Expert Opinion

Percutaneous permeation modifiers: enhancement versus retardation

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Background: The use of permeation enhancers to compromise the barrier properties of skin has been ongoing for decades. However, toxicity associated with certain xenobiotics has led to the development of permeation retardants. Since both enhancers and retardants modify the surface layer of the skin, they can be collectively referred to as penetration modifiers. *Objective*: This review attempts to outline a comparison of two types of penetration modifiers: enhancers and retardants. *Methods*: In addition to reports of enhancement and retardation by modifiers, we also provide evidence as to why we should group these compounds together, since we have found that retardants can become enhancers in different formulation environments. *Conclusion*: Since modifiers influence drug delivery, further exploration of these compounds is required to understand their modifying action on the properties of skin.

Keywords: enhancers, retardants, penetration modifiers, stratum corneum

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1. Introduction

The skin, being the largest and most accessible organ of the body, is an obvious target for drug delivery considerations. However, drug delivery across the skin is limited by the resistance offered by the topmost non-viable layer of epidermis, called the stratum corneum (SC). The remaining layers of skin, namely viable epidermis (stratum lucidum, stratum granulosum, stratum spinosum, stratum basale) and dermis, offer minimal resistance to the passage of actives across the skin (**Figure 1**). Even though several physical methods [1-3] have been introduced to compromise the barrier properties of SC, nevertheless the use of chemical penetration modifiers [4,5] dominates the transdermal drug delivery field. The transdermal delivery of several classes of drugs such as NSAIDs [6], contraceptives [7], antihypertensives [8], etc., has been significantly increased by the use of chemical permeation enhancers, some of which are also formulation vehicles. Several transdermal permeation enhancers such as surfactants, terpenes, laurocapram, dimethyl sulfoxides, fatty acids, alcohols and water are known to improve drug permeability by providing fluidity to lipid bilayers of the SC. Unlike most therapeutic agents, there are certain chemicals, including some pesticides [9], chemical warfare agents [10], sunscreens [11] and mosquito repellants [12], that unfortunately tend to pass through skin in meaningful concentrations, which results in unwanted side effects or toxicity. Some of these chemicals are classified as permeation enhancers themselves (such as DEET, diethyl *m*-toluamide) and easily permeate the skin, leading to toxic effects upon systemic exposure. The percutaneous absorption of such agents can be minimized by the use of penetration retardants. The application of these percutaneous penetration retardants

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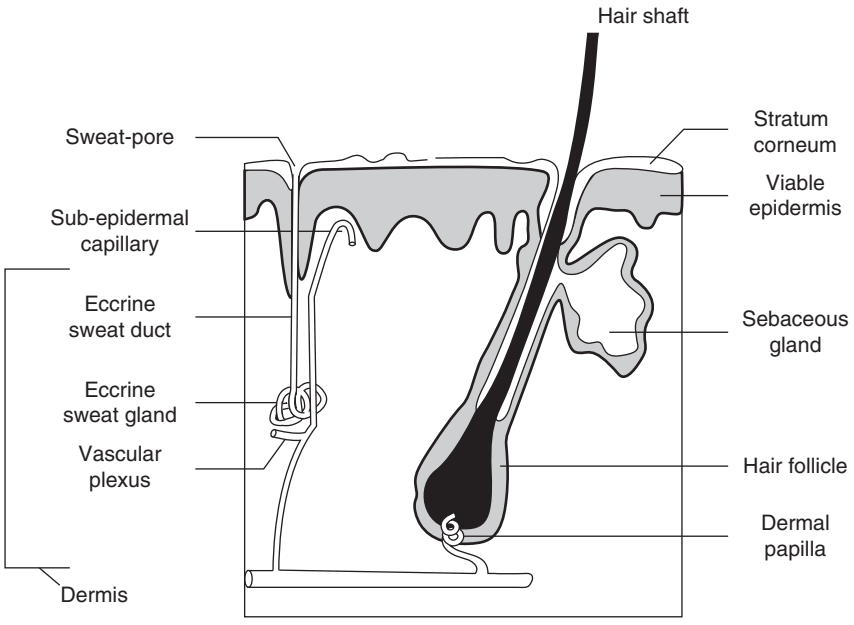

Figure 1. Diagrammatic representation of the structure of the skin, modified from [23].

