Biophysical Chemistry 187–188 (2014) 43–50

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Effect of cholesterol on behavior of 5-fluorouracil (5-FU) in a DMPC lipid bilayer, a molecular dynamics study

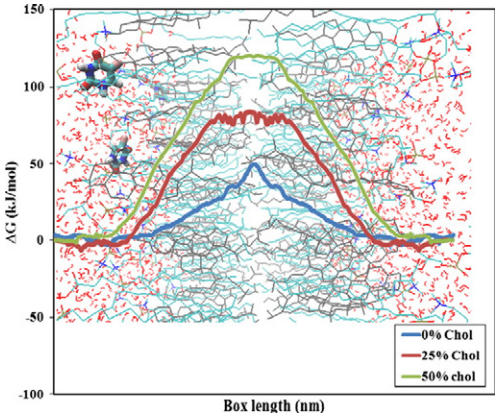
Aboozar Khajeh, Hamid Modarress *

Department of Chemical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Hafez Avenue, 15914 Tehran, Iran

HIGHLIGHTS

- Behavior of 5-FU in DMPC/cholesterol membrane was investigated by MD simulations.
- The free energy barriers for translocation of 5-FU were determined by PMF method.
- The 5-FU molecules were accumulated in the vicinity of hydrophilic part of bilayers.
- The minimum and maximum of barriers were independent of cholesterol concentration.
- The 5-FU translocation barrier enhanced with increasing membrane cholesterol.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 18 January 2014
Accepted 25 January 2014
Available online 3 February 2014

Keywords:
5-Fluorouracil (5-FU)
Cholesterol
DMPC bilayer
Molecular dynamics (MD)
Potential of mean force (PMF)
Permeation

ABSTRACT

In this work, molecular dynamics (MD) simulations were performed to investigate the effects of cholesterol on the interaction between the hydrophilic anticancer drug, 5-FU, and fully hydrated 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayer. Several structural and dynamical parameters of DMPC bilayers with varying amounts of cholesterol (0, 25, and 50 mol%) in the presence and absence of drug molecules were calculated. Moreover, the free energy barriers for translocation of one 5-FU molecule from water to the lipid bilayer were determined by using the potential of mean force (PMF). PMF studies indicated that the location of the maximum free energy barrier was in the hydrophobic middle region of bilayer, while the minimums of the barrier were located at the hydrophilic part of bilayer at the interface with water. The minimum and maximum of the free energy profiles were independent of cholesterol concentration and suggested that the drug molecules 5-FU were accumulated in the vicinity of the polar head group of lipid bilayers. Moreover, the results showed that with increasing cholesterol concentration in the bilayer, the free energy barrier for translocation of 5-FU across the bilayer also increases which can be attributed to the condensing effect of the cholesterol on the bilayer.

1. Introduction

Cholesterol is one of the most common lipid species which heterogeneously is distributed in a concentration range from 20 to 50 mol% among cellular membranes and modulates their physicochemical properties [1–3]. Cholesterol is made up of a semi-rigid hydrophobic steroid ring, a polar hydroxyl group, and a short flexible hydrocarbon chain [3,4]. The molecular interactions between the cholesterol and phospholipids and sphingolipid bilayers can greatly affect the structural stability, fluidity and permeability of a membrane [3–6]. Numerous experimental and computational studies over the past several decades have been carried out to investigate the effects of cholesterol on the properties of lipids such as their structure, dynamics and their

* Corresponding author. Tel.: +98 21 64543176.
E-mail address: hmodares@aut.ac.ir (H. Modarress).

0301-4622/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bpc.2014.01.004

molecular interactions within the membranes [7–12]. These studies indicated that cholesterol interacts favorably with the saturated lipids compared to the unsaturated lipids and therefore the effects of cholesterol on the saturated lipids are more evident [3,13,14]. In the absence of cholesterol in lipid bilayers two phases can exist [15]: the phase which exists in the lower temperature than the transition temperature is called the gel or solid-ordered phase and the phase which exists in the upper temperatures than the transition temperature is called liquid-crystalline or liquid-disordered phase. Increasing the amount of cholesterol in the lipid bilayers produces an additional phase besides these two phases which is called the liquid-ordered phase [5,15,16]. In this phase the lipids are translationally disordered and conformationally ordered [14]. The rigid sterol rings of cholesterol, as were observed by calorimetry [16], NMR spectroscopy [17] and X-ray diffraction [18] cause the ordering and condensing of the lipid bilayer in the liquid-ordered phase. As a result of ordering effect by cholesterol, the lipids exhibit a higher directional order in the hydrophobic chain and a reduction in their fluidity [1,3]. The cholesterol condensing effect reduces the surface area of a cholesterol-containing lipid bilayer to less than the sum of areas of the individual bilayer components [19]. The high degree of orientation order and packing density of the lipid bilayers in the liquid-ordered phase increase the mechanical strength of the membrane and decrease its lateral motion [3].

Molecular dynamics (MD) simulations have proven to be extremely useful in the study of cholesterol-containing lipid bilayers by employing all atom, united atom and coarse-grained molecular models [20–23]. The MD simulation can be beneficial as a supplementary of the experimental results to provide knowledge for interpretation of the interactions at molecular-level for the studied system. The MD simulations are particularly utilized to study the distribution, orientation, partitioning and permeation of various drugs across the lipid bilayer membranes [24–26]. Several articles have been published over the past decade for investigating the effect of type, composition and other physicochemical properties of the lipid and drug on the membrane properties [26–30]. These articles have been focused on exploring the precise location of the drug molecules inside the membrane and how it alters the structures of the membrane, in addition to investigating the free energy and diffusion variations of the drug and its dependence on the membrane characteristics and how the local resistance of the membrane affects the drug penetration. Despite enormous growth in the number of works on MD simulations of lipid bilayer/drug systems, the studies on the partitioning of drugs in various types of lipid bilayers containing cholesterol are rather scant. Most studies in this area are concerned with simulation of small solutes without drug applications [1,31,32] or for a limited number of drugs and lipid bilayer containing cholesterol [14,33]. Therefore, considering the important role of cholesterol in biological membranes and liposomal drug delivery systems, MD simulations can be used as an effective tool to understand the behavior of various drugs in the lipids/cholesterol systems at a molecular level.

5-Fluorouracil (5-FU, 5-fluoro-2,4-pyrimidinedione, Efudex®) is one of the most widely prescribed drug in cancer treatment of several types including colon, stomach, breast, and skin cancers [34,35]. This drug is water soluble and is known as an antimetabolite of the pyrimidine analog type [35,36]. It exerts its anticancer effect through inhibiting the normal production of the pyrimidine thymidine, which is a nucleoside required for RNA and DNA replication [37,38].

