse on the high surface area of the lung when the medicine is sent into the respiratory system. The findings of this study could be helpful in drug designing and spreading mechanism through the understanding of the prednisolone diffusion and aggregation in the lung surfactant at the molecular point view. A new treatment method may be introduced for patients who are suffering from asthma and respiratory distress syndrome.

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