is not only limited to agrochemicals (such as pesticides and household chemicals) and cosmetics (such as sunscreens and mosquito repellants) but extends to protection against exposure to lethal chemical warfare agents. Permeation retardants are usually structural analogs of enhancers that retard the diffusion of permeant by tightening and condensing the lipid arrangement of SC. Owing to the modifying action of these compounds on the barrier properties of stratum corneum, permeation enhancers and retardants are collectively termed as permeation modifiers. An extensive review of the literature has revealed that there have been numerous studies into reducing the barrier properties of skin using penetration enhancers; however there are limited reports on retarding the penetration of actives. Nevertheless, the current review attempts to outline a comprehensive comparison of two types of penetration modifiers – enhancers and retardants – with a focus on their characteristics, mechanisms of action, factors affecting their activity, techniques of characterization and their role in drug delivery.

2. Characteristics of ideal penetration modifiers

Penetration modifiers should possess certain characteristics for their safe and effective use in transdermal and topical formulations. First, ideal permeation modifiers should be pharmacologically inert and chemically stable. Secondly, they should be potent in low concentrations and compatible with all formulation components. Thirdly, penetration modifiers should possess non-toxic, non-sensitizing and non-irritant properties. Fourthly, modifiers should have a rapid and reversible onset of action and specific activity [13]. Fifthly, penetration modifiers should work in a unidirectional manner, that is, they should control the input of actives into the body and, at the same time, they should not lead to loss of endogenous material from the body. Penetration modifiers should additionally be compatible with all kinds of excipients and drugs. Beside compatibility, penetration modifiers should be cosmetically acceptable and have good aesthetic appeal [14]. Hitherto, no permeation modifier has been found that possesses all of these characteristics. One of the major issues associated with modifiers is their significant irritancy and in some cases toxicity. One study performed to evaluate the skin irritation potential of different saturated fatty alcohols after application *in vivo* on rat skin revealed that all fatty alcohols increase the transepidermal water loss (TEWL) and skin blood flow significantly. TEWL, skin blood flow and the visual scoring method were the criteria used for evaluating skin irritation potential in the study [15]. There have, however, been some encouraging reports such as that by Moffat *et al.*, which found that silicon analogs of carbon-based penetration modifiers have less irritancy potential [16]. Besides evaluation of the irritation potential of penetration modifiers, *in vitro* studies have been performed to determine toxicity associated with these compounds. For example, Song *et al.* investigated the cytotoxic effects of iminosulfuranes (penetration enhancers) on epidermal keratinocytes and dermal fibroblasts at several concentrations. The study revealed that iminosulfuranes depicted concentration-dependent cytotoxicity and in most cases negligible cytotoxicity was observed below 0.2 M [17]. Unlike short-term toxicity studies of penetration modifiers *in vitro*, there are limited reports on the evaluation of long-term toxicity of modifiers both *in vitro* and *in vivo*. One such study investigating long-term toxicity potential of laurocapram was

performed in mice, rats, guinea pigs, rabbits and monkeys and results indicated that there was no systemic or dermal toxicity observed in animals on 1 month or lifetime dermal exposure to laurocapram [18]. There are also a few studies reporting systemic absorption of penetration modifiers [19]. A pharmacokinetic study performed in healthy human volunteers to evaluate systemic absorption of laurocapram after dermal application revealed that it is poorly absorbed into the human body and the small quantity that is absorbed is readily metabolized and excreted in the urine [20,21]. Despite some concerns over irritation and the toxicity potential of chemical permeation modifiers, interest in their use in formulations is still significant since the approach is economical, relatively simple and, in most cases, reversible.

3. Theory

As mentioned above, the thin (15 – 20 μm), tough and relatively impermeable stratum corneum forms the rate controlling barrier for the passage of nearly all permeants. The dead, flattened, keratin-rich corneocytes embedded in the ordered lipid bilayer arrays are known to provide barrier properties to stratum corneum. Since stratum corneum is composed of non-viable corneocytes, the passage of permeant across it follows passive diffusion governed by physicochemical laws, with no involvement of active transport [22]. There are three major diffusion pathways involved in passage of permeant across SC, namely the appendageal route, the trans-cellular route and the intercellular route [23]. The intercellular pathway is the most prominent pathway in which the diffusant follows a tortuous route with continuous and repeated partitioning in and out of hydrophilic and lipophilic domains of intercellular lipids [24]. The intercellular lipid arrays of stratum corneum are usually a complex mixture of ceramides, cholesterol, cholesterol esters and fatty acids [23,25,26]. The diffusion via the appendageal route is the least common as it involves diffusion of actives across hair follicles, sebaceous glands and eccrine (sweat) glands, which constitute only 0.1% of the absolute skin surface area.

