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The influence of cholesterol on interactions and dynamics of ibuprofen in a lipid bilayer

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ABSTRACT

In this work, molecular dynamics (MD) simulations with atomistic details were performed to examine the influence of the cholesterol on the interactions and the partitioning of the hydrophobic drug ibuprofen in a fully hydrated 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayer. Analysis of MD simulations indicated that ibuprofen molecules prefer to be located in the hydrophobic acyl chain region of DMPC/cholesterol bilayers. This distribution decreases the lateral motion of lipid molecules. The presence of ibuprofen molecules in the bilayers with 0 and 25 mol% cholesterol increases the ordering of hydrocarbon tails of lipids whereas for the bilayers with 50 mol% cholesterol, ibuprofen molecules perturb the flexible chains of DMPC lipids which leads to the reduction of the acyl chain order parameter. The potential of the mean force (PMF) method was used to calculate the free energy profile for the transferring of an ibuprofen molecule from the bulk water into the DMPC/cholesterol membranes. The PMF studies indicated that the presence of 50 mol% cholesterol in the bilayers increases the free energy barrier and slows down the permeation of the ibuprofen drug across the DMPC bilayer. This can be due to the condensing and ordering effects of the cholesterol on the bilayer.

1. Introduction

Molecular interactions between the drug molecules and lipid membranes have always been of interest for drug delivery and drug design investigations. Drug interactions with lipid are important because of the fact that many drugs are transported in the body by drug delivery systems based on lipoproteins and liposomes [1,2]. Drugs can exert their effects via interactions with the lipid bilayer membrane, by perturbing membrane integrity, increasing the bilayer permeability, binding to transmembrane protein targets, binding to lipid signaling molecules, or altering membrane protein conformation [3]. Therefore in the design of drug and drug delivery systems knowledge about the interactions of drug with lipids has been the subject of experimental and computational studies to optimize the drug and drug delivery systems in the context of their abilities to reach intracellular targets [3].

Among the various computational approaches, molecular dynamics (MD) simulation has been frequently employed to study drug–membrane interactions because it can capture interaction details on the molecular scale, and also extract thermodynamic properties that are directly comparable to complementary analytical measurements [4–8]. MD simulations with the potential of mean force (PMF) calculations were particularly used for study partitioning and translocation of drugs from the water to the lipid membranes [9–12]. Umbrella sampling [13] which applies a biasing potential to obtain better sampling is a common method for the PMF calculations [11,12]. Although MD simulations have been widely used to study drug–lipid membrane systems, there are only a few works that study the behavior of drug molecules in the lipid membranes containing cholesterol [14–16].

The study of interaction between drug molecules with lipid membranes containing cholesterol and their transport mechanism is very important and useful, with regard to the wide distribution of cholesterol in biological membranes and its crucial effects on functional, structural, and dynamical properties of the membranes [17–21]. Therefore over the past years extensive investigations were performed to study the influence of the cholesterol on the lipid membranes experimentally by using differential scanning calorimetry (DSC), spectroscopic methods and neutron- and X-ray scattering methods or computationally by Monte Carlo (MC) and molecular dynamics (MD) simulation techniques [22–27]. The results of these investigations indicated that the incorporation of cholesterol to a lipid bilayer strongly affects its mechanical and thermodynamic properties [20,28–30] by a) broadening and eventually eliminating the gel to liquid-crystalline phase transition; b) inducing new phase regions above the phase transition as the liquid-disordered, the liquid-ordered and the coexistence of liquid-ordered and liquid disordered phases; c) increasing the area per molecule in the gel phase, and reducing the area per molecule in a liquid-crystalline phase; d) affecting the orientational ordering of the hydrocarbon chains and e) decreasing the passive permeability of the bilayer above and increasing this property below the main transition temperature.

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Ibuprofen (Ibu) is one of the most widely nonsteroidal antiinflammatory (NSAID), analgesic, and antipyretic drugs used for treatment of rheumatic disorders, pain, and fever and can be considered as a potent drug against various cancers, Alzheimer's disease and heart disease [3]. It works by decreasing production of cyclo-oxygenase (COX)-1 and COX-2 derived prostanoids in the blood [31]. Based on the results obtained from molecular dynamics simulations the ibuprofen has a high partition coefficient in the DPPC bilayer from the water and the solubility-diffusion mechanism is the likely mechanism of the permeation of ibuprofen molecules in the neutral form [9].

The aim of this work is to investigate the behavior of ibuprofen as a hydrophobic drug inside the fully hydrated DMPC bilayer containing 0, 25 and 50 mol% cholesterol with atomistic detail by using molecular dynamics (MD) simulations. Several analyses such as the area per lipid, density distributions, deuterium order parameters, mean square displacement and tilt angle were performed to elucidate the role of cholesterol on the structural and dynamic properties of drug molecules and lipid bilayers and also to explore the localization of the drug and effects of its presence on bilayers. Moreover, translocation of the Ibu from the water to the DMPC bilayer with various cholesterol concentrations was determined by using the potential of the mean constrained force method. The main energy barrier in translocation of an Ibu molecule across the membrane was obtained from the free energy profile.

