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Assessment of penetration of Ascorbyl Tetraisopalmitate into biological membranes by molecular dynamics

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ABSTRACT

The present work, involves the simulation of the transport of a vitamin C derivative, Ascorbyl Tetraisopalmitate (ATI), through human skin by molecular dynamics. Percutaneous absorption of the ATI molecule through the infundibulum, an important route of absorption into the hair follicle of the human skin, has been modeled and compared with the stratum corneum membrane. The comparative study was done using molecular dynamics with Martini force field. In infundibulum, a single ATI molecule require more time to penetrate, and the data obtained suggested that a high concentration of ATI molecule accelerated the process of penetration. In conclusion, the ATI molecule was found to have more affinity towards the stratum corneum as compared with the infundibulum, and it followed a straight pathway to penetrate (until 600 ns of simulation). In the infundibulum, it showed less affinity, more mobility and followed a lateral pathway. Thus, this work contributes to a better understanding of the different molecular interactions during percutaneous absorption of active molecules in these two different types of biological membranes.

1. Introduction

One current challenge faced by cosmetic/pharmaceutical sciences is the permeation of active molecules within the biological membranes. It becomes even more difficult in case of skin due its natural barrier function [1,2]. Although its functions as a barricade to the penetration of potentially dangerous substances is important, still there is a deliberate increase in the approaches aimed at controlling this barrier and, allowing percutaneous absorption of drugs and cosmetics [3–8].

Percutaneous absorption is the transport of chemicals from the outer surface of the skin into the skin appendages, which may/may not convey them to the systemic circulation. This is often divided in, penetration (the entry of a substance into a particular layer or structure), permeation (the penetration through one layer into a second layer) and resorption (the uptake of a substance into the skin lymph or local vascular system, which may lead to the systemic circulation [9].

There are three potential routes for percutaneous absorption: across the continuous stratum corneum, through the hair follicles with their associated sebaceous glands and via sweat ducts [1,2]. The stratum corneum, exhibits a unique morphology that consist of dead flattened cells called corneocytes (bricks) embedded into a complex intercellular lipid matrix (mortar). This organization makes the permeation of molecules difficult through this route [10–13]. Whereas, the hair follicle, located close to the channel of the sebaceous glands lacks keratinized cells. In this region, known as infundibulum, the permeation of molecules is facilitated [3–8].

Fig. 1 depicts the follicular penetration of the hydrophobic substances applied to the skin through infundibulum membrane. The assumed model shows that the infundibulum membrane facilitates permeation of the substances into the skin whereas, the stratum corneum primarily composed of ceramides, fatty acids and cholesterol, delays such permeation.

Due to the emergence of skin as a potential route for the delivery of active molecules to escape the drawbacks of other routes [3,4], it has become necessary that all possible ways of penetration/permeation through skin be studied and exploited in order to, optimize and develop new methods for the efficient delivery of active molecules into various depths of the skin and allow its functional nutrition.

The role of stratum corneum as the foremost route of percutaneous absorption is well supported by several experimental studies however; the assessment of the role of hair follicles in percutaneous absorption remains difficult [3]. As the

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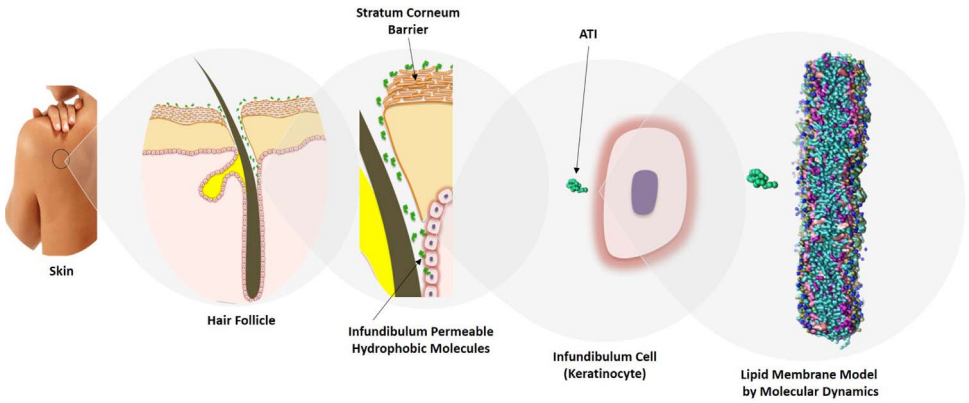

Fig. 1. Schematic representation of a complex system (skin) by a representative model of percutaneous absorption of a molecule through the infundibulum of hair follicles, by molecular dynamics.

infundibulum offers a possible route for the penetration, permeation and resorption [3–8] therefore, it is necessary studies to predict its rule into skin permeation.

The permeation of the active molecules can been studied experimentally by various *in-vitro* and *in-vivo* techniques. For instance, using the Franz diffusion cells with artificial/natural skin [14,15] and Confocal Raman Spectroscopy [16,17]. However, nowadays the molecular dynamics has emerged as a powerful alternative to experimental techniques with a potential advantage of modeling million atoms in the same biological systems and attaining a better understanding of penetration of molecules into membranes [18]. Moreover, it allows the study of specific thermodynamic conditions by introducing parameters such as pressure and skin temperature. Such characteristics result in a more accurate representation of reality.