Even though the skin is a complex, heterogeneous membrane, simple Fick's laws of diffusion are often used to describe percutaneous absorption of permeants. Since transdermal delivery involves the application of drug formulation for a long period of time, Fick's first law of diffusion is the most relevant law to describe the steady-state of the diffusant across stratum corneum [27].

$$J = \frac{KD}{h}(Co - Ci) \quad (1)$$

Equation 1 is the mathematical representation of Fick's first law of diffusion where J is the flux per unit area, K is the SC formulation partition coefficient of the permeant, D is the diffusion coefficient in the SC of path length h; Co is the concentration of permeant applied to the skin surface and Ci is the concentration of permeant inside the skin. Practically Co >> Ci, therefore Equation 1 simplifies to:

$$J = k_p Ci \quad (2)$$

Where k_p (= DK/h) is the permeability coefficient that accounts for both partition and diffusion characteristics of the permeant.

Even though permeability across SC is the inherent property of the molecule (based on its partition coefficient and molecular size), percutaneous diffusion of permeants can be altered by the use of permeation modifiers that increase/decrease percutaneous absorption. In the case of permeation enhancers, skin permeability is altered via several mechanisms (**Figure 2**) [28]. Chemical permeation enhancers, especially laurocapram, dimethyl sulfoxide, etc., significantly improve the flux, J (Equation 1), of the permeant by increasing its diffusing coefficient, D (Equation 1), via fluidizing the lipid environment of SC. Similarly, enhancers such as surfactants and terpenes tend to increase diffusion coefficient (D) of the permeant by interacting with intracellular proteins present in SC. Other enhancers such as propylene glycol, lower alcohols, Transcutol® and *N*-methyl pyrollidone increase the permeant flux by increasing partitioning and solubility of the drug in the stratum corneum [24,29]. Unlike permeation enhancers, the exact mechanism for the action of retardants is not clear. However, it is hypothesized that retardants decrease the diffusion coefficient of the drugs by providing order to the lipid–lipid arrangement in the SC [30].

4. Factors affecting the performance of transdermal permeation modifiers

4.1 Molecular shape and size

The shape of a permeation modifier greatly affects its activity as an enhancer or a retardant. For example, graphic molecular representation (**Figure 3**) of laurocapram indicates that it exists in 'soup spoon' or 'bent spoon' conformation [31,32]. In the 'soup spoon' conformation, the carbonyl moiety is oriented in such a way that it moves away from the skin lipid bilayer into the polar region of the skin membrane. Also, laurocapram has a cross-sectional area of 60 Å^2, which corroborates the bent spoon conformation generated by molecular graphics. When laurocapram is applied on the skin surface, it inserts itself into the intercellular lipid bilayer arrays by pushing apart the head groups of ceramides. This disturbance in lipid arrangement creates free volume in the alkyl chain region of the lipid domain that facilitates the diffusion of drugs whose percutaneous absorption was initially limited by slow intercellular diffusion. In contrast, when a potent retardant, N0915 (3-dodecanoyloxazolidin-2-one), is applied topically, it inserts itself easily into the ceramide bilayer structure but,