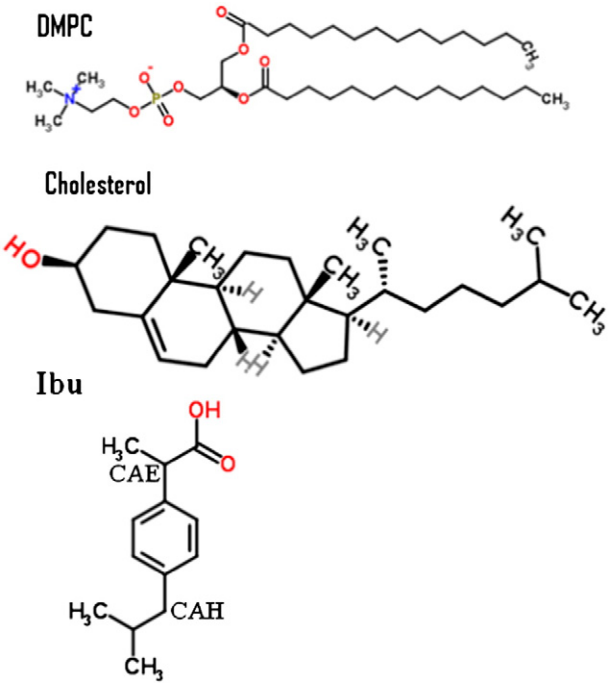

Fig. 1. Molecular structures of DMPC lipid, cholesterol and Ibu.

2. Simulation methods

In this work molecular dynamics (MD) simulations were performed on fully hydrated dimyristoylphosphatidylcholine (DMPC) lipid bilayers containing 0, 25 and 50% cholesterol (Chol) mol fractions. All preparation steps and simulations were carried out using the GROMACS v5.0.4 software package [32–34]. The pure lipid bilayer consists of 128 DMPC lipids which are arranged in a bilayer, 64 lipid molecules per leaflet, parallel to the x–y plane with three-dimensional periodic boundary conditions. Then the bilayers with the appropriate cholesterol concentrations were created by replacing the suitable number of lipids by cholesterol molecules. The number of lipid molecules and water in the simulation systems is shown in Table 1. The prepared bilayer was equilibrated by MD simulation with 20 ns time length before insertion of the drug molecules. Following insertion of 4 drug molecules (Ibu) in one side of the lipid membrane, the unit cell was filled with SPC [35] water. In all simulations, for the DMPC molecules, the partial charges and force field parameters of the Berger et al. [36] were used. The force field parameters and the topology of the cholesterol were based on the work of Holtje et al. [37]. The topology of the neutral Ibu molecule with the interaction parameters corresponding to the GROMOS 43A1 force field was obtained from the PRODRG server [38]. The atomic charges for the Ibu molecule were taken from Reference [9]. The structures of DMPC, cholesterol and Ibu molecules are shown in Fig. 1.

Each system was initially minimized using the steepest descent algorithm to remove any unfavorable contacts and interactions, followed by an equilibration simulation of 200 ps with the NVT ensemble. The 100 ns production simulation was run at constant pressure (P = 1 bar) and temperature (T = 323 K) with a 2 fs time step in the NPT ensemble and the leapfrog algorithm for integrating the equations of motion. By using the LINCS algorithm [39] all bond lengths and angles in the systems were constrained, allowing the 2 fs integration time step. The neighbor list for computing nonbonded pair forces was updated every 10 steps with a list cutoff of 1.2 nm. The Particle-Mesh-Ewald summation method [40] with a direct space cutoff of 1.2 nm, a grid spacing of 0.16 nm and an interpolation order of 4 was used to compute long-range electrostatic interactions. The system pressure was maintained at 1 bar by means of the Parrinello-Rahman barostat [41] with a coupling time constant of 1.0 ps. The temperature was maintained at 323 K, using the Nosé–Hoover thermostat scheme [42] with a coupling time constant of 0.5 ps. In this temperature DMPC bilayers with 0, 25, and 50 mol% cholesterol are in the liquid-disordered, the coexistence of liquid-ordered and liquid-disordered, and the liquid-ordered phases, respectively [43,44]. Snapshots from the production simulations of four Ibu molecules in membranes containing 0, 25 and 50 mol% cholesterol are depicted in Fig. 2.

Table 1
Volume of simulation box V (nm^3), number of water, DMPC and cholesterol in the simulation systems: system 1 (0 mol% Chol), system 2 (25 mol% Chol), and system 3 (50 mol% Chol).