In this context, our focus is to derive maximum information about the mechanism of penetration/permeation of an active molecule through the hair follicle route, the aim of this study is to assess and compare the two routes, stratum corneum and hair follicles via computer simulation by molecular dynamics. To this end, the active molecule selected was a derivative of vitamin C, Ascorbyl Tetraisopalmitate (ATI). ATI is the hydrophobic and non-oxidizable form of vitamin C used in cosmetic formulations to overcome its drawbacks such as poor penetration due to its hydrophilicity and oxidation by air. Once absorbed ATI is converted into Vitamin C in the cells through the enzymatic reaction of cytosolic esterase [19–22]. The present research article portrays molecular dynamics as an effective mean to derive an insight into the mechanism of interaction between Ascorbyl Tetraisopalmitate (ATI) and the biological membranes of skin.

2. Material and methods

Molecular dynamics simulations involves two representations, i.e., the simulation at the atomic scale known as Fine-grained (FG) [23] and the simulation by grouping atoms called Coarse-grained (CG) [24–26]. In the Fine-grained approach, each atom is considered in the interaction process. Studies with FG representations allow the interactions between molecules that are more refined but involve greater computational requirements and short time scale evolution [25,26]. The CG approach involves mapping whereby, computational efficiency and chemical representability [27]. In mapping of four to one, on average four heavy atoms and its associated hydrogen are represented by a single interaction center in a single bead, considering four main types of the interaction sites: polar (P), nonpolar (N), apolar (C), and charged (Q) [26]. The current work includes four to one mapping to convert fine-grained molecules to coarse-grained molecules. Further transformations were made using the g_fg2cg algorithm [28] implemented in the GROMACS 3.3.1 package [29] in conjunction with the Gromos53a6 force field [30]. Molecular Dynamic simulations in coarse-grained were performed with GROMACS 5.0.6 and 4.5.7 [29,31–35] package using the Martini force field [25,26]. The initial system was energy minimized for 50000 steps using the steepest descent method. A time step of integration of 20 fs was used. The bonds lengths were constrained with LINCS [36]. Neighbor searching were calculated using a Verlet scheme [35,37] and updated every twenty steps. Electrostatic interactions were calculated using a Reaction-Field with a cutoff of 1.4 nm and a dielectric constant of 15. Van der Waals interactions were also calculated using cutoff potential with Potential-shift-verlet (vdw-modifier) with a cutoff of 1.4 nm and a switch at 0.9 nm. Temperature control was set using the V-rescale [38] at 310 K. Each component of the system was included in separated heat bath. Pressure control was implemented using the Berendsen barostat [39], with a reference pressure of 1 bar and isothermal compressibility of 3.0×10^{-4} bar^{-1}. Periodic boundary condition was used in x, y and z directions.

To start with, the initial structure of the ATI molecule was obtained from the PRODRG server [40]. The molecule was energy minimized and equilibrated in NPT (number of particles, pressure and temperature constant) ensemble. The temperature was maintained at 300 K using the Berendsen thermostat [39] with a time constant of 0.1 ps. Pressure control was implemented using the Parrinello–Rahman [38], with a reference pressure of 1 bar with a coupling time constant of 2 ps of relaxation time and isothermal compressibility of 4.5×10^{-5} bar^{-1}. The steepest descent minimization algorithm was use for integration. Long-range electrostatic interactions were treated using the Particle-Mesh Ewald algorithm [41]. Bond lengths were constrained using the LINCS algorithm [36]. The FG was then parameterized and transformed to CG as specified in the previous paragraph.

The infundibulum membrane model was constructed according to the lipid composition of the human epidermis as suggested by Lampe et al. [42]. In order to build the lipid membrane model, we selected fourteen lipids out of 63 lipids as described by Ingólfsson et al. [43]. These lipids represent the basal layer (epidermis) according to Lampe et al. [42]. The stratum corneum membrane model was constructed based on Martin and Huzil [44,45]. The comparative composition of the lipids representing infundibulum and stratum corneum are specified in Table 1. The components and their concentration in the membranes represent their characteristic model towards the penetration of the hydrophobic substances in the most realistic way.

The membrane models were built using CELLmicrocosmos

Table 1
Composition of the simulated infundibulum and stratum corneum membranes. Source: Infundibulum lipids based on Lampe et al. [42] and Ingólfsson et al. [43]. Stratum corneum lipids based on Martins et al. and Huzil et al. [44,45].

Infundibulum lipids	**%**
Polar lipids	
Phosphatidylcholine (POPC and PIPC)	17.0
Phosphatidylserine (POPS and PAPS)	17.0
Phosphatidylethanolamine (POPE and PAPE)	18.8
Phosphatidylinositol (POP1, POP2 and POP3)	6.9
Sphingomyelin (DPSM and PNSM)	9.8
Cholesterol (CHOL)	24.4
Cholesterol Sulphate (CHOLS)	1.5
Ceramide type II (CERII)	4.6
Stratum corneum lipids	**%**
Fatty acids 24 carbons (FA24)	39.0
Cholesterol (CHOL)	36.0
Ceramide type II (CERII)	25.0

2.2 program [46]. Periodic boundary conditions were applied in the systems. All the images were obtained with VMD program [47].

3. Results and discussion

3.1. Infundibulum membrane model

Fig. 2 postulates the infundibulum membrane model. As shown in the figure, the model was built in a box of 19.837 nm, 19.820 nm and 9 nm in (*x*, *y* and *z*). Periodic boundary conditions were applied into the system. The membrane model was immersed in 15.495 CG beads of water and 667 Na^+ ions (in order to balance the negative charge presented by membrane lipids).