The aim of this work is to use molecular dynamics (MD) simulations to study the interaction of 5-FU as a hydrophilic drug with the dimyristoylphosphatidylcholine (DMPC)/cholesterol mixed membranes. The effects of wide range concentration of cholesterol in the presence of 5-FU on DMPC bilayer in three phases including liquid-ordered, liquid-disordered and the coexistence phases were determined by calculating various structural properties of the bilayers such as the area per lipid, deuterium order parameters and density distributions. Moreover, to study the permeation of a 5-FU molecule from water to the interior of a lipid bilayer, by using umbrella sampling method, the potential of mean forces (PMF) was computed. The PMF provides valuable information about the preferred location of the drug molecule in the bilayer and the main energy barriers in translocation of the drug, across the membrane, with different cholesterol concentrations. The results of this work can be used to understand the therapeutic properties of the 5-FU drug as a common anticancer and exploring the role of cholesterol in the liposomal drug delivery systems of this drug, as well as its penetration and retention into the cellular membranes.

2. Simulation methods

Molecular dynamics (MD) simulations were performed using the GROMACS v5.0.4 software package [39–41] on hydrated DMPC/cholesterol mixtures with 0%, 25% and 50% cholesterol (Chol) concentrations. The bilayers containing 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 [42,43]. The pure lipid bilayer consists of 128 DMPC lipids which 64 lipids were arranged in each leaflet. For the creation of the bilayer with 25% cholesterol, 32 DMPC lipids in each leaflet were randomly selected and replaced with the cholesterol molecule. In the bilayer with 50 mol% cholesterol where the concentration of cholesterol was high, to prevent cholesterol clustering the 64 DMPC lipids in each leaflet were sequentially selected and replaced with the cholesterol molecule. In both bilayers the substitution was performed for the location of cholesterol hydroxyl groups at approximately the same distance from the bilayer center as the ester carbonyl groups of the DMPC were located. The initial simulated position of molecules in the bilayers with 25 and 50 mol% cholesterols is shown in Fig. 1. This figure indicates that there is no or few cholesterol clustering in the bilayers which is consistent with the results obtained by the study of Dai et al. [44] who showed that cholesterol clusters in phosphatidylcholine (PC) bilayers are very unstable and readily disperse into cholesterol monomers. Moreover they stated that the cholesterols have no tendency to forming clusters in lipid bilayers with regard to unfavorable cholesterol–cholesterol interaction [44]. In addition, each of these systems also contained 4 neutral 5-FU molecules and an appropriate amount of water molecules have been added using tools included in the GROMACS package. The number of water and lipid molecules in the simulation systems is shown in Table 1. The Berger et al. [45] force field parameters were used for the phospholipid molecules and the force field parameters for the cholesterol were based on the work of Holtje et al. [46]. For water molecules, the simple point charge (SPC) [47] model was applied. The topology files for the neutral form of 5-FU molecule with the interaction parameters corresponding to the GROMOS 43A1 force field were obtained from the PRODRG server [48]. The atomic charges for the drug molecule (5-FU) were recalculated at the Hartree–Fock 6-31G* level by using Spartan software (Wavefunction, Irvine, CA). The structures of DMPC, cholesterol and 5-FU are depicted in Fig. 2.

To eliminate any bad contacts between the atoms, all initial structures went through energy minimization using the steepest descent algorithm. Then, after equilibrating the system at constant volume in a NVT ensemble, a simulation at constant pressure ($P = 1$ bar) and temperature ($T = 323$ K) was performed. Upon completion of the two equilibration phases, for data collection a 40 ns MD simulation was performed with three-dimensional periodic boundary conditions in the NPT ensemble for each system. First 20 ns of simulation time was performed for bilayer equilibration and the second 20 ns was performed after adding drug molecules to the systems. The temperature was set to 323 K, using the Nose–Hoover thermostat scheme [49] with a coupling time constant of 0.5 ps. The system pressure is regulated by means of the Parrinello–Rahman barostat [50] with a coupling time constant of 1.0 ps. All bonds in the system were constrained by using the LINCS algorithm [51], to make it possible to use a longer time step. It is reported that at a time step up to 4 fs the LINCS algorithm does not deteriorate the energy conservation [40], therefore we used a time step of 2 fs to

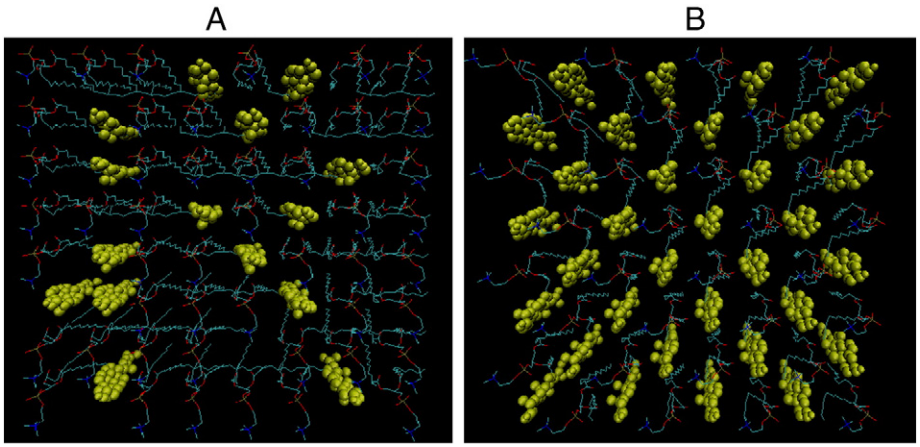


Fig. 1. The initial snapshots of the cholesterol distribution in bilayers (A) 25 mol% and (B) 50 mol%. The molecules are shown as: DMPC (line) and cholesterol (van der Waals spheres).

assure the accuracy of the results. The long-range electrostatic interactions were estimated using the Particle-Mesh-Ewald summation method [52] with a direct space cutoff of 1.2 nm and a grid spacing of 0.16 nm. Fig. 3 shows snapshots from the production simulations of four 5-FU molecules in membranes containing 0, 25 and 50 mol% cholesterol.

In order to examine the interactions between a hydrophilic drug and membranes containing cholesterol and its effect on partitioning and permeation of drug, the free energy profile of a 5-FU drug molecule from bulk water to the interior of a DMPC lipid bilayer was calculated by using the potential of mean force (PMF) method. The center of mass of 5-FU was constrained at the z distance between the center of mass (COM) of the drug and DMPC bilayer (along the membrane normal direction), and the drug 5-FU was allowed to rotate and translate freely in the x–y plane. The constraint force, F_z, acting on the drug molecule at a particular location z along the bilayer normal was collected and then the free energy was calculated by integrating between z_i and z_f as follows:

$$\Delta G = G_{z_f} - G_{z_i} = -\int_{z_i}^{z_f} \langle F_z \rangle_z dz \tag{1}$$

where $\langle F_z \rangle$ is the mean force acting on the drug at a particular location z along the bilayer normal, z_i represents the normal position of drug at the interface of bilayer with the bulk water and z_f represents the normal position of drug at the middle of the bilayer. In this work the potentials of mean force (PMFs) were calculated using the umbrella sampling (US) method [53]. The drug molecule was placed in the bulk water, and then it was pulled into the DMPC bilayer along the z-direction using a harmonic spring with the force constant of 2000 kJ/(mol nm^2) and a pulling rate of 0.01 nm/ps. From the obtained trajectory the frames with distance change of ~0.2 nm between the center of mass of bilayer and the 5-FU drug molecule were extracted. For each system, 13 z-locations or frames of the 5-FU molecule per monolayer ranged from the bulk water to the middle of the bilayer were explored. In each frame, which is referred as a window, the z distance between the COM of the drug and the bilayer was constrained using a biasing harmonic potential with a force constant of 2000 kJ/(mol nm^2). Each window was simulated for 4 ns and based on the results obtained for all 13 simulations, the weighted histogram analysis method (WHAM) [54,55] was used to obtain the potential of mean force (PMF) profile across the monolayer. In several studies [30,32,56,57] it is shown that this simulation time is sufficient for equilibration and accurate in calculating the PMF profiles. With regard to the symmetric structure of bilayer, the PMF profile for half part of bilayer, or one monolayer, can be applied to another half part.