	System 1	System 2	System 3
V (nm^3)	277.95	206.39	170.84
N_{H_2O}	4491	2725	2103
N_{DMPC}	128	96	64
N_{Chol}	0	32	64

The free energy profiles as the potential of mean force (PMF) for translocation of one Ibu molecule across the lipid bilayers were calculated using umbrella sampling [13] and the weighted histogram analysis method (WHAM) [45,46]. Starting configurations were obtained by positioning the center of mass (COM) of an Ibu molecule in the bulk water, and then pulling it into the bilayer center along the z-axis using the umbrella method. A harmonic spring with a force constant of 2000 kJ/(mol nm^2) and a pulling rate of 0.01 nm/ps was applied on COM of the drug molecule. These force and rate constants are sufficient for translocation of drug molecule across the lipid bilayers without disturbing the bilayer structure. From the obtained trajectory, 14 adjacent umbrella windows with a distance change of ~0.2 nm were selected which spanned the complete space between the bulk water and the center of bilayer. Each window was simulated for 8 ns where the z distance between the center of mass (COM) of the drug and DMPC bilayer (along the membrane normal direction) was constrained, and the drug Ibu was allowed to rotate and translate freely in the x–y plane. In several studies [9,12,47,48] it is shown that this simulation time for each window is sufficient for equilibration and accurate calculation of the free energy profiles. The PMF profile across the monolayer was calculated by using

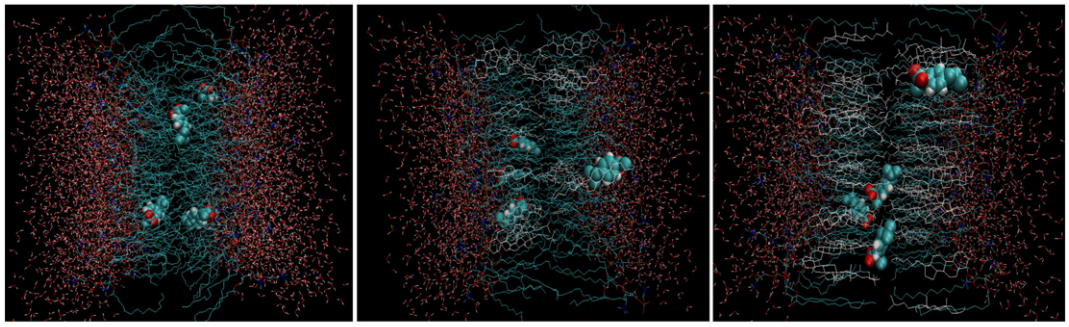

Fig. 2. Snapshots of the Ibu molecules in (A) 0 mol%, (B) 25 mol% and (C) 50 mol% cholesterol membranes.

the weighted histogram analysis method (WHAM) and assumed that it can be applied to the other monolayer with regard to the symmetric structure of bilayer.

3. Results and discussion

3.1. Area per lipid

The area per lipid molecule is an essential parameter in characterizing the bilayer structure, which is related to other physical quantities of membranes, such as the dynamics of lipids and ordering of hydrocarbon chains. The procedure used here for calculating the area per lipid is according to Hofsa et al. [49], using the following equations:

$$A_{\mathrm{DMPC}} = \frac{2A}{N_{\mathrm{DMPC}}}\left(1 - \frac{N_{\mathrm{Chol}}V_{\mathrm{Chol}}}{V - N_{\mathrm{w}}V_{\mathrm{w}}}\right) \quad (1)$$

$$A_{\mathrm{Chol}} = \frac{2A - N_{\mathrm{DMPC}}A_{\mathrm{DMPC}}}{N_{\mathrm{Chol}}} \quad (2)$$

where A_{DMPC} and A_{Chol} are the average area per DMPC and cholesterol lipids, respectively. A and V are the area and the volume of simulation box. N_{DMPC}, N_{w} and N_{Chol} stand for the number of particular molecules in the simulation box (DMPC, water and cholesterol, respectively). V_{w} and V_{Chol} are the volume occupied by a water molecule and a cholesterol molecule, respectively. The volume of a water molecule, V_{w}, is 0.031546 nm^3 [28] and that of cholesterol molecule, V_{Chol}, is 0.593 nm^3 [27,28].

The volume per DMPC lipids and the average thickness of the lipid bilayer (h) were calculated by the following equations:

$$V_{\mathrm{DMPC}} = \frac{V - N_{\mathrm{w}}V_{\mathrm{w}} - N_{\mathrm{Chol}}V_{\mathrm{Chol}}}{N_{\mathrm{DMPC}}} \quad (3)$$

$$h = \frac{V - N_{\mathrm{w}}V_{\mathrm{w}}}{A}. \quad (4)$$

The average values for the area per lipids, bilayer thickness and the volume per DMPC for membranes containing various cholesterol concentrations in the presence of Ibu molecules over the last 10 ns of simulation time were calculated and presented in Table 2. The average areas per lipid are consistent with the previous experimental and computational data, which justifies the equilibration and the accuracy of the simulations [50]. As can be seen in this table with increasing the content of cholesterol in the membrane the areas per lipid decrease while the bilayer thickness increases. This can be related to the rigid structure of cholesterol which causes less perturbation and more packing and ordering in the lipid chains.