The membrane model showed structural integrity and movements after its stabilization. The hydrophilic content (solution) was structured in two layers.

3.2. ATI molecule models

Fig. 3 represents the process of transformation of the ATI molecule from FG to CG and optimization of its structure in water.

3.3. Interaction between infundibulum membrane model and ATI molecule

In order to observe the existing relationship (if any) between extent/route of penetration of vitamin through infundibulum membrane, different models were built by varying the number of ATI molecules added on the optimized structure of membrane. Fig. 4 displays three simulations over the time with a variation in the number of molecules.

As shown in Fig. 4a, it was noticed that a single ATI molecule penetrated the membrane near 320 ns. While in case of three molecules, the first molecule penetrated in the first 10 ns and the other two molecules combined with each other and penetrated in 100 ns (Fig. 4b). On increasing the number of molecules further (i.e., nine), the structural changes in the ATI molecule due to clustering of groups was observed. Two ATI molecules combined and penetrated together the membrane in the first 10 ns, other 4 ATI molecules grouped together (~22 Å) and penetrated in 30 ns whereas, the last three ATI molecules (~19 Å) penetrated in 110 ns (Fig. 4c). Probably these structural changes were related to solvent (water) and ATI's hydrophobicity. The advantage of these small clusters was the reduction in the contact area with the hydrophobic environment.

It was observed that, the ATI molecules penetrated the first layer of the bilayer membrane, and remained at that position for at least 1000 ns of simulation, in each case however, the cholesterol molecules showed the flip-flop between the layers.

Therefore, since a single ATI molecule showed delayed penetration into the infundibulum membrane and the higher number of ATI molecules showed earlier penetration, it could be suggested that the high concentrations are an important factor for speeding up the penetration process.

For showing the localization of the molecules in the models with 1 ATI and 9 ATI molecules, the molecular density was plotted as shown in Fig. 5.

3.4. Comparative study: selective penetration between stratum corneum and infundibulum membrane models

As stated earlier, a comparative study was conducted to

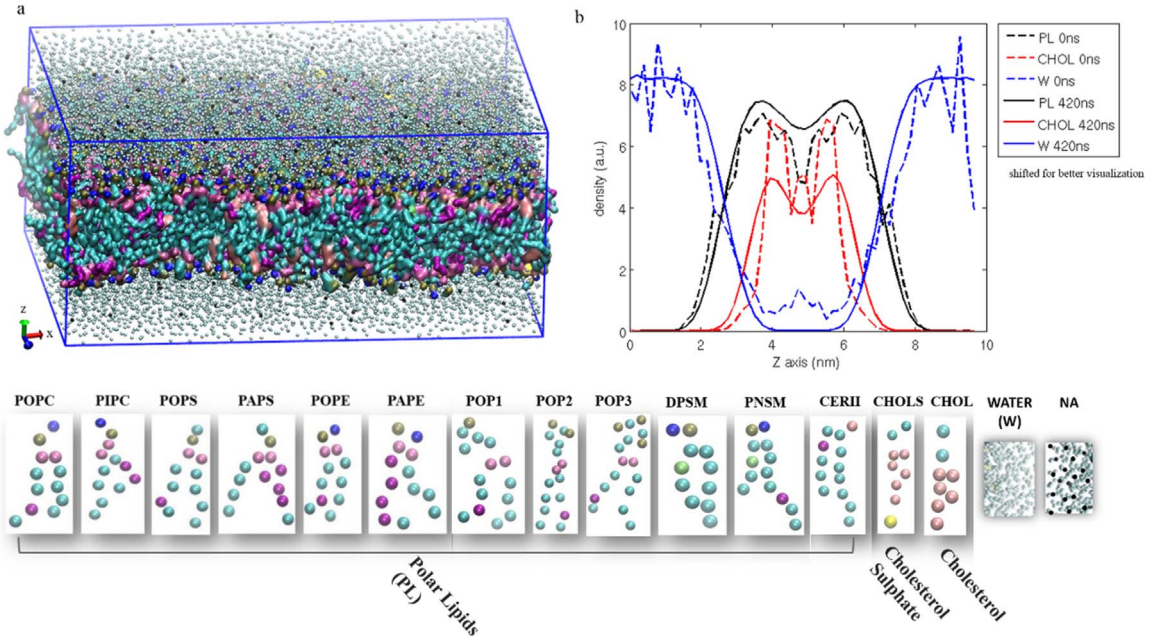

Fig. 2. Lipid Membrane Model: (a) lipid membrane model in solution confined in a box and (b) lipid membrane density plot.

