Pathways for Skin Penetration

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ABSTRACT: *This paper reviews recent findings about three skin penetration pathways (including a "polar pathway") and four types of penetration enhancers (enzymes, vesicular systems, ceramides and chemical enhancers).*

This article will review possible ways for compounds to penetrate the skin and interact with it. Four families of enhancers will be discussed, along with their possible mechanisms of action and limitations. This is written in an attempt to trigger creative thinking about possible research work for better understanding the skin and the design of formulations to treat and protect it.

At the very early stages of understanding the roles of the body's organs, we understood that the skin functions as a barrier between our body and our surroundings. Therefore it was clear that the skin would challenge penetration of compounds and repel outside insults. Having such a nature it was not surprising to discover that skin's upper layer, the stratum corneum (SC), is a subtissue that very efficiently limits penetration of compounds.

Over the years scientists have attempted to find compounds or systems that will allow overcoming this barrier and interaction with deeper subtissues or tolerating permeation to the circulation system. After years of research, it is now clear that there are ways to allow permeation of compounds to and through the skin. The focus has shifted toward understanding the microstructure of the skin, as well as the mechanism of action of these enhancers.

Because skin penetration enhancers provoke structural changes in the SC, they often trigger undesired immune system reactions such as irritation, allergy, or inflammation. Most of the enhancers are not specific and will allow penetration of any compound that is small and lipophilic enough to penetrate. When dealing with cosmetic formulations this means that compounds such as fragrance components and preservatives will penetrate in conjunction with the active compound. Moreover, the skin is a very viable tissue. It includes many metabolic systems that were originally aimed to drive biochemical processes such as desquamation, creation of extracellular lamellar sheets, programmed cell death (apoptosis) and sebum or sweat secretion. These enzymes may attack active or inactive compounds as they penetrate, and convert them into an inactive, active or toxic form.

It is therefore believed that by understanding the details of the interaction of penetration enhancers with the skin, one can find ways to prevent their drawbacks or overcome their limitations.

Penetration between SC corneocytes is the pathway by which most compounds penetrate the skin.

When designing a formulation for topical application, one must understand the possible interactions between the formulation and the skin. This article attempts to provide an initial understanding of this matter. Skin penetration differs from skin permeation. The former describes the passage of an ingredient into the skin, we hope, to the target skin layer. The latter describes the passage of an ingredient through the skin, to the circulatory system such as in

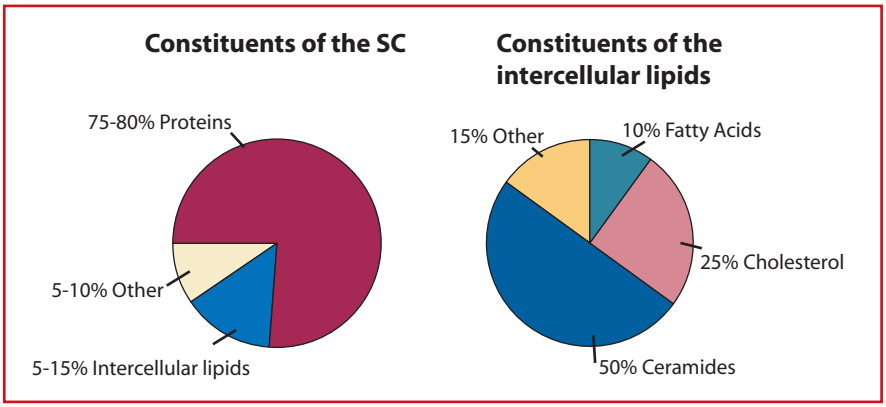

Figure 1. Percentage (by dry weight) of ceramides within the composition of the SC

pharmaceutical transdermal systems. It is however difficult to differentiate between the 2 because once a compound passed the limiting step of the SC and reached to the living epidermis it is almost impossible to prevent its partitioning into skin subtissues, including the blood vessels that lead to the circulation. Both terms will be encountered in this article, and used according to these definitions.