3.2. Mass densities

To evaluate the influence of the cholesterol concentration on the distribution of drug molecules in the lipid bilayer, the mass density profiles of different molecular components along the axis perpendicular to the bilayer computed and the results were shown in Figs. 3–5. These figures indicate that the most probable locations of the Ibu molecules were in the hydrophobic region of the systems, at the hydrocarbon tails of lipids. The hydrophobic nature of the Ibu molecules implies that they may be located outside the polar head groups of the lipid molecules and water. The previous studies indicated that hydrophilic nature of the drug molecules, such as psoralen [51], 5-fu [52], ciprofloxacin [53] and hypericin [54], causes an accumulation of the drugs in the vicinity of the polar head group of lipid bilayers. Whereas the mass density profiles of the Ibu molecules in this study indicates that the hydrophobic molecules have lower tendency for accumulation in hydrophilic part of the bilayers, and diffuse into the hydrophobic part of the bilayer at the hydrocarbon tails of lipids. Moreover, these figures indicate that the cholesterol molecules do not significantly alter the distribution position of the Ibu molecules along the membrane normal. It is seen that increasing the cholesterol concentration increases the distance between the maxima (head to head spacing) of bilayer and thickens it. The extension of the flat regions between the peaks and the minimums of the density profiles together with the reduction of the density of the middle of the bilayer indicates the ordering of bilayer with increasing the cholesterol concentration [50].

3.3. Order parameter

The chain conformational flexibility of lipid can be characterized by order parameter profiles, which in NMR experiments and computational studies are determined by measuring the deuterium order parameters S_{cd}. S_{cd} is defined as:

$$S_{cd} = \left\langle \frac{3}{2}\left(\cos^2\theta\right) - \frac{1}{2} \right\rangle \quad (5)$$

Table 2
The average area per lipid (A_{DMPC} and A_{Chol}), the average thickness (h) of the lipid bilayers, and the volume per DMPC (V_{DMPC}) evaluated for studied simulation systems: System 1 (0 mol% Chol), System 2 (25 mol% Chol), and System 3 (50 mol% Chol).

Systems	A_{DMPC} (nm^2)	A_{Chol} (nm^2)	h (nm)	V_{DMPC} (nm^3)
1	0.63 (0.01)	–	3.40 (0.06)	1.06 (0.01)
2	0.54 (0.01)	0.30 (0.00)	3.94 (0.06)	1.06 (0.01)
3	0.51 (0.01)	0.29(0.00)	4.11 (0.04)	1.04 (0.01)

where θ is the angle between the CD bond and the bilayer normal, and the angular brackets indicate the ensemble averaging over time and over all CD bonds. The computed order parameters of the DMPC Sn-1 and Sn-2 acyl chains are shown in Fig. 6. From this figure it can be concluded that increasing the cholesterol content in the bilayer increases ordering of the hydrocarbon chains of the DMPC lipid. This ordering effect of the cholesterol can be due to relatively rigid structure of the cholesterol molecules and also various types of interactions such as hydrogen bonding with the DMPC molecules [17,18]. This figure indicates that incorporation of Ibu molecules leads to a noticeable ordering effect of both acyl chains of DMPC lipids in pure bilayer. Previous studies showed that the presence of various drugs in the phospholipid bilayer can induce different effects on the order parameters of the lipid tails. For example inclusion of articaine [7], pyrene [16] and 5-fluorouracil [52] into the membrane leads to an increase of the order parameters while benzocaine [55] and prilocaine [56] cause a decrease of the order parameters. In this study accumulation of the Ibu molecules in the region of acyl chains forces them to be slightly more ordered. However the presence of Ibu molecules in the membrane with 50% cholesterol content, which is in the liquid order state, induces a chain disordering of DMPC lipids. The low free volume in this region of bilayer may cause the presence of the Ibu molecules to perturb the flexible chains of DMPC lipids and lead to a local reduction of the acyl chain order parameter. Such perturbation effects of compound presence in the bilayers containing cholesterol, were in the liquid order state, have been reported in both computational [57] and experimental observations [58–60], which can be related to the lipid molecules' dynamics and conformal perturbation due to the presence of other molecules.

3.4. Mean square displacement (MSD)

Computing the mean square displacement (MSD) is a common way to evaluate the movements of molecule inside the bilayer. MSD can be obtained from the MD trajectories by using the following equation:

$$\mathrm{MSD}(t) = \left\langle [r(t+t_0) - r(t_0)]^2 \right\rangle \tag{6}$$

where r is a vector defined by the center of mass of a molecule and the angle bracket denotes averaging over all possible initial times t_0 and molecules of the given type. The MSD profiles of the DMPC and the drug molecules along the z-axis with different cholesterol concentrations in the bilayer are displayed in Figs. 7 and 8. Fig. 7 reveals that the lateral motion of the DMPC molecules in the membrane was decreased with increasing the cholesterol content in the membranes. This is related to condensing and ordering effects of cholesterol on the lipid bilayer. The minimum MSD of the drug molecules is in the membrane with 25 mol% cholesterol which might correspond to the point of percolation for the L_d phase in the $L_d + L_o$ coexistence region. However, in the membrane with 50 mol% cholesterol, the movement of DMPC molecules is low, and the presence of high free volume caused the free motion of Ibu molecules, especially at the hydrocarbon tails of lipids where the Ibu molecules were located.