Table 1
Volume of simulation box V (nm^3) number of water, DMPC and cholesterol in the simulation systems: system1 (0 mol% Chol), system2 (25 mol% Chol), system3 (50 mol% Chol).

	System 1	System 2	System 3
V (nm^3)	278.7193	206.6977	170.8899
N_{H20}	4542	2760	2129
N_{DMPC}	128	96	64
N_{Chol}	0	32	64

3. Results and discussion

3.1. Area per lipid

The area per lipid is one of the most fundamental characteristics for describing the state of molecular packing within a lipid bilayer. The average area per lipid for different molecules in the bilayer containing cholesterol can be obtained by using the following equations [58]:

$$A_{DMPC} = \frac{2A}{N_{DMPC}}\left(1 - \frac{N_{Chol}V_{Chol}}{V - N_w V_w}\right) \tag{2}$$

$$A_{Chol} = \frac{2A - N_{DMPC}A_{DMPC}}{N_{Chol}} \tag{3}$$

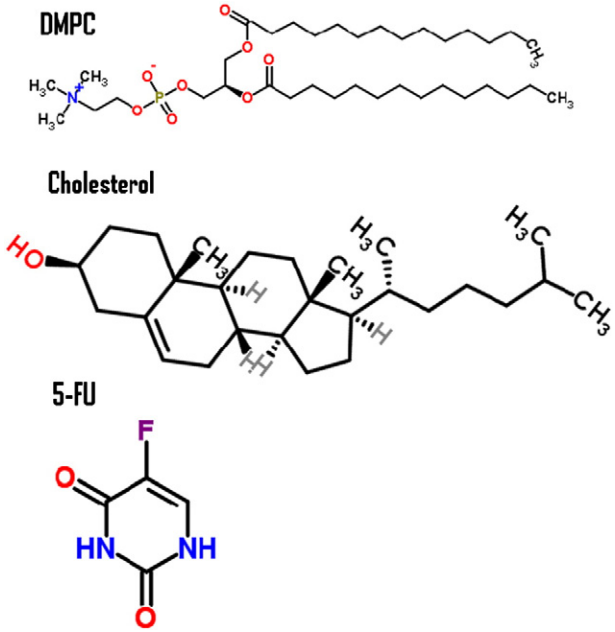


Fig. 2. Molecular structures of DMPC lipid, cholesterol and 5-FU.

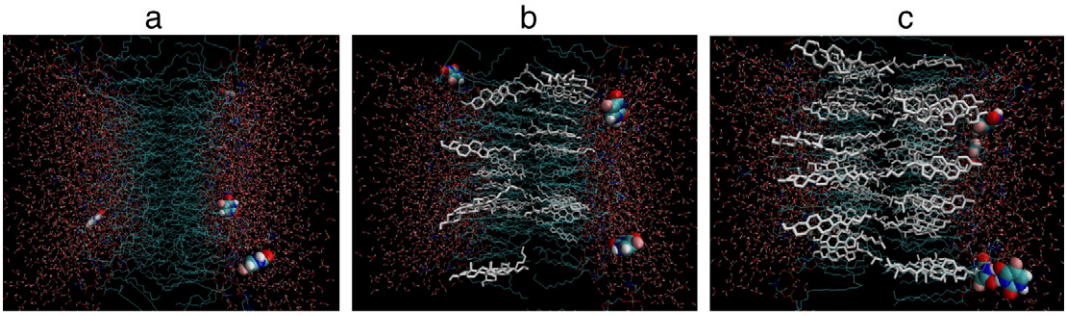


Fig. 3. Snapshots of the 5-FU molecules in (a) 0 mol%, (b) 25 mol% and (c) 50 mol% cholesterol membranes. The molecules are shown as: water, white and red lines; DMPC, cyan lines; cholesterol, white cylinders; 5-FU, van der Waals spheres.

where A_{DMPC} and A_{Chol} are the average area per DMPC lipids and cholesterol lipids, respectively. A is the area of simulation box. N_{DMPC} stands for the number of DMPC lipids in the simulation box, and V is the volume of the simulation box. N_w is the number of water molecules, and N_{Chol} is the number of cholesterols. V_w is the volume of a water molecule and V_{Chol} is the volume of a cholesterol molecule. The volume of a cholesterol molecule, V_{Chol}, is 0.593 nm^3 and that of water, V_w, is 0.0305 nm^3 [12].

The average thickness (h) of the lipid bilayer was calculated by the following equation:

$$h = \frac{V - N_w V_w}{A}. \quad (4)$$

The area per lipid is a proper criterion for evaluating the equilibration and the stability of bilayer simulations. The time evolution of the area per lipid for membranes containing various cholesterol concentrations in the presence of 5-FU over the last 10 ns of simulation time was calculated and displayed in Fig. 4. No significant fluctuations are observed in Fig. 4 in the area per lipid profiles, which justifies the equilibration of the simulated systems. The average values for the area per lipid and bilayer thickness for different systems are presented in Table 2. The values of the area per lipid and bilayer thickness computed for pure DMPC reported in Table 2 are fairly close to the obtained values in the X-ray diffraction experiment, 60.6 nm^2 and 3.55 nm, respectively [59]. As it is seen in Table 2, with increasing cholesterol concentration in the bilayer the area per lipid decreases while the bilayer thickness increases. This is known as condensing effect of cholesterol on lipid bilayers as has been reported in other simulation and experimental studies on the hydrated lipid bilayer [9,19,60]. The condensing effect of

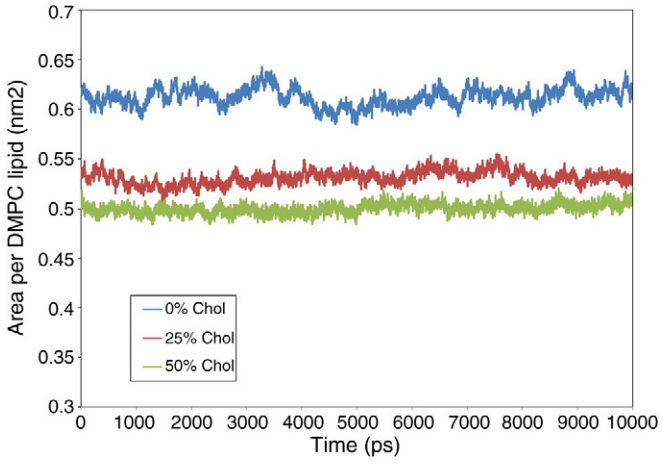


Fig. 4. The time evolution of the area per DMPC lipid for the simulated systems.