Factors Affecting Skin Penetration

The SC is a heterogeneous structure. Microscopically, the upper cells of the epidermis, the corneocytes, are arranged in pillars to form clusters. The SC consists of alternating cell and lipid layers and is only 6-10 µm thick, which is equivalent to about 14-18 cellular layers. Corneocytes are typically 200-300 nm thick, 30-50 µm in diameter and hexagonal or polygonal in shape. The barrier nature of the SC is governed by its constituents: 75%-80% are proteins, 5%-15% lipids, and 5%-10% unidentified elements (Figure 1). Such a composition favors the absorption of certain lipid-soluble compounds by the skin.[1]

In order to simplify the calculation of a compound's permeation profile through skin, scientists have adopted models for simple diffusion to explain the flux of compounds through SC lipid domains. When using this model one is assuming that interaction between a given compound and the skin is physicochemical in nature, with the multi-layer structures of the corneocytes within the SC organized horizontally. The majority of molecules that cross the epidermis will permeate between the cells via the intercellular route. To penetrate, a compound must partition into the SC before diffusing across the viable epidermis. Therefore, the major pathway for a compound is highly dependent upon its partition coefficient. Hydrophilic compounds may preferably partition into the intracellular domains, while lipophilic ones may cross the SC through the intercellular route. In fact, although the nature of the compound may dictate its preferred route of penetration, most molecules penetrate this layer through both pathways simultaneously.

Factors that may affect penetration include the size of the molecule, its affinity to the surface of the skin, and its compatibility with the intercellular lipids. Also of significance for penetration are general skin condition, moisture content, temperature, thickness of the SC (that can differ between races and body parts), and physical integrity. SC integrity can be affected by age, exposure to solvents, skin care routine, health condition and environmental factors.

Often a correlation can be made between the value obtained for a compound partitioning between octanol and water (i.e., partition coefficient) and its postulated route of penetration through skin. Study results suggested that while mannitol, for example, permeated via a polar route, hydrocortisone permeated mainly through the lipid route and progesterone via a lipid pathway with the aqueous layers affecting its permeation rate. These results correlated with the compound's octanol-water partition coefficient.[2]

> Human skin appendages, hair follicles, and sebaceous glands, constitute a significant route for penetration of steroids and thus probably for other chemicals of similar molecular properties.

When considering polar and non-polar pathways for penetration, it is usually assumed that polar compounds will penetrate through polar routes, while non-polar compounds will favor lipophilic routes.

The intercellular pathway: Penetration between SC corneocytes is the pathway by which most compounds penetrate the skin. Since corneocytes are not stacked parallel to one another in the layers, when penetrating between them, a compound has a sinuous way to pass. This pathway is considered to enable free volume diffusion through lipid bilayers present between the cells.

Most skin penetration enhancers were found to affect the intercellular lipid bilayers of which this route consists. Skin penetration accelerators such as dimethylsulphoxide (DMSO), laurocapram (Azone), glycols and surfactants enhance penetration into the skin by reversibly decreasing the diffusional resistance of its intercellular lipid bilayers.[3] These enhancers may not only act as solvents that solubilize the intercellular lipids but can also affect intercellular desmosomal connections or interfere with metabolic activity necessary for creation of an intact barrier. In normal skin conditions the effect on lipid bilayer structure is reversible. The decrease in the lipid bilayers' resistance to penetration can be due to a thermodynamic effect of fluidization (decrease in lipid's transition temperature) or due to phase separation of lipids in the intercellular spaces.

The intrafollicular pathway: The amount of sebaceous glands on the total skin surface represents not more than 0.1%. Therefore there are scientists who believe that this route is not a significant penetration pathway for most molecules. Others claim that the appendages can bypass the low diffusivity of the SC and may act as diffusional shunts. When the follicle is the site of action, such as in acne, scientists find ways to target a compound to this site, by developing delivery systems with specific physicochemical properties.[4]

When considering these openings as a possible route for penetration, it is important to understand the variations in follicle distribution among different body locations.[4] The highest hair follicle density and percentage of follicular orifices were found on the forehead. The highest average size of the follicular orifices was measured in the calf region. The forehead and the calf regions were also found to exhibit the highest infundibular volume and therefore the highest potential for creation of a reservoir. The lowest values for all parameters were found on the forearm. The plantar and palmar regions of the skin are the only sites completely devoid of sebaceous glands.

It is difficult to study penetration via follicles because of the lack of suitable animal models. A technique was developed to differentiate between penetration through a shunt route and bulk trans-epidermal permeation. This method compares delivery through the epidermis as a membrane versus delivery through a sandwich of SC and epidermis.