3.5. Tilt angle

Computing the average tilt angle of the lipid molecules with respect to the bilayer normal can provide valuable information about the effects

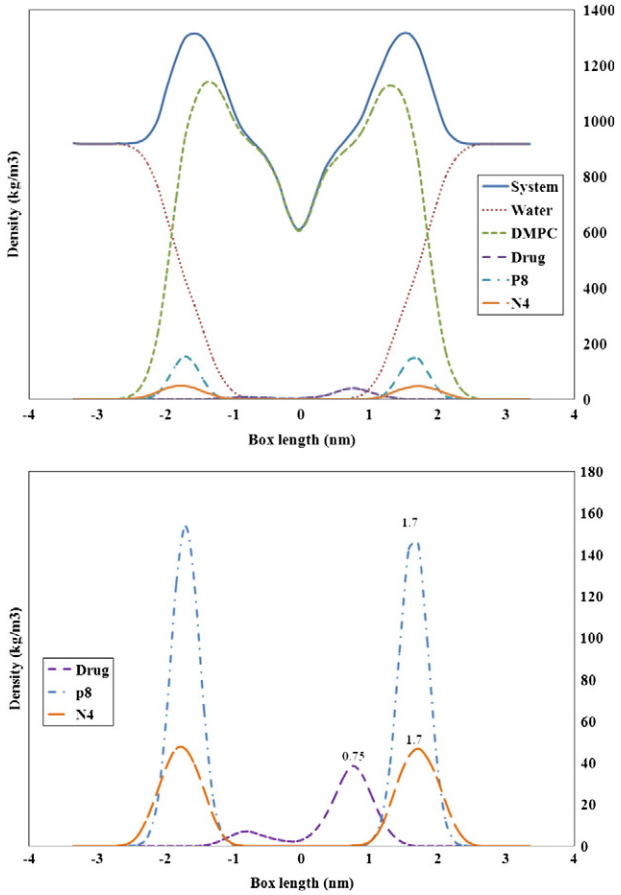

Fig. 3. Density profile of the Ibu in the DMPC lipid bilayer.

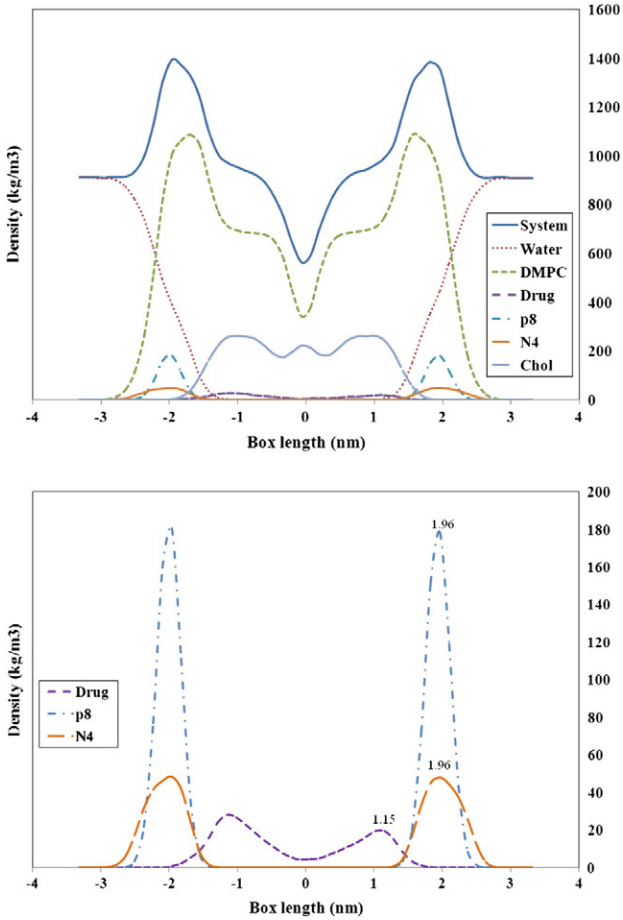

Fig. 4. Density profile of the Ibu in the DMPC bilayer with 25% cholesterol.