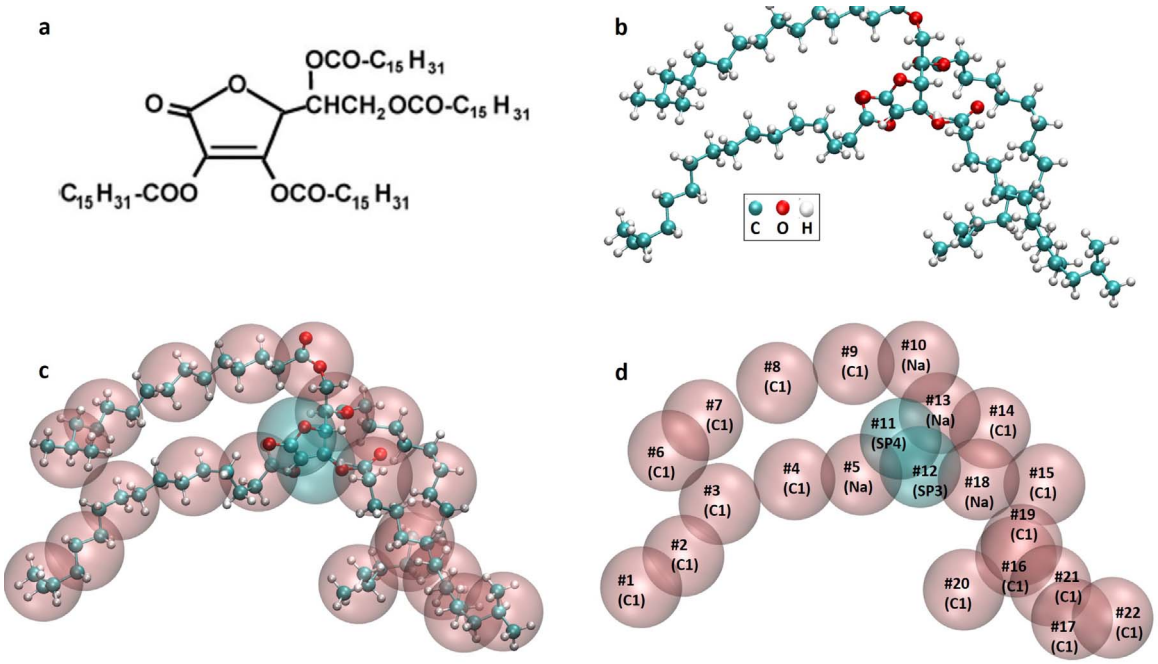

Fig. 3. ATI molecule: a) chemical structure [20], b) representation of the molecule using FG methodology and (c and d) representation of the molecule according to Marrink et al. [25,26] in CG, with 3 types of beads, N (nonpolar), C (apolar) and SP (polar).

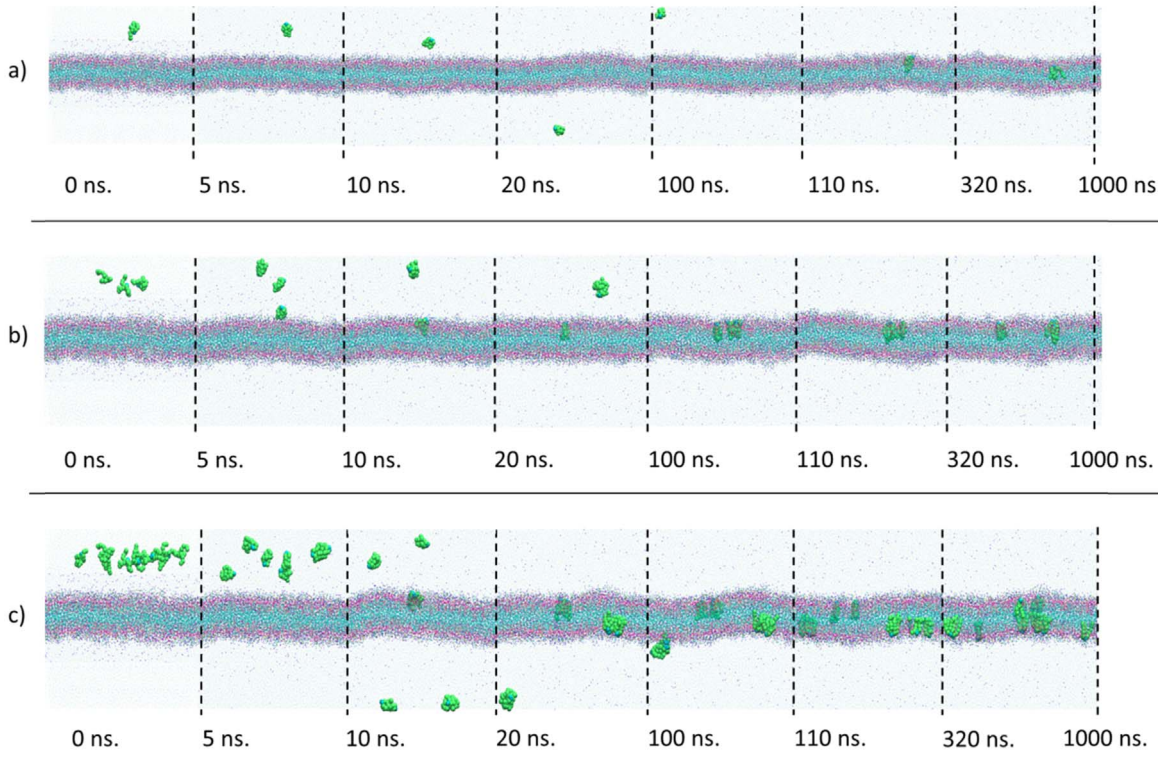

Fig. 4. Membrane Model with ATI molecule/molecules during the simulation process. a) the model with 1 ATI molecule dispersed in 41523 beads of water and 667 Na+ ions. b) the model with 3 ATI molecules dispersed in 41450 beads of water and 667 Na+ ions and c) the model comprised of 9 ATI molecules dispersed in 41271 beads of water and 667 Na+ ions. Run time equals 1000 ns in each case.

determine the affinity of the ATI molecule towards the stratum corneum and infundibulum membranes. For that purpose, stratum corneum membrane was also simulated. For conducting the comparative study, the two membrane models (stratum corneum and infundibulum) were positioned opposite to each other and the ATI molecules were placed between the two membranes in the same system (Fig. 6). Two types of system were modeled to ascertain their non-biased affinity of the ATI molecule towards the two membranes. The model 1 was constructed with a thin layer (~50 Å in *z*-axis) of water between two membranes whereas; the model 2 contained a thick layer (~90 Å in *z*-axis) of water.