Table 2
The average area per lipid (A_{DMPC} and A_{Chol}), the average thickness (h) of the lipid bilayers, free energy (ΔG) and lateral diffusion coefficient of drug evaluated for studied simulation systems: system1 (0 mol% Chol), system2 (25 mol% Chol), system3 (50 mol% Chol).

Systems	A_{DMPC} (nm^2)	A_{Chol} (nm^2)	h (nm)	ΔG (kJ/mol)	D_{lat} (10^5 cm^2/s)
1	0.6123	–	3.5769	52.8973	0.3174
2	0.5317	0.2923	4.0578	90.3768	0.1694
3	0.5006	0.2794	4.2446	122.6103	0.0562

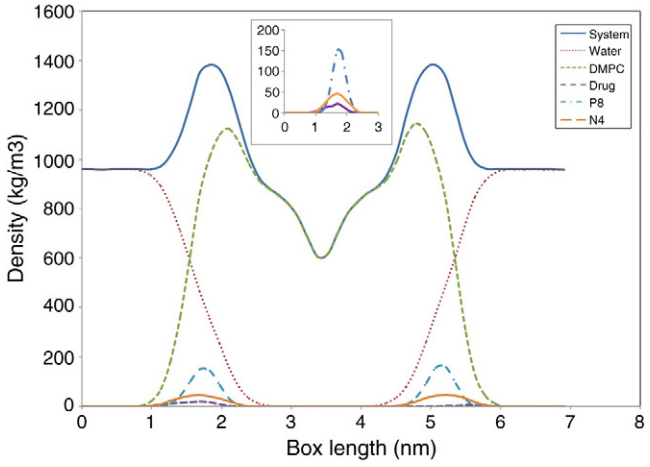


Fig. 5. Density profile of the 5-FU in the DMPC lipid bilayer.

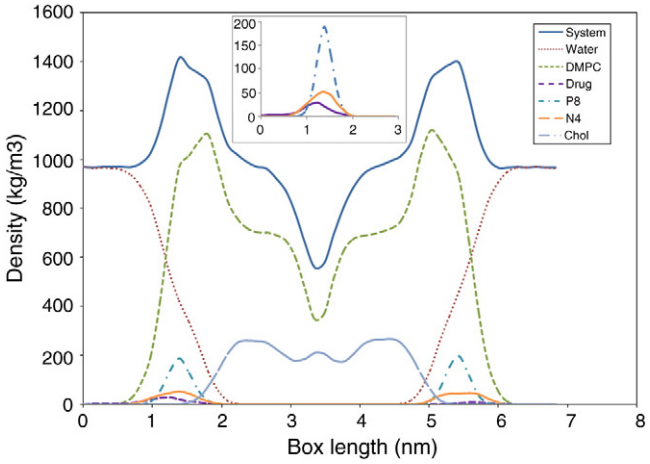


Fig. 6. Density profile of the 5-FU in the DMPC bilayer with 25% cholesterol.

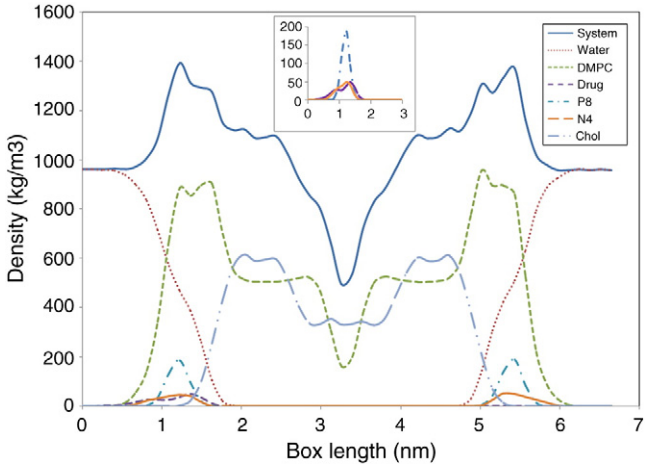


Fig. 7. Density profile of the 5-FU in the DMPC bilayer with 50% cholesterol.

cholesterol on the lipid bilayers is related to the rigid structure of cholesterol which causes the compression and ordering of the chains of lipid molecules [19,60].

3.2. Mass densities

In order to determine the influence of cholesterol on the distribution of the 5-FU drugs in the lipid bilayer, the mass density profiles were calculated for all components of the simulated systems and were shown in Figs. 5–7. These figures indicate that for all studied systems, the 5-FU molecules predominately are located in the more dense region of the membrane, close to the polar lipid head groups. Due to the hydrophilic nature of the 5-FU molecules, no diffusion occurs into the apolar center of the bilayer and as these molecules are attracted by the polar head groups of bilayer which are located at the membrane–water interface. Therefore 5-FU molecules form strong hydrogen bonds with the head groups of the lipid molecules and water. Moreover, these figures indicate that the cholesterol molecules do not alter the 5-FU molecule distribution along the *z*-axis which is considered perpendicular to the bilayer. The asymmetric distribution of the drugs in two sides of the lipid bilayers, in Figs. 5–7, is due to the application of periodic boundary conditions and jumping of the drug molecule from one side of the lipid bilayer to the other side during the MD simulations. However, the bilayer thickness is substantially increased with the enrichment of cholesterol.