Follicles are present in layers and only rarely are superimposed. This model assumes the possibility of follicles to be superimposed is diminutive, and therefore the bottom of the skin's top layer blocks the openings of the follicles. It therefore allows one to distinguish between the 2 ways of penetration. If penetration through follicles is dominant, the passage through the sandwich model will be significantly reduced in comparison to passage through the epidermis membrane alone.[5]

Another method to evaluate penetration through shunts was based on the comparison between scarred and normal skin. The main modifications observed in scarred skin include the absence of hair follicles and sebaceous glands and thinning of the collagenous fibers.[6] In this study, the percutaneous absorption of 4 steroids through scarred skin and normal skin was observed. Results of the experiments demonstrated that human skin appendages, hair follicles and sebaceous glands, constitute a significant route for penetration of steroids and thus probably for other chemicals of similar molecular properties. The results also showed that steroids tend to create larger reservoirs in the SC of an appendage-free scarred skin in comparison to normal skin. Steroids concentrations appearing in the epidermis and dermis were greater in normal skin, where openings played a role in allowing deeper penetration.

Particulate delivery systems may play a role in targeting molecules to follicles. Depending on the formulation and the compound's intrinsic properties, certain compounds can enter faster into shunts than through a different route in the SC. It was demonstrated, for example, that microspheres with an optimal size of around 1.5 µm showed 55% penetration into hair follicles.[7]

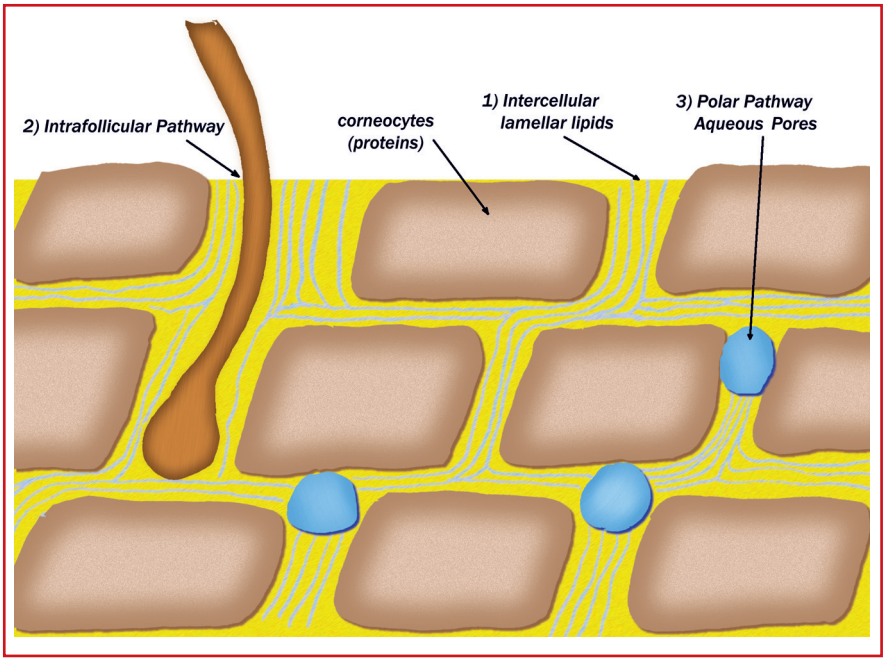

Figure 2. The "Polar Pathway"

Two types of liposomes (classic multilamellar vesicles and flexible ultradeformable liposomes) were found to contribute significantly to penetration through shunt routes.[8]

The "polar pathway": This route is believed to be hydrophilic in nature. It is composed of aqueous regions surrounded by polar lipids that create the walls of microchannels (see Figure 2). It is known to have a high penetration resistance to lipophilic compounds but low resistance to hydrophilic compounds. It is also thought to be the route by which water evaporates through the skin. The localization of the hydrophilic pores is unclear. While some scientists claim compounds permeating through this route will penetrate between the corneocytes clusters through imperfections that create openings comprising of water, others think the intracellular keratin provides this pathway.[1] It may be that both pathways exist and that the preferred route for penetration is a function of molecular properties and the test model used.

> Different enzymes, depending on their biological function, can affect metabolism and biochemical cascades in the skin that eventually allow penetration of compounds.