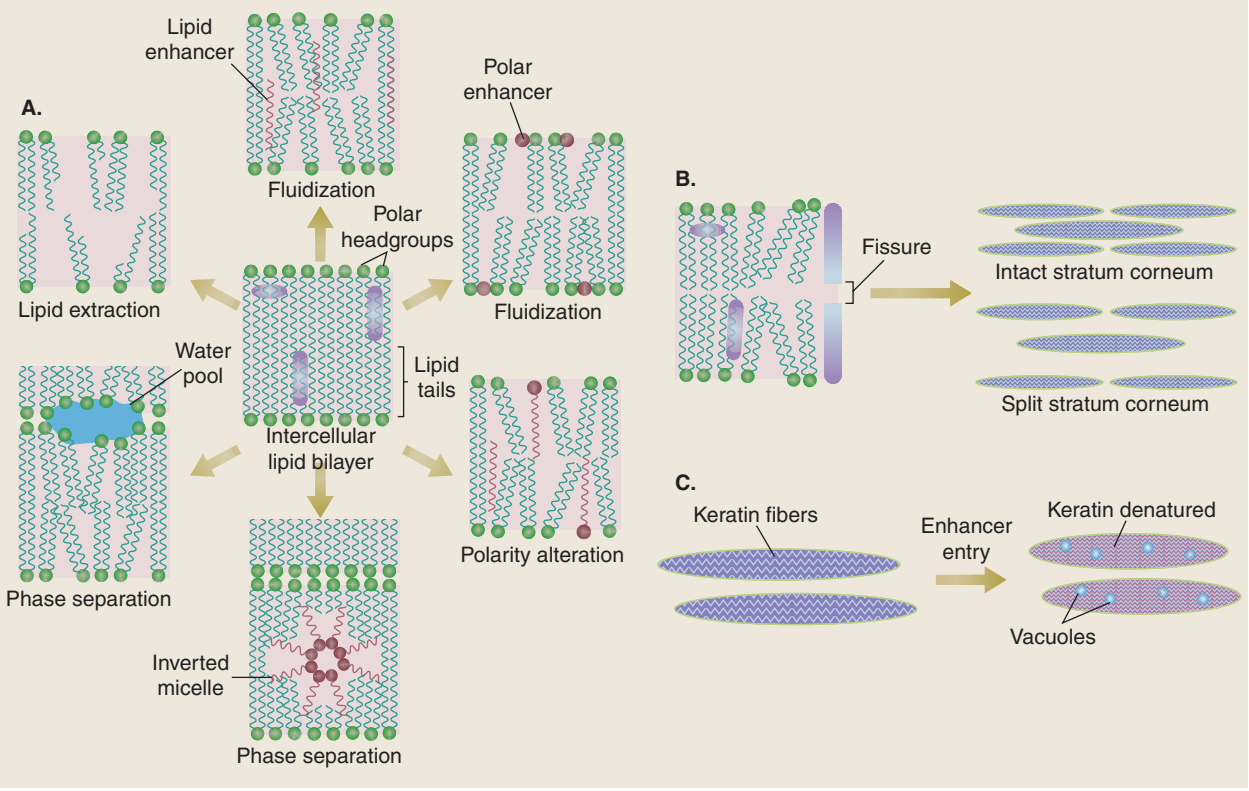

Figure 2. Different mechanisms of action of permeation enhancers. A. Action at intercellular lipids domain of the stratum corneum. **B.** Interaction with protein structures leads to a splitting of the stratum corneum that is clinically unacceptable. **C.** Action within corneocytes leads to swelling, keratin denaturation and vacuolation within the individual horny layer cells of stratum corneum [28].

due to its linear conformation and relatively smaller size (compared to laurocapram), is unable to push the head groups of ceramide molecules apart, leading to no facilitation of diffusion of molecules across the stratum corneum. Furthermore, its insertion into the ceramide arrays strengthens the intercellular lipid arrangement, which subsequently retards the permeation of the diffusant [33,34].

4.2 Hydrogen bonding potential and polarity

Although the high energy 'bent spoon' conformation hypothesis justifies the transdermal enhancement resulting from the use of laurocapram, it cannot explain the favorability for existence of this high energy conformation in the bilayer structure of the packed ceramide molecules of SC [30]. Therefore, an alternative hypothesis for the penetration modifier action of laurocapram and its analogs has been proposed. This hypothesis is based on the H-bonding potential of penetration modifiers with stratum corneum cerebrosides and more importantly on the polarity of penetration modifiers. According to this concept, the formation of an H-bond favors the feasibility of the penetration modifier for organization in the intercellular lipid array of SC and its unipolar or bipolar nature decides its activity as an enhancer or retardant. Among the various cerebrosides present in SC, ceramide 6 is the predominant one. Studies show that it possesses bipolarity into its head group region owing to four secondary alcohol groups and one secondary amide group. It is hypothesized that laurocapram on insertion into the intercellular lipid domain forms an H-bond with these ceramides from one side of the molecule. Since its ring structure is uniformly positive in nature (unipolar), it tends to force apart the ceramide molecules present on either side due to electrostatic repulsion. In contrast, N0915, which is smaller in size than laurocapram and possesses a bipolar ring structure, undergoes a favorable electrostatic interaction with ceramide molecules that pulls the intercellular lipids together and the subsequent condensed state leads to a decrease in skin permeability. **Figure 4** shows that two oxygen containing groups on both sides of N0915 enable it to form hydrogen bonds with groups present in the ceramide molecule and therefore imparts order to the skin lipid bilayer. In contrast, laurocapram has only one oxygen containing group and is only capable of forming a hydrogen bond with ceramide groups from one side, thus providing disorder to the intercellular lipid bilayer [25,30].