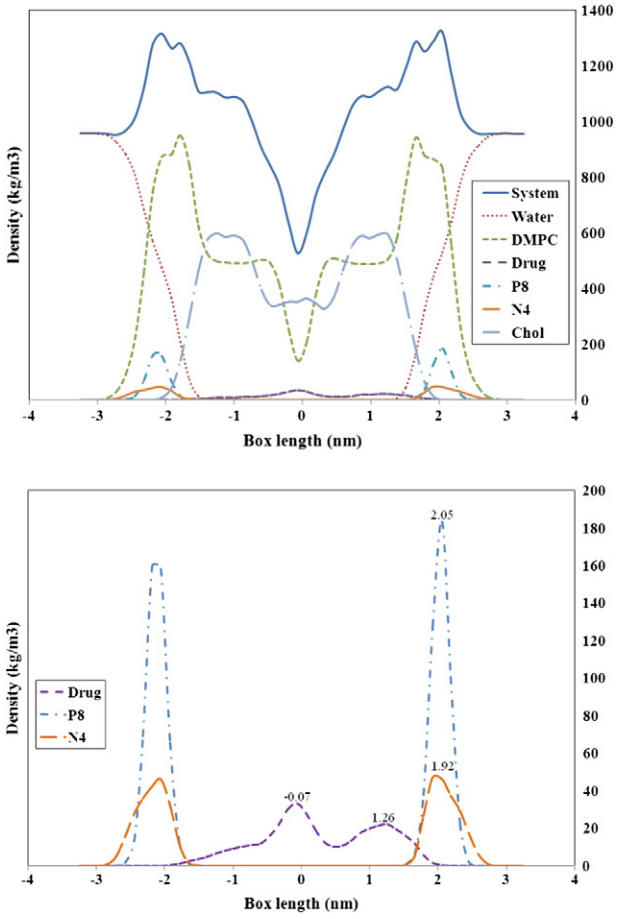

Fig. 5. Density profile of the Ibu in the DMPC bilayer with 50% cholesterol.

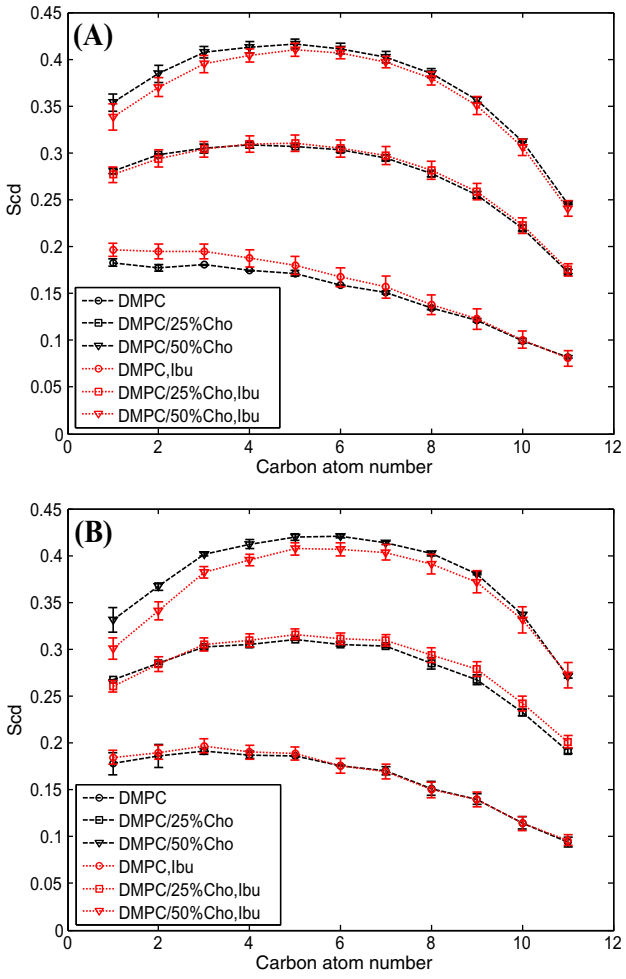

Fig. 6. Deuterium order parameter S_{cd} calculated for the Sn-1 (A) and Sn-2 (B) of DMPC acyl chains in the simulated systems.