Variation and repetition of simulation was done in the random seeds to see different ways of penetration (if any). Thirty seeds were generated for thirty new simulations. Fig. 7 shows the

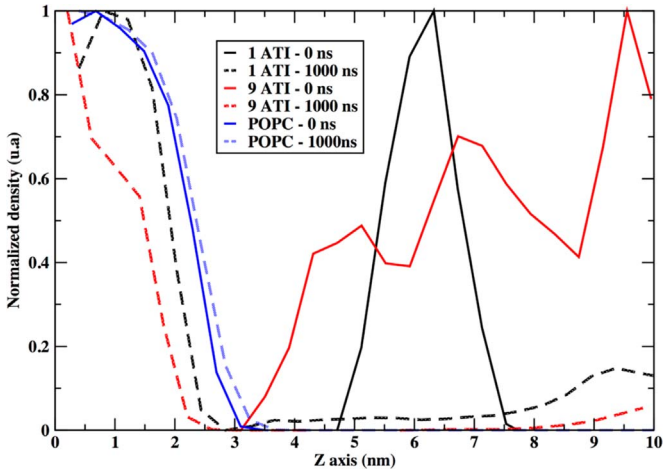

Fig. 5. Molecule density plot: showing the location area of the molecules in model with 1 ATI (black lines) and model with 9 ATI (red lines), before (0 ns) and after (1000 ns) the stabilization. The *Z* axis denotes the size of the box, where zero corresponds to the center of the membrane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

results.

In model 1, it could be seen that the ATI molecule showed preference for the stratum corneum, i.e., 65.55% against 34.45% for infundibulum. In model 2, ATI molecule showed non-significant difference in the preference towards the two membranes, i.e., 52.22% for stratum corneum against 47.88% for infundibulum. Here, the reason could be the neighbor searching. In model 1, the ATI molecule could feel the strong electrostatic attraction of the both membranes. Thus, the affinity of the ATI molecule depends on such electrostatic attractions in this model, but in model 2, with a thicker water layer, the ATI molecule cannot experience the strong electrostatic attraction by the membranes, due to water molecules. Its neighborhood within a cutting radius is initially confined with the water molecules (polar). Therefore, much of the early trajectory involves a “dance” repulsion between ATI and water molecules until it randomly finds an attractive force from any of the membrane. So, by this model, it can be said that the affinity of the ATI molecule is based only on randomness.

These finding establish the aptness of model 1 over model 2 for displaying the real behavior of the ATI molecule towards the membranes.

To compare the mobility of molecules in stratum corneum and infundibulum, the lipid lateral diffusion was calculated from the mean square displacement (MSD) of the two molecules in the plane membrane for 600 ns. The representative molecules were head bead (SP1) for a cholesterol molecule and head bead (SP3) for an ATI molecule in both the membranes. The MSD is a distance traveled in the lateral directions (*x*, *y* and *z*-axis), between times *t* and $t+t0$. The GROMACS gmx_msd tool was used for calculating the MSD curves. Here, average ‘*D*’ value and linearity of curve denote a normal diffusion.

The cholesterol molecule and ATI molecule showed low diffusion (Fig. 8a and c) in all axis in the stratum corneum membrane, whereas, the same molecules showed greater diffusion (Fig. 8b and d respectively) in the infundibulum membrane (up to 600 ns).

This mobility behavior can be clearly seen in Fig. 9, whereby, same molecules of ATI and cholesterol have been expanded during trajectories in *x*, *y* and *z*-axis. It can be visualized that, in stratum corneum, ATI and cholesterol molecules are in same region, while they moved freely in infundibulum model during the 600 ns.

This affinity and lateral diffusion of ATI molecule suggests that the composition of SC (ceramides, cholesterol and fatty acid) is most important, mainly due to ceramides, which play a key role in structuring and maintaining its barrier function [11,48–53]. There are at least eleven different classes of ceramides in human stratum corneum [54], with slight variations of the head groups and, ceramide two (used in present study) contains a fatty acid tail containing 24 carbon atoms. Thus, ceramides along with their long asymmetric tails regulate the main distinguishing characteristics of SC lipid membranes as compared to other biologically relevant membranes [11,48–55] and can be held responsible for the selective penetration of a molecule through it, as compared to the phospholipids in infundibulum.