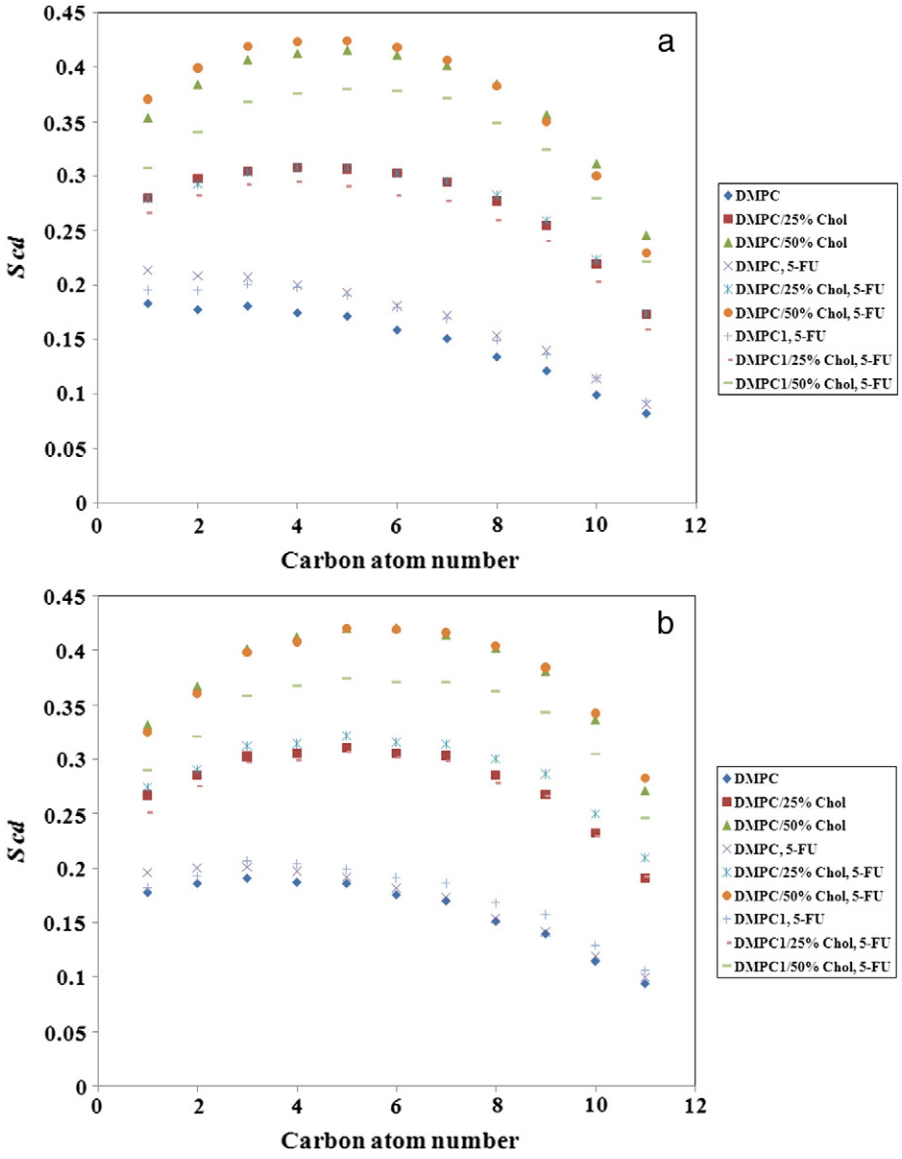


Fig. 8. Deuterium order parameter S_{cd} calculated for the sn-1 (a) and sn-2 (b) of DMPC acyl chains in the simulated systems.

3.3. Order parameter

Incorporation of cholesterol into phospholipid membranes can affect the ordering of lipid hydrocarbon tails. The ordering of hydrocarbon tails in bilayers is usually measured experimentally by the NMR spectroscopy, which can be quantified using deuterium order parameter, S_{cd}. The deuterium order parameter S_{cd} in computer simulations is defined as:

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

where θ is the angle between the carbon–deuterium (CD) bond and the bilayer normal (z-axis), and the angular bracket denotes an ensemble average over time and all CD bonds. In Fig. 8, the deuterium order parameters of the DMPC hydrocarbon chains for the leaflet inside the drug molecules extracted from simulations were plotted. A significant increase in ordering of the hydrocarbon chains of DMPC lipid can be observed with cholesterol content. The effect of cholesterol on ordering of the lipids is further visualized in Fig. 9. This could be due to the relatively rigid nature of the cholesterol molecules and also various types of interactions such as hydrogen bonding with the DMPC molecules which has been approved by experimental and simulation methods [9,60]. It is seen in Fig. 8 that the presence of the 5-FU molecules does not drastically alter the ordering of the lipid chains. This can be justified by accumulation of 5-FU molecules in the lipid–water interface of the membrane which as a result has no effect on hydrocarbon chains of the DMPC inside the bilayer. However, for bilayer without cholesterol the slight increase in the order parameters (S_{cd}) of carbon atoms of the acyl chains, as it is seen in Fig. 8, can be due to the 5-FU interaction with DMPC molecules. In order to examine the effect of deeper insertion of the 5-FU on the order parameter of the membranes, for each cholesterol concentrations (0, 25 and 50%), one of the 4 ns MD simulation in the umbrella sampling that the 5-FU was located at the middle of lipid tail was analyzed. The results are depicted as DMPC1 in Fig. 8 which indicate that the 5-FU induces a strong chain disordering in the membrane containing cholesterol, especially for the membrane with 50 mol% cholesterol which is in the liquid order state. This disordering can be related to the lipid molecules' dynamics and conformal perturbation due to the presence of 5-FU molecules in the membranes containing cholesterol. These perturbation effects of compound presence in bilayers have been reported in both computational [61] and experimental observations [62–64].

3.4. Lateral diffusion coefficient

The interactions between the cholesterol and various lipids may change the dynamic properties of a drug in the lipid membrane [6–11]. A common way to evaluate the dynamic properties of a drug is to calculate the mean square displacement (MSD) of the drug molecules. The lateral diffusion coefficient, D_{lat}, can be determined from molecular dynamics simulations through the Einstein relation by the slope of the average mean square displacement at long times:

$$D_{lat} = \lim_{t\to\infty} \frac{1}{4}\frac{d}{dt}\left\langle [r(t+t_0) - r(t_0)]^2 \right\rangle_{t_0} \tag{6}$$

where r is a vector defined by the center of mass of a molecule and the angle bracket denotes the averaging positions of molecules over all possible initial times t_0.

The MSD profiles for the 5-FU molecules along the z-axis with different cholesterol contents are displayed in Fig. 10. From this figure it can be concluded that by increasing the amounts of cholesterol in the lipid bilayer, the lateral motion of the drug molecules considerably decreases. The values of lateral diffusion coefficient for 5-FU in systems composed of 0%, 25%, and 50% cholesterol were [0.3174 ($\pm$0.0529), 0.1694 ($\pm$0.0744) and 0.0562 ($\pm$0.0165)] (10^5 cm^2/s), respectively. These values indicate that the lateral diffusion of 5-FU drug molecule decreases by a factor of 2–3 over the concentration interval covered in this study. This can be explained by the increase in the order, thickness, and rigidity of the bilayer due to the presence of cholesterol molecules.

3.5. Free energy analysis

The free energy profiles for transferring a 5-FU molecule from bulk water to the interior of the DMPC/Chol bilayer membrane, for several cholesterol concentrations, were calculated by using the potential of mean force as outlined in Section 2. The calculated free energy profiles are shown in Fig. 11 which indicates that, by moving the 5-FU molecule from the water/bilayer interface to the bilayer middle, the free energy increases. This increase in the free energy is attributed to the transfer of the hydrophilic drug 5-FU molecule from hydrophilic head of lipid