of drug insertion on the orientation of lipid chains. The angles between the bilayer normal and the vectors connecting atoms P8–N4, C29–C17 and C46–C34 were defined as the tilt angles of the headgroup and Sn-1 and Sn-2 acyl chains of the DMPC, respectively. The ring part and side-chain tilt angles of cholesterol were defined as the angles between the vectors connecting C21–C5 and C27–C21 of the cholesterol molecule and the bilayer normal. An angle of 0° corresponds to the vector aligned parallel to the bilayer normal, while an angle of 90° corresponds to the vector perpendicular to the bilayer normal. The average tilt angles for various parts of lipids in the membrane with different cholesterol concentrations in the presence and absence of the drug molecules are presented in Table 3. As seen in this table with increasing the cholesterol concentration in the membrane, the DMPC tails tilt angles and cholesterol tilt angles decrease which imply that the lipids have more orientation order and as a result the bilayer is more packed. Moreover, the results of this table indicate that the presence of Ibu molecules in the bilayer without cholesterol content decreases the average tilt angles which can be related to the compression of lipids and reduction of the in-plane spanning. For the membrane with 50 mol%, insertion of Ibu molecules causes an increase in the tilt angles of DMPC tails. This is in agreement with the obtained results by order parameter analysis as mentioned in Section 3.3. The orientation of Ibu molecules in a bilayer was measured by calculating the angles between the vectors connecting CAH and CAE of the Ibu molecule and the bilayer normal. The average values of this angle are 68.75, 54.90 and 49.96 for Ibu molecules in the membranes with 0, 25, and 50 mol% cholesterol, respectively. It is indicated that with increasing the cholesterol concentration in the membrane, the average tilt angles of Ibu decrease which implies that the drug was aligned parallel to the bilayer normal. This can be explained by increasing the ordering of the lipids and decreasing the space and compression of lipids due to the presence of cholesterol molecules which cause lipid chains forcing drugs to be slightly more ordered.

3.6. Free energy analysis

The free energy profiles calculated by the potential of mean force for transferring an Ibu molecule from the bulk water to the center of the DMPC bilayer containing various cholesterol concentrations are shown in Fig. 9. As seen in this figure the free energy decreases from the water to the inside of the bilayer which is attributed to the transfer of Ibu molecule as a hydrophobic drug from the water to the hydrophobic region of bilayer. A similar decrease of the free energy for transferring the Ibu molecule from the water to the middle of phospholipid bilayer was reported in other PMF studies [9,10]. As indicated in these studies [9,10] low polarity of ibuprofen molecules causes a low Coulomb interaction between drug and water or drug and polar groups of lipids, and also the number of hydrogen bonding between Ibu and water and lipids is negligible, especially in deeper part of bilayer. For these reasons, there aren't large energy barriers to overcome when passing Ibu molecule from the water to the middle of phospholipid bilayer. Previous studies [47,51,52] showed that by transferring hydrophilic drugs from the

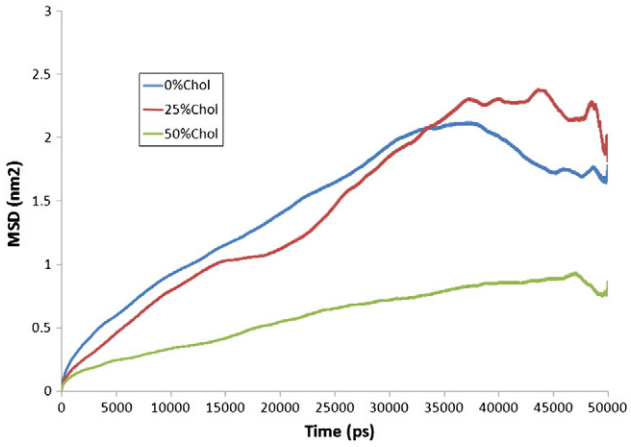

Fig. 7. The mean square displacement of the DMPC lipids in the simulated systems.

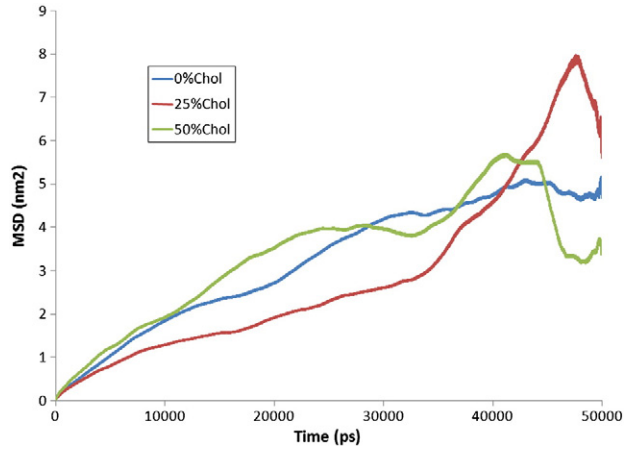

Fig. 8. The mean square displacement of the Ibu in the simulated systems.