All ceramides and the fatty acids found in stratum corneum are rod or cylindrical in shape, and this physical attribute makes these lipids suitable for the formation of highly ordered gel phase membrane domains. Gel phase domains have less fluidity, and thereby, less permeable than their liquid crystalline counterparts [49]. Moreover, the findings can be supported by the studies of Moghimi and Schneider that consider the SC to be a lipoidic compartment and the underlying living tissue to be a more

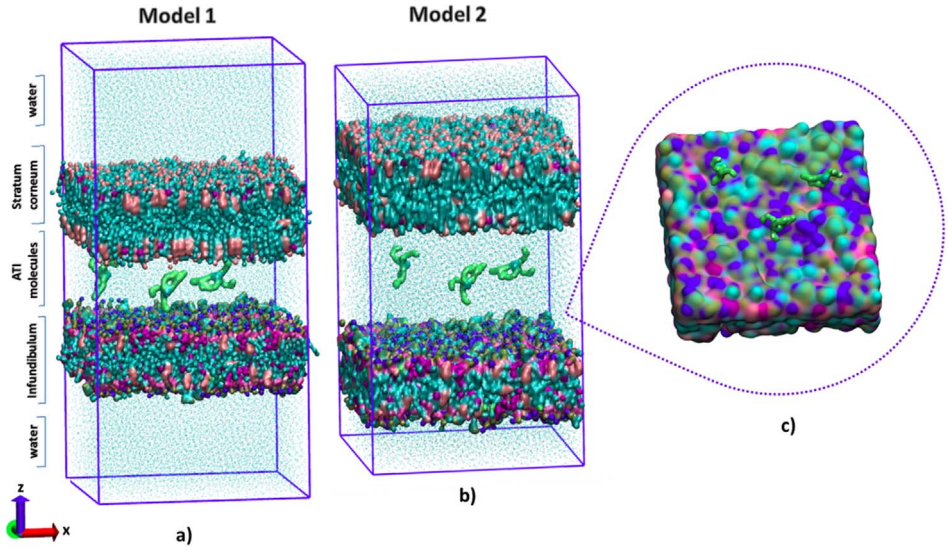

Fig. 6. Positioning of both membranes in a box of 15 nm, 15 nm and 30 nm in (*x*, *y* and *z*-axis respectively): three ATI molecules in the middle, surrounded by water, during 600 ns. a) Model with a thin layer of water and b) model with a thick layer of water. c) A top view of the infundibulum with 3 ATI molecules to show the localization of each ATI molecule.

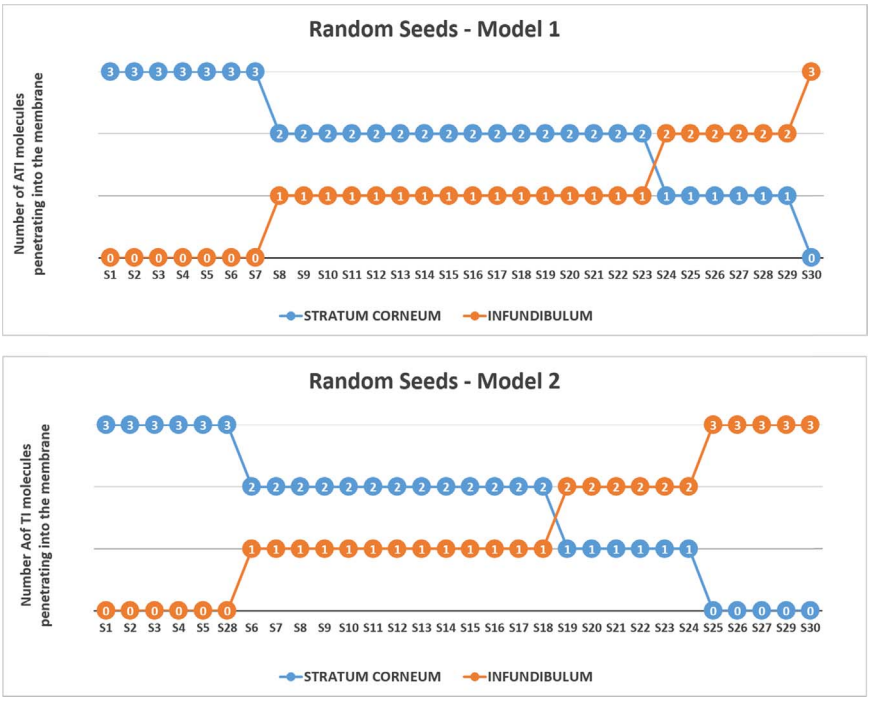

Fig. 7. Thirty random seeds (S) created to study the affinity of ATI molecules between both types of membranes (stratum corneum or infundibulum) in two different models (model 1 with a thin layer of water and the model 2 with a thick layer of water between two membranes). Model 1 represents the preference of the ATI molecule towards stratum corneum membrane.

