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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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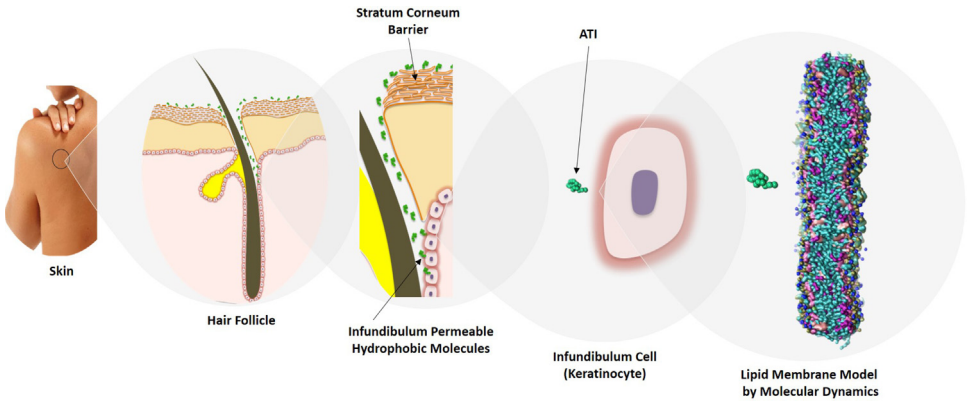

Fig. 1. Schematic representation of a complex system (skin) by a representative model of percutaneous absorption of a molecule through the infundibulum of hair follicles, by molecular dynamics.

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