Supporting the first theory, a study conducted on the percutaneous penetration of baclofen through cadaver skin suggested that the polar pathway is intercellular and is made up of aqueous regions surrounded by polar lipids.[9]

A technique developed using fluorescent ultra deformable lipid vesicles with confocal laser microscopy allowed for visualization of the penetration pathway in intact skin.[1] It was observed that 3-10 corneocytes create "columns" that form a cluster. Corneocytes edges inside each cluster were found to intercalate extensively, but neighboring clusters were separated by gaps of a few micrometers. Lipid packing in the inter-cluster region showed less regularity in comparison to the packing within clusters. This technique also allowed the quantification of 2 different hydrophilic pathways: the inter-cluster route with low penetration resistance made up of around 1% of the total or about 20% of the pathway area of the skin. The inter-corneocyte pathway that showed higher penetration resistance exhibited about 3% of the skin, or 80% of the pathway area. This later route was

found to be twisted as it goes between the corneocytes in a cluster and traces the irregularities between the intercellular lipid lamellae and/or the adjacent corneocytes envelopes that may act as virtual channels in the skin.

Most research indicates that classic multi-layered phospholipid liposomes create a skin reservoir and would not be the carrier of choice for transdermal delivery.

Ways to Enhance Skin Penetration

There is more than one way to differentiate and classify skin penetration enhancers. They can be classified according to their ability to carry different molecules or according to their mechanism of enhancement. Another differentiation would be their preferred route of penetration. This classification divides penetration enhancers into three main categories:[2]

- Solvents that enhance penetration through both polar and non-polar pathways such as 2-pyrrolidone, N-methyl pyrrolidone, N-methylformamide and propylene glycol in combination with azone
- Enhancers that preferentially affect the polar route, such as propylene glycol, in combination with decylmethylsulfoxide, and
- Enhancers that mainly modify the non-polar route, such as propylene glycol and oleic acid, propylene glycol alone and to limited extent water.

Penetration enhancers enhance transport of polar molecules by three major mechanisms:[9]

- Extracting the SC lipids
- Inducing a relaxation of the polymeric structure of the cytoplasmic matrix in keratinocytes, and
- Changing the solvent properties of the SC.

Surfactants, for example, were shown to enhance transport of polar molecules by solubilization and removal of intercellular lipids and binding to keratin filaments of the intracellular matrix. This resulted in cell order disruption. Non-ionic surfactants were shown to demonstrate fluidizing effect on the SC. Skin pretreated with gels containing various non-ionic surfactants showed a loosely layered SC and wide intercellular spaces.[10]

Skin penetration enhancers differ in structure, properties and mechanism of action, as will be shown in the following discussion of enzymes, chemical enhancers, vesicular systems, and ceramides.

Enzymes: The approach of using enzymes to affect the barrier properties of the SC refers to either affecting activity of enzymes present in the skin, or to enzymes applied to it from the outside.

Each of the three key lipid classes in the SC (fatty acids, cholesterol and ceramides) is required for normal barrier

function. Inhibition of the generation of any of these components can delay the barrier recovery after barrier insult.[11] Inhibition of fatty acid synthesis (by 5-(tetradecyloxy)-2- furancarboxylic acid), or cholesterol (by fluvastatin) resulted in increased percutaneous absorption of lidocaine. Modulation of epidermal lipid biosynthesis, following application of conventional chemical penetration enhancers, can cause further increase in a compound's delivery across the skin creating a simultaneous effect of interference with barrier homeostasis and thermodynamics.

The same effect of creating a controlled metabolic interference provides the rationale for the application of enzymes onto the skin. Different enzymes, depending on their biological function, can affect metabolism and biochemical cascades in the skin that eventually allow penetration of compounds. For example, an application of papain, a proteolytic enzyme that was conjugated to SC-glucan revealed an enhancement of percutaneous absorption. This application triggered structural changes in the SC that led to an increase in thickness of the SC and the living epidermis. Application of the enzyme also induced subtissues phase separation, lamellar bodies formation and created disorder in lamellar structures in the SC. It is hypothesized that these structural changes were induced by the hydrolysis of the cross-linkage between corneocyte envelopes and intracellular proteins. The SC-glucan-papaine conjugate showed no irritation when applied to the skin.[12]

> It was concluded that branching of the alkyl chain reduces the ability of the enhancer to affect lipid fluidization in the SC lipid lamellae at the target site.