4.3 Chemical structure

Although chemical structure influences the property of the molecule such as log P and solubility, there are no reports that relate the behavior of the permeation modifier as

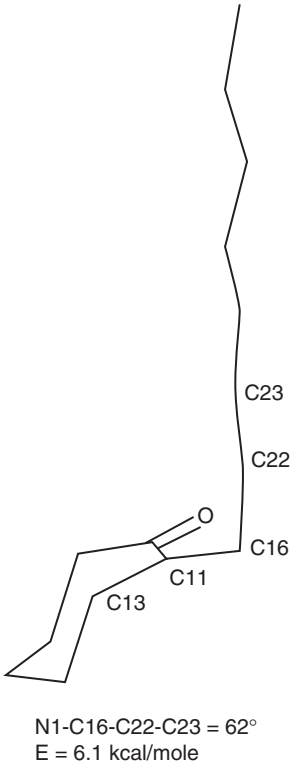

Figure 3. Bent spoon conformation of laurocapram. This figure is modified from [30] where N1 represents nitrogen atom in laurocapram ring and C13, C16, C22 and C23 represent carbon atoms at 13, 16, 22 and 23 positions respectively. N1, C16, C22 and C23 form an angle of 62° that leads to energy of conformation, E equal to 6.1 kcal/mole.

enhancer or retardant with consideration of these physical properties. However, the replacement of one substituent group in the same class of penetration modifiers has been reported to alter their property as enhancer or retardant. For example, Kim *et al.* reported that *S,S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane and *S,S*, dimethyl-*N*-(4-nitrobenzoyl)iminosulfurane, which belong to the class of *N*-aroyliminosulfuranes, enhanced and retarded respectively the permeation of hydrocortisone across the hairless mouse skin [35]. The replacement of bromo group in *S,S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane by a nitro group lead to retardation of the active permeant by threefold. Similarly, Hadgraft *et al.* reported that replacement of the cycloheptane ring of laurocapram by an oxazolidine-2-one moiety changed its activity from an enhancer to a retardant. [30]. In addition, replacement of a more electronegative group by a less electronegative group changed the intensity of the penetration modifier activity. For example, structural activity analysis of laurocapram and its analogs showed that on replacing oxygen by sulfur in the laurocapram structure, a reduced partial net negative charge developed on the molecule, ultimately leading to a loss of its enhancement activity [30].

4.4 Formulation effect

Formulation plays an important role in affecting the degree of enhancement/retardation caused by a penetration modifier. Recent *in vitro* studies in our laboratory on human cadaver skin investigated the effect of three permeation modifiers (laurocapram, N0915, *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane) that were dissolved/suspended in various vehicles: water, ethanol, propylene glycol (PG) and polyethylene glycol 400 (PEG 400), on permeation of diethyl-*m*-toluamide (DEET). The study revealed that solvent systems markedly affect the behavior of permeation modifiers in either enhancing or retarding the permeation of the active across the skin. Results showed that the activity of laurocapram in enhancing DEET permeation decreased from a modifier ratio (MR) of 9.4 in PG to 3.9 in PEG 400. Likewise, N0915 acted as a retardant for diffusion of DEET with ethanol (MR = 0.5) and PEG 400 (MR = 0.5), but not with water (MR = 5.0) or PG (MR = 1.6). Similarly, *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane was a retardant with ethanol (0.9) and PEG 400 (0.9), but not with water (4.6) or PG (2.2) [36,37].