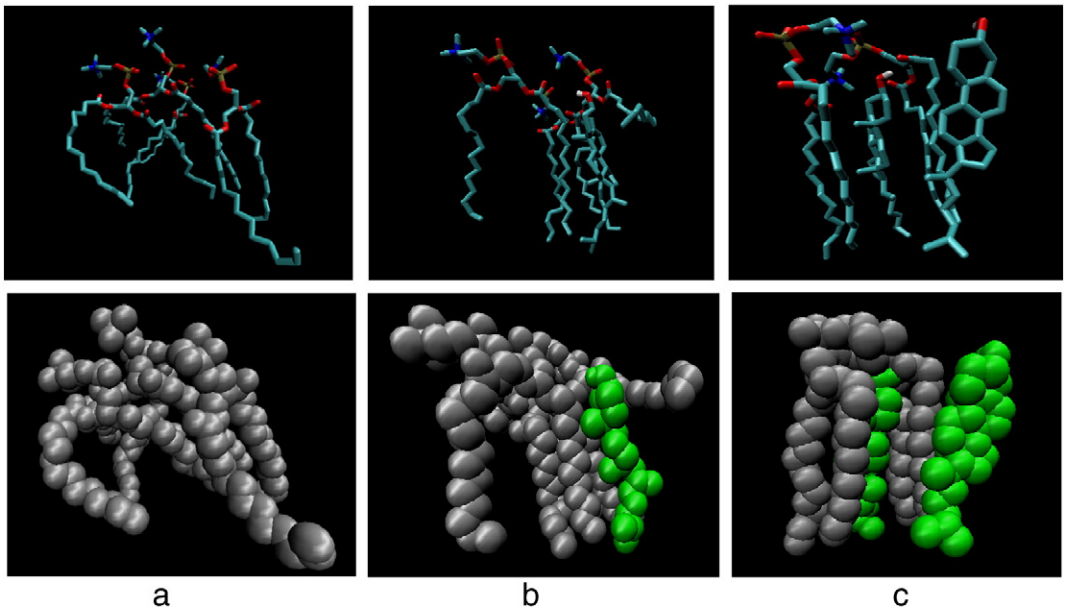


Fig. 9. Ordering of lipids in (a) 0 mol%, (b) 25 mol% and (c) 50 mol% cholesterol membranes.

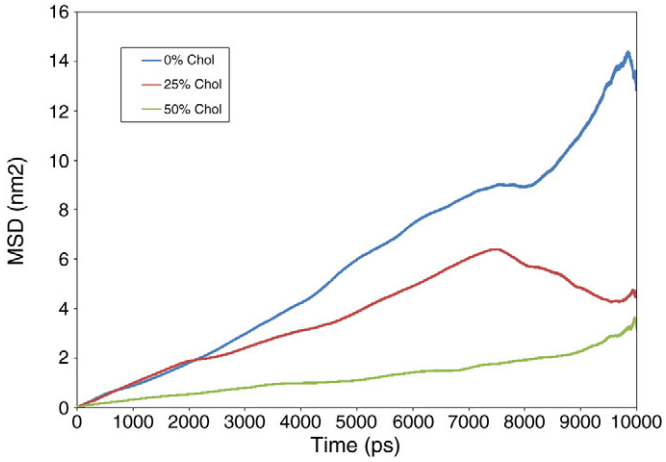


Fig. 10. The mean square displacement of the 5-FU in the simulated systems.

bilayer at the interface with water to the hydrophobic tail region of the bilayer. The low value of free energy in the polar head group region of the bilayer indicates that the 5-FU molecule is more likely to accommodate in this region of the bilayer, whereas the free energy high values in the middle of bilayer represent the lower tendency of the 5-FU molecule to diffuse into the hydrophobic part of the bilayer. This is in agreement with the analyses of mass density profiles shown in Figs. 5–7. Moreover Fig. 11 indicates that the free energy barrier of the DMPC/Chol membranes increases with the cholesterol concentration, which implies a decrease in the 5-FU transport across the membranes. Subczynski et al. [65] based on the experimental results explained that incorporation of cholesterol to the DMPC bilayer increases the hydrophobicity of the membrane interior, which increases the hydrophobic barrier to the permeation of the small polar molecules, including water. Therefore considering the result of our work for the bilayer with the cholesterol, it can be stated that the increase in the free energy barriers for permeation and translocation of 5-Fu as a polar hydrophilic molecule can be due to the increase in the hydrophobic barrier at the middle region of the bilayer and also the condensing effect of cholesterol on the membrane which has been justified by various analyses in Sections 3.1 to 3.4 and compared experimental studies [66]. Decreasing the permeation of the 5-FU and increasing the barriers suggest that incorporation of the cholesterol to the DMPC liposome-based drug carriers can increase the encapsulation of hydrophilic drugs into the carriers and decrease the leakage and release of drugs. The free energy for transferring the 5-FU molecule from water to the center of the bilayers with the 0%, 25%, and 50% cholesterol compositions is ~50 kJ/mol, 90 kJ/mol and

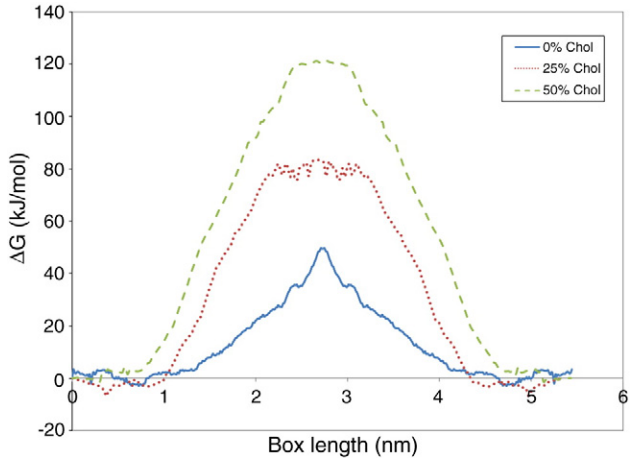


Fig. 11. Free energy profiles of 5-FU across the DMPC/Chol membrane at different cholesterol concentrations.

120 kJ/mol, respectively. The measured activation energy by NMR spectroscopy for translocation of 5-FU across an egg phosphatidylcholine membrane was 57 kJ/mol [67] which is comparable with the 52.8973 kJ/mol calculated in this work. Comparison between the error of the calculated free energies estimated by bootstrapping analysis which is within ±3 kJ/mol and the observed values validates the accuracy of free energy profiles.

4. Conclusion

In this work, molecular dynamics (MD) simulations were employed to investigate the behavior of the hydrophilic anticancer drug 5-FU in hydrated DMPC bilayer with different amounts of cholesterol (0, 25 and 50 mol%). The umbrella sampling technique was used to calculate the potential of mean force (PMF) for the transferring of drug molecule across the DMPC/Chol membranes. The minimum free energy barriers determined by PMFs for the transferring of 5-FU from water to the middle of bilayers were calculated and were shown to be located in the vicinity of polar head groups in the interface region of lipids and water. This implies that the 5-FU molecule preferentially was accommodated in this region of the membrane. The mass density profiles extracted from the unconstrained simulations validated the variation of calculated free energy across the membranes. Moreover, from PMF calculations it was shown that the free energy barrier of bilayers increased by the enhancement of the cholesterol concentrations in the bilayers. This increase can be related to the molecular condensing effect of cholesterol on the lipid bilayer. The results of MD simulations justify that the thickness, ordering, and rigidity of the bilayers as increased by cholesterol cause a decrease in the lateral diffusion of drug molecule in the membrane.