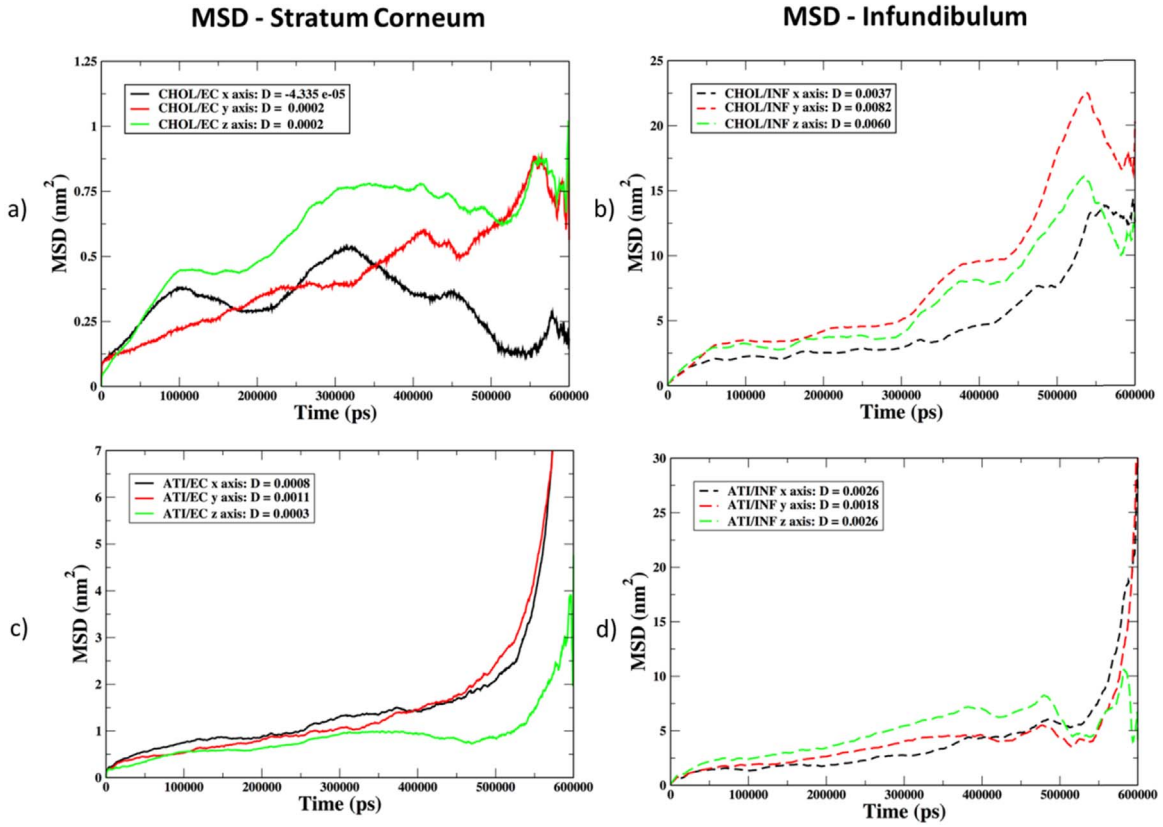

Fig. 8. MSD curves of stratum corneum (a and c) and infundibulum (b and d) of the head bead (SP1) of cholesterol and head bead (SP3) of ATI molecule. *D* denotes average diffusion in 10–5 cm^2/s.

aqueous environment. Hence, lipophilic molecules can distribute more easily into the SC and penetration is facilitated. Diffusion into the living tissue, however, is in favor of polar molecules and objects restricting the invasion of too lipophilic compounds [56,57]. The physicochemical properties of the molecules have an effect on the contribution of the follicular and non-follicular routes