Previous studies by Kim *et al.* have shown that penetration modifiers, namely *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane) behave as retardants on diffusion of hydrocortisone across hairless mouse skin [35]. However, current investigations by Kaushik and Michniak demonstrate both enhancement and retardation effects by these compounds depending on the vehicle in which they were incorporated [36,37]. In the case of N0915, Hadgraft *et al.* reported it to be a retardant of DEET and metronidazole permeation [30], but its activity changed when it was incorporated in PG and water [37]. Therefore, it can be proposed that for a penetration modifier to be regarded as an enhancer, it should always depict enhancement of the permeant, irrespective of the formulation in which it is incorporated and the converse also holds true for the retardants.

5. Characterization of penetration modifiers

Most *in vitro* permeation studies are performed using Franz diffusion cells, which is a classic yet still the most relevant technique for quantifying the degree of enhancement/retardation of percutaneous diffusion of drugs in the presence of penetration modifiers. Besides, there are numerous analytical techniques that are used to characterize the effect of penetration modifiers on intercellular lipids of stratum corneum. A good insight into the different analytical methods used for characterizing penetration modifiers is given by Potts and Guy [38]. Also Touitou *et al.* [39] showed the quantification of drugs in different skin layers using techniques such as autoradiography and ATR–FTIR (Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy) remittance, fluorescence and photo-thermal spectroscopy. Some common techniques that are used to characterize the effects of permeation modifiers in skin are summarized in **Table 1**.