Chemical enhancers: This is probably the largest and most studied group of permeation enhancers mostly to allow transdermal delivery. Most chemical enhancers affect the intercellular lipid bilayers in the SC. They lead to a reversible deformation in the bilayer structure that allows the creation of various types of "openings" in the bilayers. The nature of these "openings" can vary. It can be triggering of a thermodynamic imbalance within the lipid domains leading

CERAMIDE NS

Ceramide NS, commonly referred to as ceramide 2, and its analogues are being used in formulations for topical application to treat dry, flaky skin. It is also claimed to slow down skin aging. Formulations containing ceramide NS are believed to support and reinforce the cutaneous barrier properties. All major sub-fractions of stratum corneum ceramides are to some extent generated from lamellar body derived glucosylceramides. Yet, it was shown that sphingomyelin-derived ceramides are required for normal barrier homeostasis. Moreover, sphingomyelin can be available for the generation of ceramides from two sources: the plasma membrane and the lamellar bodies. The epidermal sphingomyelin SM-1 (Figure 3) is an important precursor for ceramide NS.[22]

Ceramide NS was shown to inhibit cell proliferation and induce apoptosis. The hydrolysis of sphingomyelin was shown to lead to the generation of ceramide NS that is responsible for the formation of apoptotic bodies. Apoptosis is an active process of programmed cell death. It requires metabolic activity by the dying cell, and is often characterized by cleavage of the DNA into fragments. Induction of apoptosis is necessary when mature cells reach the end of their life cycle. Since the skin is a continuously renewing tissue, it is essential to maintain a "birth-death" cycle to clear the way for new, fresh cells.[23]

A synthetic analogue of ceramide NS, pseudo-ceramide 2 (N-stearoyl-DL-erythro-sphinganine), was shown to significantly decrease TEWL values when applied after stripping or treatment with sodium lauryl sulfate. The analogue was applied at 0.5% or 1% levels in a cream. The results of this study suggest that these NS ceramide analogues participate in the restructuring of the stratum corneum.[24]

It is known that while cleansing the skin, detergents may remove valuable skin lipids, hence, disrupting the epidermal barrier function and elevating TEWL.[25] Reduction in TEWL following an application of monoglycerides, squalene, cholesterol ester and pseudo-ceramide 2 was tested after the application of 5% sodium lauryl sulfate. Among the compounds tested, monoglyceride and ceramide 2 demonstrated the best results in restoring barrier function. It was concluded that the addition of ceramide NS into skin cleanser formulations could have a beneficial effect in the prevention of detergent induced barrier disruption. When applied to the skin, ceramide NS can be enzymatically converted to other ceramides and hence, may provide enhanced barrier properties. To achieve effective enzymatic recognition, it is crucial that ceramide NS will be in its pure optically active form.

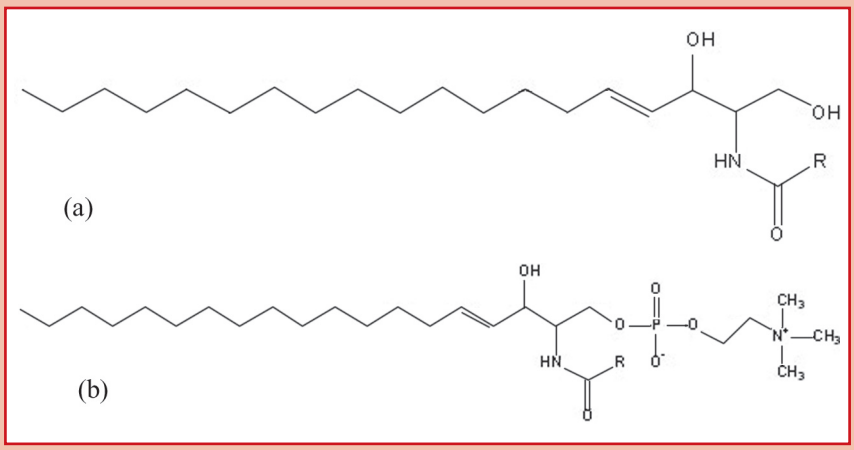

Figure 3. Chemical structures of ceramide (a) and sphingomyelin (b)

to increased lipid fluidity or creation of actual microscopically visual pores.