water into the bilayer, the free energy increases in different manner compared to the Ibu molecule. This increase is proportional to the size and hydrophobicity of the solute [47]. A comparison of free energy profiles shown in Fig. 9 indicates that the magnitude of the free energy change for transferring of Ibu molecule from the water to the bilayer with 50 mol% cholesterol, is about 25 kJ/mol, which is lower than that of the bilayers with 0 and 25 mol% cholesterol, which is about 55 kJ/mol. The direct relation between drug partitioning into the membrane and the free energy change for transferring of drug molecule from the water to the membrane [9,47] suggests that partitioning of Ibu molecule in the bilayers with 0 and 25 mol% cholesterol is higher than partitioning in the bilayer with 50 mol% cholesterol. In the DMPC membrane with 50 mol% cholesterol the lipid tails are highly ordered and the favorable van der Waals interactions are formed between the nearby cholesterol and other lipid tails [47]. Therefore, upon the insertion of Ibu molecule, strong van der Waals contacts are broken and large voids are formed above and below the drug molecule. Whereas in the DMPC membrane with 0 and 25 mol% cholesterol, low ordering of the lipid tails and the weak interactions facilitate the Ibu molecule transfer into the membrane. The PMF profiles indicate that an Ibu molecule for penetration into the membrane should pass a free energy barrier located in the middle of the bilayer. The height of this barrier can be measured as the difference between the absolute free energy minimum and the maximum of free energy in the middle region of bilayer which are 8.9, 3.7 and 14.3 kJ/mol for membranes with 0, 25, and 50 mol% cholesterol. In a previous experimental study [61], it is shown that incorporation of cholesterol to the DMPC bilayer increases the hydrophobicity of the membrane interior, and increases the hydrophobic barrier to the permeation of the polar molecules. With regard to nonpolarity of Ibu molecules and its hydrophobic nature, incorporation of 25 mol% cholesterol to the DMPC bilayer doesn't alter its barrier to the Ibu permeation, while for membrane with 50 mol% cholesterol the barrier was increased significantly. This increasing for membrane with 50 mol% cholesterol can be related to the existence of membrane in the liquid order state and high ordering and compactness of lipids in the membrane. Therefore the values of free energy barriers indicate that the existence of phospholipid bilayer in the liquid order state slows down the Ibu or other hydrophobic molecules transport across the membranes and decrease their permeability.

4. Conclusion

In this work, molecular dynamics (MD) simulations were carried out to investigate the behavior of ibuprofen as a hydrophobic nonsteroidal antiinflammatory drug in fully hydrated 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayer with varying amounts of cholesterol (0, 25 and 50 mol%). Several analyses were performed to study the precise location of the ibuprofen molecules inside the membrane and how it alters the structural and dynamic properties of the membrane and also the effects of the cholesterol. The results indicated that ibuprofen molecules were preferentially accumulated in the hydrophobic acyl chain region of the lipid bilayers which causes packing and ordering of lipids decreasing their lateral motion. However, for bilayer with 50 mol% cholesterol, the presence of ibuprofen molecules in the bilayer induces some disordering in the DMPC lipid tails. The free energy profiles for the permeation of the ibuprofen molecule across the DMPC/Chol membranes were determined by the potential of the mean force (PMF) method. The free energy for transferring of Ibu molecule as a hydrophobic drug from the water to the middle of bilayer decreases. From the free energy profile, it was concluded that the existence of the 50 mol% cholesterol in the bilayer increases the free energy barrier and slows down the translocation of the ibuprofen molecule across the DMPC bilayer.

Table 3
The average tilt angles evaluated for lipid segments in studied simulation systems in the presence and absence of drug molecules: System 1 (0 mol% Chol), System 2 (25 mol% Chol), and System 3 (50 mol% Chol).

Systems	P–N angle	Sn-1 angle	Sn-2 angle	Chol ring part angle	Chol side chain angle
1 without drug	80.90 (12.92)	33.88 (6.15)	36.45 (6.63)	–	–
2 without drug	80.65 (18.69)	21.54 (4.46)	22.23 (5.01)	21.82 (2.75)	28.50 (3.69)
3 without drug	81.15 (14.86)	11.82 (1.68)	12.84 (1.89)	11.64 (1.30)	19.54 (1.71)
1 with drug	80.08 (8.09)	32.48 (2.54)	33.92 (2.87)	–	–
2 with drug	80.45 (10.65)	21.41 (3.34)	22.75 (2.71)	19.95 (1.75)	29.56 (2.36)
3 with drug	81.45 (7.59)	13.16 (1.03)	13.02 (1.09)	11.76 (1.26)	20.35 (1.71)

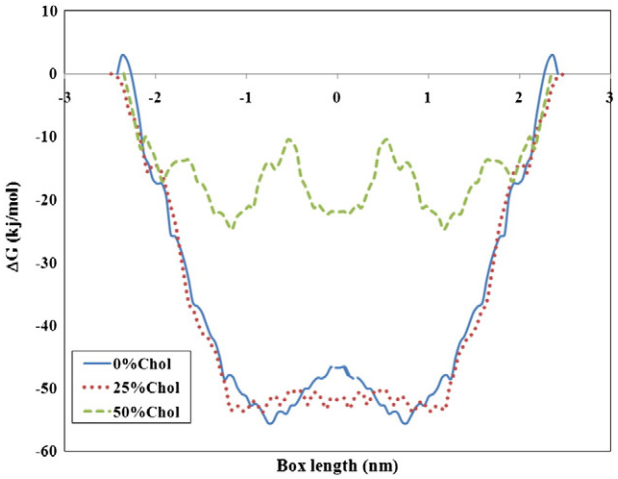

Fig. 9. Free energy profiles of Ibu across the DMPC/Chol membrane at different cholesterol concentrations.

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