References

[1] H. Saito, W. Shinoda, Cholesterol effect on water permeability through DPPC and PSM lipid bilayers: a molecular dynamics study, J. Phys. Chem. B 115 (2011) 15241–15250.
[2] E. Sackmann, in: R. Liposwski, E. Sackmann (Eds.), In Structure and Dynamics of Membranes, Elsevier, Amsterdam, 1995.
[3] H. Ohvo-Rekila, B. Ramstedt, P. Leppimaki, J.P. Slotte, Cholesterol interactions with phospholipids in membranes, Prog. Lipid Res. 41 (2002) 66–97.
[4] H. Martinez-Seara, T. Róg, M. Karttunen, I. Vattulainen, R. Reigada, cholesterol induces specific spatial and orientational order in cholesterol/phospholipid membranes, PLoS ONE 5 (2010) e11162.
[5] W.D. Bennett, J.L. MacCallum, D.P. Tieleman, Thermodynamic analysis of the effect of cholesterol on dipalmitoylphosphatidylcholine lipid membranes, J. Am. Chem. Soc. 131 (2009) 1972–1978.
[6] T. Róg, M. Pasenkiewicz-Gierula, Non-polar interactions between cholesterol and phospholipids: a molecular dynamics simulation study, Biophys. Chem. 107 (2004) 151–164.
[7] F. Foglia, D.J. Barlow, F.C. Szoka Jr., Z. Huang, S.E. Rogers, M.J. Lawrence, Structural studies of the monolayers and bilayers formed by a novel cholesterol–phospholipid chimera, Langmuir 27 (2011) 8275–8281.
[8] A. Ivankin, I. Kuzmenko, D. Gidalevitz, Cholesterol–phospholipid interactions: new insights from surface X-ray scattering data, Phys. Rev. Lett. 104 (2010) 108101.
[9] G. Khelashvili, G. Pabst, D. Harries, Cholesterol orientation and tilt modulus in DMPC bilayers, J. Phys. Chem. B 114 (2010) 7524–7534.
[10] J. Pan, S. Tristram-Nagle, J.F. Nagle, Effect of cholesterol on structural and mechanical properties of membranes depends on lipid chain saturation, Phys. Rev. E. 80 (2009) 021931.
[11] T. Róg, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, Ordering effects of cholesterol and its analogues, Biochim. Biophys. Acta 1788 (2009) 97–121.
[12] M.L. Berkowitz, Detailed molecular dynamics simulations of model biological membranes containing cholesterol, Biochim. Biophys. Acta 1788 (2009) 86–96.
[13] T. Róg, M. Pasenkiewicz-Gierula, Cholesterol effects on a mixed-chain phosphatidylcholine bilayer: a molecular dynamics simulation study, Biochimie 88 (2006) 449–460.
[14] E.S.E. Erikssonand, L.A. Eriksson, The influence of cholesterol on the properties and permeability of hypericin derivatives in lipid membranes, J. Chem. Theory Comput. 7 (2011) 560–574.
[15] J.H. Ipsen, O.G. Mouritsen, M. Bloom, Relationships between lipid membrane area, hydrophobic thickness, and acyl-chain orientational order. The effects of cholesterol, Biophys. J. 57 (1990) 405–412.
[16] M.R. Vist, J.H. Davis, Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: deuterium nuclear magnetic resonance and differential scanning calorimetry, Biochemistry 29 (1990) 451–464.