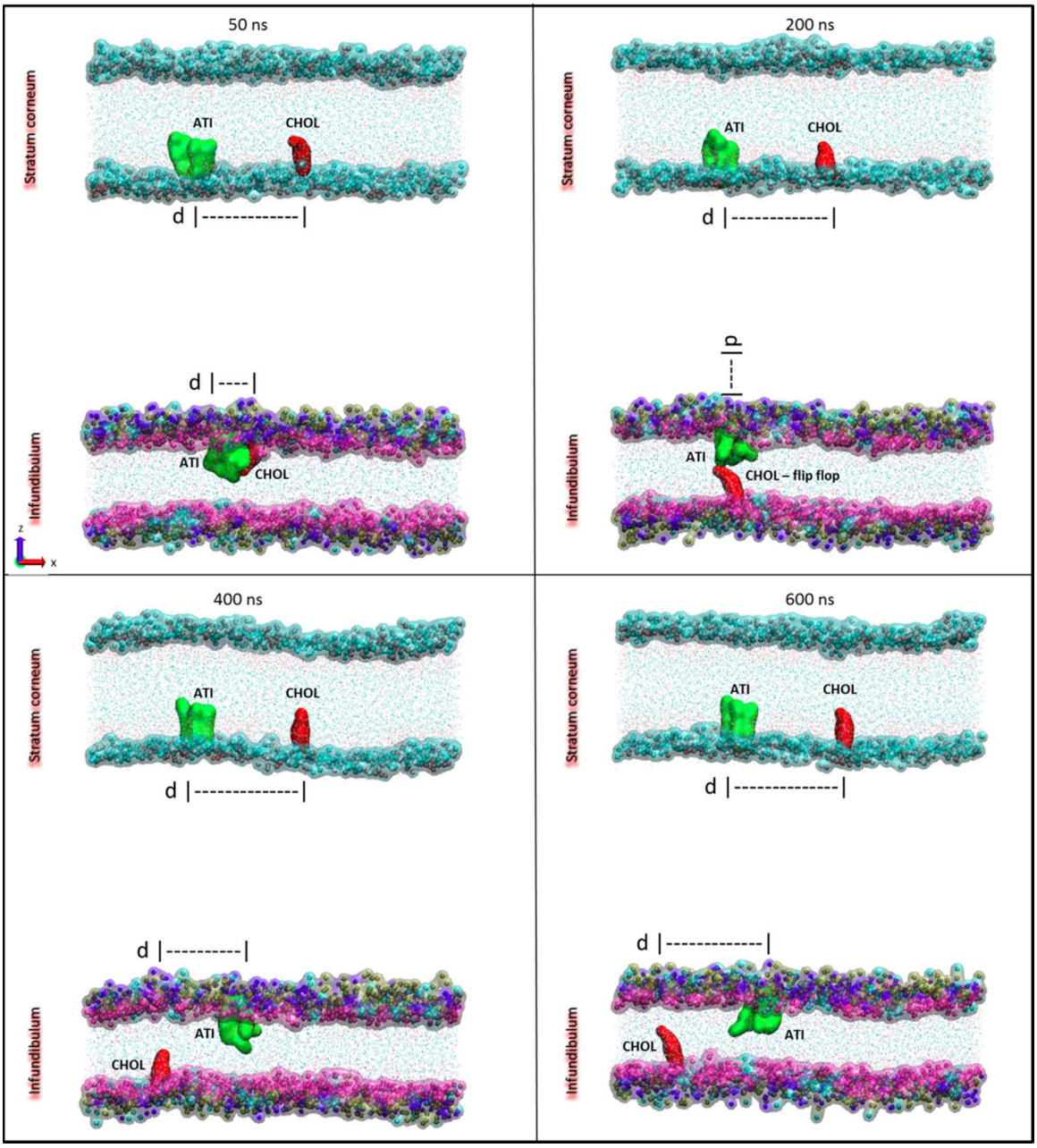

Fig. 9. Lateral mobility of ATI and cholesterol molecules in both the membranes. In stratum corneum ATI (green) and cholesterol molecules (red) are confined in same area. Whereas, in infundibulum they moved freely, with cholesterol showing flip flop movement. "*d*" denotes the distance between the ATI and cholesterol molecule and we can observe variations in this distance in infundibulum. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of penetration [58,59].

Furthermore, the follicular environment, although more hydrophilic, is amphiphilic due to high concentrations of lipids coming from sweat glands. So, the ATI molecule being both lipophilic and slightly polar may penetrate both membranes, but preferably the stratum corneum.

However, a different type of activity was observed between these two membranes: the time-based variability in the mobility of the ATI molecules. In infundibulum, it was clearly seen that the ATI molecules showed mobility at initial stages (75 ns) of the simulation. They moved in *x* and *y*-axis. While in stratum corneum, such mobility (distance between ATI molecule and closer cholesterol) was not visible until the end of simulation (150 ns). For explanation, the role of cholesterol comes into sight. Cholesterol is a ubiquitous membrane lipid and is capable either of fluidizing membrane domains or of making them more rigid, depending on the physical properties of the other lipids and the proportion of cholesterol relative to the other components [49].

The function of cholesterol in the epidermal barrier is probably to provide a degree of fluidity to what could otherwise be a rigid, possibly brittle membrane system (this may be necessary for pliability of the skin) [49]. Thus, it can be suggested, although the CHOL present in the SC provides fluidity to it, but still, the infundibulum (present in this study) has higher fluidity imparted by the diversity of components and their proportions. This property can be associated to the low mobility of the ATI molecule within the SC membrane as compared to infundibulum.

One must be aware that, the deeper localization (between epidermis and dermis) and capillary vasculature of the infundibulum, assists it to transport the molecule into the deeper layers [4]. However, the SC does not contain any capillary vasculature, so the chemicals that pass through the epidermis must pass

partially through the dermis to reach the bloodstream, i.e., through a continuous and tortuous, intercellular network between the keratinocytes.

Moreover, for the conversion of ATI molecule to ascorbic acid (for the purpose of its availability to the skin), it is essential that this molecule reaches the cytosol of living cells, here perhaps, the follicular route, may be the most straightforward [20–22].

All in all, penetration processes in the skin are not yet fully understood, so there is a growing search for alternatives that contribute to a better understanding of these processes. The use of animal models, often used to predict skin absorption in humans, is flawed and too complex to be directly correlated to the human beings, mainly due to differences in percutaneous absorption of different animal species. Therefore, several experimental and theoretical modeling approaches [57,60] have made progress by developing appropriate alternative methods, based on the human skin. One of the advantages of in-silico models over experimental models is the low cost of study, therefore, computer simulations, mathematical models, human skin equivalent, chromatography-based methods and synthetic membranes have emerged as the potential skin mimic systems.

4. Conclusions

It was possible to simulate the membrane model of the infundibulum and stratum corneum membranes using molecular dynamics with CG methodology. The model was able to reach the study's goal because it was receptive to the hydrophobic molecules and it responded repulsively towards hydrophilic molecules. This property is due to the biochemical interaction between the components. The model remained intact in bilayers during simulations. Thus, this model presents itself to be good to simulate the interactions between different molecules and to analyze them.

The data obtained by interactions between infundibulum membrane models and different concentrations of ATI molecules suggest that the greater concentrations of ATI molecules is important for speeding-up the process of penetration.

Comparison of the affinity of the ATI molecules towards the two types of membranes (stratum corneum and infundibulum), presented the stratum corneum to be more receptive for the penetration (up to 600 ns) of the ATI molecule. The infundibulum showed less receptivity to penetration; however, the ATI molecules that penetrated had high lateral mobility (up to 600 ns) as compared to that in stratum corneum. This high mobility in infundibulum model suggests that the permeation (into deeper layers) is more likely to happen in infundibulum than in stratum corneum.

In this work, we successfully achieved a minimal model of biological membranes and ATI molecule with further possibility to undergo several other characteristic refinements related to in-vivo environment. This model can be used further to understand the process of interaction between biological membranes and candidate/active molecules in cosmetic formulations and topical dosage forms. In addition, it is able to exclude a range of substances with less efficiency before experimental trials, focusing on effectively promising substances.

Conflict of interest statement

None declared.

Acknowledgments

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