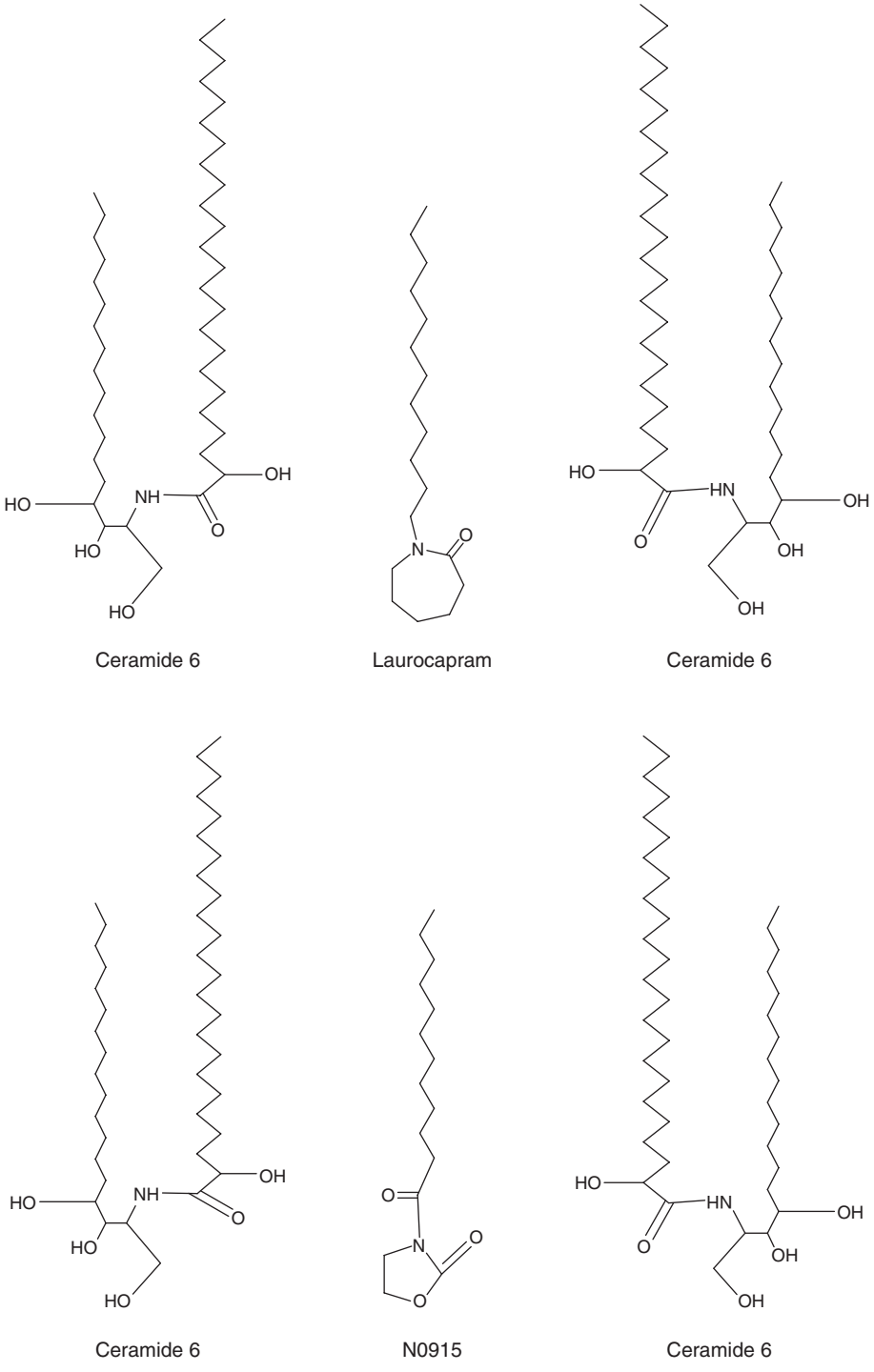

Figure 4. Diagrammatic representation of interaction of penetration modifiers with ceramide 6.

Table 1. Techniques for the characterization of penetration modifiers.

Technique	Description	Ref.
Microscopy	Direct and indirect visualization of the effects of permeation modifiers in the skin	[38,47-49]
Infrared (IR) spectroscopy (especially ATR–FTIR)	Peak heights and areas of symmetric and asymmetric C–H absorbances of the skin are observed before and after penetration modifier treatment	[38,39,48,50-52]
Differential Scanning Calorimetry (DSC)	Thermodynamic parameters of stratum corneum like heat capacity and phase transition temperature are recorded as a function of temperature (thermograms) with and without penetration modifiers	[30,38,49,52]
Impedance spectroscopy	Electrical resistance (impedance) of different layers of skin is measured as a direct function of the modifier effect	[38,53]
Fluorescence spectroscopy	Fluorescence of selected skin layer (e.g., dermis) in the presence of light absorbing drug or the drug itself (in the case of fluorescent molecule) is quantified with the help of carboxyfluorescein assays using a spectrometer, in the presence and absence of permeation modifier	[39,49]
Raman spectroscopy	Modifications induced by permeation modifiers to the lipid layer in the skin are observed as a function of stretching, twisting, scissoring -CH, $-CH_2$ and -C–C vibrations	[17,50,54]
Cytotoxicity assays	Determines *in vitro* toxicity of chemical permeation modifiers on skin cells (dermal fibroblasts and epidermal keratinocytes)	[17,55]
Molecular modeling	The modifier effect is predicted on computer simulated skin using mathematical algorithms	[30,33]

6. Penetration modifiers in drug delivery

Penetration modifiers consist of a diverse group of agents with varying physiochemical properties, structure–activity relationships and mechanisms of action. While penetration enhancers have been studied extensively, little information is available on penetration retarders and their mechanisms. **Table 2** details classes of penetration modifiers with representative compounds and their penetration modifying effects on various agents. This list, although not comprehensive, attempts to summarize the most investigated and important dermal penetration modifiers in the literature.

7. Expert opinion and conclusion

The transdermal approach has appeared attractive to drug delivery scientists not only because of the large surface area of the skin that is potentially available, but also due to the fact that actives that are transported through the skin bypass first pass metabolism, do not face the challenges of gastrointestinal pH changes and can attain sustained drug release. In spite of these advantages, the success of transdermal drug delivery is limited by the barrier properties of the stratum corneum. Both physical and chemical enhancement techniques have been introduced to overcome the resistance offered by the skin, the use of latter being more dominant due to its simplicity and low cost. However, many candidate enhancers have not successfully made the transition from purely research studies to the market, due to skin irritancy issues. Some chemical enhancers have been introduced and commercialized such as SEPA009® (Macrochem), NexAct88® (NexMed) and SR38® (Pharmetrix) [25]. Besides the development of enhancers, compounds termed retardants have emerged that have the potential to condense the lipids of stratum corneum and retard the permeation of the active. Hitherto, the known retardants such as N0915 and *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane are structural analogs of known enhancers laurocapram and dimethyl sulfoxide. The activity of a penetration modifier as an enhancer or retardant is based on its shape, H-bonding potential, polarity, chemical structure and the accompanying formulation. Recent studies in our laboratory revealed that the solvent in which the enhancer/retardant is formulated markedly affects its behavior as an enhancer or retardant. For instance, our results indicated that N0915 acted as a retardant of DEET permeation when formulated in ethanol and PEG 400, whereas it enhanced DEET permeation when formulated in water and PG. These results suggest that enhancers/retardants should be referred to as dermal permeation modifiers, as they are liable to change their permeation activity depending on their formulation [36].