[17] T.H. Huang, C.W.B. Lee, S.K. Das Gupta, A. Blume, R.G. Griffin, A 13C and 2H nuclear magnetic resonance study of phosphatidylcholine/cholesterol interactions: characterization of liquid–gel phases, Biochemistry 32 (1993) 13277–13287.
[18] S.W. Hui, N.B. He, Molecular organization in cholesterol–lecithin bilayers by X-ray and electron diffraction measurements, Biochemistry 22 (1983) 1159–1164.
[19] M. Alwarawrah, J. Dai, J. Huang, A molecular view of the cholesterol condensing effect in DOPC lipid bilayers, J. Phys. Chem. B 114 (2010) 7516–7523.
[20] D. Hakobyan, A. Heuer, Phase separation in a lipid/cholesterol system: comparison of coarse-grained and united-atom simulations, J. Phys. Chem. B 117 (2013) 3841–3851.
[21] M. Nishizawa, K. Nishizawa, Coarse-grained simulations of branched bilayer membranes: effects of cholesterol-dependent phase separation on curvature-driven lipid sorting, J. Biophys. Chem. 2 (2011) 268–284.
[22] A. Choubey, R.K. Kalia, N. Malmstadt, A. Nakano, P. Vashishta, Cholesterol translocation in a phospholipid membrane, Biophys. J. 104 (2013) 2429–2436.
[23] S.O. Yesylevskyy, A.P. Demchenko, How cholesterol is distributed between monolayers in asymmetric lipid membranes, Eur. Biophys. J. 41 (2012) 1043–1054.
[24] D. Bemporad, C. Luttmann, J.W. Essex, Behaviour of small solutes and large drugs in a lipid bilayer from computer simulations, Biochim. Biophys. Acta 1718 (2005) 1–21.
[25] M. Orsi, J.W. Essex, Permeability of drugs and hormones through a lipid bilayer: insights from dual-resolution molecular dynamics, Soft Matter 6 (2010) 3797–3808.
[26] W. Kopeć, J. Telenius, H. Khandelia, Molecular dynamics simulations of the interactions of medicinal plant extracts and drugs with lipid bilayer membranes, FEBS J. 280 (2013) 2785–2805.
[27] A. Yousefpour, S. Amjad Iranagh, Y. Nademi, H. Modarress, Molecular dynamics simulation of nonsteroidal antiinflammatory drugs, naproxen and relafen, in a lipid bilayer membrane, Int. J. Quantum Chem. 113 (2013) 919–1930.
[28] E.H. Mojumdar, A.P. Lyubartsev, Molecular dynamics simulations of local anesthetic articaine in a lipid bilayer, Biophys. Chem. 153 (2010) 27–35.
[29] A. Kyrychenko, I.Y. Sevriukov, Z.A. Syzova, A.S. Ladokhin, A.O. Doroshenko, Partitioning of 2,6-Bis(1H-Benzimidazol-2-yl)pyridine fluorophore into a phospholipid bilayer: Complementary use of fluorescence quenching studies and molecular dynamics simulations, Biophys. Chem. 154 (2011) 8–17.
[30] M.B. Boggara, R. Krishnamoorti, Partitioning of nonsteroidal antiinflammatory drugs in lipid membranes: a molecular dynamics simulation study, Biophys. J. 98 (2010) 586–595.
[31] P. Jedlovszky, M. Mezei, Effect of cholesterol on the properties of phospholipid membranes. 2. Free energy profile of small molecules, J. Phys. Chem. B 107 (2003) 5322–5332.
[32] C.L. Wennberg, D. van der Spoel, J.S. Hub, Large influence of cholesterol on solute partitioning into lipid membranes, J. Am. Chem. Soc. 134 (2012) 5351–5361.
[33] T.J. Yacoub, A.S. Reddy, I. Szleifer, Structural effects and translocation of doxorubicin in a DPPC/Chol bilayer: the role of cholesterol, Biophys. J. 101 (2011) 378–385.
[34] D. Paolino, D. Cosco, R. Muzzalupo, E. Trapasso, N. Picci, M. Fresta, Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer, Int. J. Pharm. 353 (2008) 233–242.
[35] V.S. Shenoy, R.P. Gude, R.S. Murthy, In vitro anticancer evaluation of 5-Fluorouracil lipid nanoparticles using B16F10 melanoma cell lines, Int. Nano Lett. 2 (2012) 14–24.
[36] K.K. Gupta, N. Pal, P.K. Mishra, P. Srivastava, S. Mohanty, P. Maiti, 5-Florouracil-loaded poly(lactic acid)–poly(caprolactone) hybrid scaffold: potential chemotherapeutic implant, J. Biomed. Mater. Res. Part A. (2014), http://dx.doi.org/10.1002/jbm.a.34932.
[37] D.B. Longley, D.P. Harkin, P.G. Johnston, 5-Fluorouracil: mechanisms of action and clinical strategies, Nat. Rev. Cancer 3 (2003) 330–338.
[38] C.G. Park, M.H. Kim, M. Park, J.E. Lee, S.H. Lee, J.H. Park, K.H. Yoon, Y.B. Choy, Polymeric nanofiber coated esophageal stent for sustained delivery of an anticancer drug, Macromol. Res. 19 (2011) 1210–1216.
[39] H.J.C. Berendsen, D. van der Spoel, R. van Drunen, GROMACS: a message-passing parallel molecular dynamics implementation, Comput. Phys. Commun. 91 (1995) 43–56.
[40] B. Hess, C. Kutzner, D. Van Der Spoel, E. Lindahl, GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation, J. Chem. Theory Comput. 4 (2008) 435–447.
[41] D.V.D. Spoel, E. Lindahl, B. Hess, G. Groenhof, A.E. Mark, H.J.C. Berendsen, GROMACS: fast, flexible, and free, J. Comput. Chem. 26 (2005) 1701–1718.
[42] P.F. Almeida, W.L. Vaz, T.E. Thompson, Lateral diffusion in the liquid phases of dimyristoylphosphatidylcholine/cholesterol lipid bilayers: a free volume analysis, Biochem. 31 (1992) 6739–6747.
[43] A. Filippov, G. Orädd, G. Lindblom, The effect of cholesterol on the lateral diffusion of phospholipids in oriented bilayers, Biophys. J. 84 (2003) 3079–3086.
[44] J. Dai, M. Alwarawrah, J. Huang, Instability of cholesterol clusters in lipid bilayers and the cholesterol's umbrella effect, J. Phys. Chem. B 114 (2009) 840–848.
[45] O. Berger, O. Edholm, F. Jähnig, Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure and constant temperature, Biophys. J. 72 (1997) 2002–2013.
[46] M. Holtje, T. Forster, B. Brandt, T. Engels, W. von Rybinski, H.D. Holtje, Molecular dynamics simulations of stratum corneum lipid models: fatty acids and cholesterol, Biochim. Biophys. Acta 1511 (2001) 156–167.
[47] H.J.C. Berendsen, J.P.M. Postma, W.F. van Gunsteren, J. Hermans, Interaction models for water in relation to protein hydration, in: B. Pullman (Ed.), Intermolecular Forces, Reidel, Dordrecht, 1981, pp. 331–342.
[48] A.W. Schuttelkopf, D.M.F. van Aalten, PRODRG: a tool for high-throughput crystallography of protein–ligand complexes, Acta Cryst. 60 (2004) 1355–1363.
[49] W.G. Hoover, Constant–pressure equations of motion, Phys. Rev. A 34 (1986) 2499–2500.
[50] M. Parrinello, A. Rahman, Crystal structure and pair potentials: a molecular dynamics study, Phys. Rev. Lett. 45 (1980) 1196–1199.
[51] B. Hess, H. Bekker, H. Berendsen, LINCS: a linear constraint solver for molecular simulations, J. Comput. Chem. 18 (1997) 1463–1472.
[52] U. Essmann, L. Perera, L.G. Pedersen, A smooth particle mesh Ewald method, J. Chem. Phys. 103 (1995) 8577–8593.
[53] G.M. Torrie, J.P. Valleau, Non-physical sampling distributions in Monte-Carlo free-energy estimation: umbrella sampling, J. Comput. Phys. 23 (1977) 187–199.
[54] S. Kumar, D. Bouzida, J.M. Rosenberg, The weighted histogram analysis method for free-energy calculations on biomolecules. 1. The method, J. Comput. Chem. 13 (1992) 1011–1021.
[55] J.S. Hub, B.L. de Groot, D. van der Spoel, g_Wham—a free weighted histogram analysis implementation including robust error and autocorrelation estimates, J. Chem. Theory Comput. 6 (2010) 3713–3720.
[56] C.F. Chew, A. Guy, P.C. Biggin, Distribution and dynamics of adamantanes in a lipid bilayer, Biophys. J. 95 (2008) 5627–5636.
[57] S. Samanta, S. Hezaveh, G. Milano, D. Roccatano, Diffusion of 1, 2-dimethoxyethane and 1, 2-dimethoxypropane through phosphatidycholine bilayers: A molecular dynamics study, J. Phys. Chem. B 116 (2012) 5141–5151.
[58] C. Hofsa¨ß, E. Lindahl, O. Edholm, Molecular dynamics simulations of phospholipid bilayers with cholesterol, Biophys. J. 84 (2003) 2192–2206.
[59] N. Kucerka, Y. Liu, N. Chu, H.I. Petrache, S. Tristram-Nagle, J.F. Nagle, Structure of fully hydrated fluid phase DMPC and DLPC lipid bilayers using x-ray scattering from oriented multilamellar arrays and from unilamellar vesicles, Biophys. J. 88 (2005) 2626–2637.
[60] J. Czub, M. Baginski, Comparative molecular dynamics study of lipid membranes containing cholesterol and ergosterol, Biophys. J. 90 (2006) 2368–2382.
[61] R. Reigada, Influence of chloroform in liquid-ordered and liquid-disordered phases in lipid membranes, J. Phys. Chem. B 115 (2011) 2527–2535.
[62] L. Zhao, S.S. Feng, N. Kocherginsky, I. Kostetski, DSC and EPR investigations on effects of cholesterol component on molecular interactions between paclitaxel and phospholipid within lipid bilayer membrane, Int. J. Pharm. 338 (2007) 258–266.
[63] K.Y.Y. Pang, K.W. Miller, Cholesterol modulates the effects of membrane perturbers in phospholipid vesicles and biomembranes, Biochim. Biophys. Acta 511 (1978) 1–9.
[64] K.W. Miller, K.-Y.Y. Pang, General anaesthetics can selectively perturb lipid bilayer membranes, Nature 263 (1976) 253–255.
[65] W.K. Subczynski, A. Wisniewska, J.J. Yin, J.S. Hyde, A. Kusumi, Hydrophobic barriers of lipid bilayer membranes formed by reduction of water penetration by alkyl chain unsaturation and cholesterol, Biochem.J. 33 (1994) 7670–7681.
[66] W.C. Hung, M.T. Lee, F.Y. Chen, H.W. Huang, The condensing effect of cholesterol in lipid bilayers, Biophys. J. 92 (2007) 3960–3967.
[67] N. Yoshii, E. Okamura, Kinetics of membrane binding and dissociation of 5-fluorouracil by pulsed-field-gradient¹⁹F NMR, Chem. Phys. Lett. 474 (2009) 357–361.