Various esters and fatty alcohols are known to enhance skin penetration. Examples are isopropyl myristate and isopropyl alcohol. Both were shown to increase permeation through skin. When applied together, they demonstrated a synergetic enhancement of permeation. Their mechanisms of action were found to be different. While isopropyl myristate generated disordered bilayers in the corneocyte-bound lipids, isopropyl alcohol resulted in fluidization and disorder in the free bilayer structures of the intercellular lipids.[13]

When combining 2 or more skin penetration enhancers to improve skin permeation, it is recommended to choose enhancers with different mechanisms of action. This will prevent competition at the site of action and has a better probability of not provoking irritation.

Oleic acid was found to increase epidermal permeability through a mechanism involving the perturbation of the SC lipid bilayers.[14] A technique using electron microscopy with osmium or ruthenium tetroxide allowed for ultra structural examination of the SC after the application of oleic acid. It was observed that marked alteration in the SC occurred specifically in the intercellular spaces.

Vesicular systems: Vesicles are microscopic spheres, usually composed of amphiphilic molecules. Classic liposomes are composed of phospholipids. Topically applied vesicles can either mix with the SC lipid matrix or penetrate the SC by using the lipid-water interface of the intercellular matrix. A major force driving vesicle penetration through the skin may be the water gradient across the epidermis.[15]

When comparing the effect of classic lecithin-derived vesicles with SC derived vesicles, scientists claim 2 different opposing effects. Most research indicates that classic multi-layered phospholipid liposomes create a skin reservoir and would not be the carrier of choice for transdermal delivery.[16] SC lipid-based liposomes, on the other hand, were found to deliver a greater amount of radiolabeled marker to the deep layers of the skin (epidermis and dermis) when compared to classic lecithin liposomes. Classic liposomes were also shown to significantly reduce systemic absorption of the marker and reduced organ distribution. Liposome size was found to be a crucial factor in penetration. The larger the mean size, the poorer the penetration to SC layers.[17]

Contradicting evidence was found in a study were liposomes formed from phospholipids were compared with liposomes composed of lipids imitating the SC (ceramides, cholesterol, palmitic acid and cholesteryl sulfate).[18] In this study, phospholipids-composed liposomes were found to increase SC lipid bilayer disorder and increase percutaneous permeation. SC lipid-derived liposomes demonstrated high affinity to the SC and generated a reservoir. These liposomes were also observed to increase the order of lipids in the intercellular lamellae.

Ceramides: SC ceramides are fundamental to maintaining the skin barrier. Most ceramides and their analogues contribute to repair of a disturbed skin barrier and hence decrease skin permeability (see sidebar on ceramide NS).

The understanding of the effect created by application of ceramides to the skin is crucial to achieve desired results. While application of ceramides, in specific concentrations, and especially in combination with fatty acids and cholesterol, can contribute to an improvement in skin barrier resistance, certain ceramides, in certain concentrations, were shown to create an imbalance in the intercellular lamellar organization and to enhance skin penetration.

In a study conducted to understand the correlation between the chemical structure of enhancers and their enhancement efficacy, it was found that in some cases the polar head of an enhancer is responsible for the penetration and anchoring of a molecule into the SC.[19] This study, conducted with branched-chain alcohols as skin permeation enhancers, suggested that the branched-chain alcohols have lower enhancement capability than the primary alcohols of the same molecular weight. It was concluded that branching of the alkyl chain reduces the ability of the enhancer to affect lipid fluidization in the SC lamellae at the target site.

A similar study conducted with a series of ceramide analogues further supports this finding.[20] These analogues included different polar head groups and different chain lengths. Here, ceramides having the same chain length exhibited enhanced activity that is only dependent on their permeability coefficients. It was found that the hydrogen bonding ability of the ceramide is inversely related to the enhancement capacity. The same group of scientists also studied transdermal L-serine- and glycine-based ceramide analogues as permeation enhancers that are related to SC ceramides. The glycine-based ceramide analogue was shown to significantly enhance skin permeation of theophylline through human skin in vitro.[21]

Summary and Future Research

Although, as described in this paper, one can distinguish between different routes for penetration with different properties, most compounds applied to the skin will permeate through more than one pathway. A certain compound, however, depending on its characteristics and its vehicle may exhibit a preferred route of penetration. Still, there is a continuous need for models that mimic the SC layer and techniques that will enable the tracking of compounds applied to it.

When studying the penetration properties of a compound it is important to understand its possible penetration pathways. Changing its molecular properties, choosing a delivery system or a carrier formula, can alter its preferred route of penetration. Understanding the possible routes for penetration can provide tools for the design of an appropriate system that will deliver the molecule to the desired target of action.

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