Besides acting as a protective barrier to the body, the skin also plays an extensive role as a metabolizing organ, which is corroborated by reports of successful delivery of prodrugs across the skin [40,41]. Studies performed in isolated [42] or cultured keratinocytes [43] have revealed that most of the biotransformation activity occurs within the viable epidermis. Some of the biotransformation activity is also exhibited by sebocytes [44], fibroblasts and melanocytes, but the enzymatic activity is relatively lower than that depicted in viable epidermis [45]. Biotransformation occurring in the viable epidermis corresponds to Phase I and Phase II type reactions, with cytochrome P450 involved in Phase I reactions. Studies performed by Zhu *et al.* on rat whole skin and cultured

Table 2. Selected penetration modifiers with their mechanisms of action and penetration modifier ratios (PMR).

Penetration modifiers used (category and examples)	Suggested mechanism of action	Drugs tested (enhancer/retardant, *PMR**)
Enhancers		
Alcohols, fatty alcohols and glycols		
Alcohols and fatty alcohols: (ethanol, butanol, propanol, octanol, lauryl alcohol)	Alcohols with carbon chains C1 – C6 act as solvents or alter other thermodynamic properties of the molecule Alcohols with carbon chains > C6 act by increasing diffusion due to extraction of lipids	Mefanamic acid (ethanol 10%, *2.42*) [56] Melatonin (octanol 5%, *4.22*) [57] Melatonin (lauryl alcohol 5%, *3.82*) [57]
Glycols: propylene glycol	Solvate the keratins in SC, resulting in occupation of hydrogen binding sites Alter the thermodynamic activity of the drug in the vehicle, increasing diffusion	Bupranolol (propylene glycol 10%, *2.5*) [58]
Sulfoxides		
Dimethylsulfoxide (DMSO), Decylmethylsulfoxide (DCMS)	DMSO: Changes keratin confirmation from α helical to β sheet, interacts with lipid domains in SC to distort the packing geometry and also acts as a solvent within the skin tissue	Diclofenac sodium (DMSO 10%, *1.02*) [59]
DMSO-related compounds (iminosulfuranes) *S,S*-Dimethyl-*N*-(4-chlorbenzenesulfonyl) iminosulfurane *S,S*-Dimethyl-*N*-(5-nitro-2-pyridyl)iminosulfurane		Hydrocortisone (*S,S*-Dimethyl-*N*-(4-chlorbenzenesulfonyl) iminosulfurane, *21.03*) [35]
Fatty acids		
Oleic, linoleic, linolenic, lauric, myristic, stearic, undecanoic acids	Interact with the lipid domains leading to perturbation of the bilayers Oleic acid also increases lipid fluidity by decreasing their phase transition temperatures Higher concentrations of oleic acid are postulated to exist as a separate phase within the lipid bilayers, leading to permeability defects	Melatonin (Oleic Acid 5%, *5.35*) (linoleic acid 5%, *6.58*) (linolenic acid 5%, *7.54*) (lauric acid 5%, *6.11*) (undecanoic acid 5%, *8.57*) [60]
Surfactants		
Anionic (sodium lauryl sulfate) (SLS)	Penetrate into the skin and extract water soluble agents that act as plasticizers Interact with and bind to epidermal proteins Cause damage to the skin leading to high irritation potential	Lorazepam (SLS 5%, *11.66*) [61]
Cationic (cetyl trimethyl ammonium bromide (CTAB), benzalkonium chloride)	Interact with the proteins, lipid lamellae and other components of the SC Cause more damage to the skin than anionic surfactants	Lorazepam (CTAB 5%, *10.16*) [61]

*Penetration modifier ratios (PMR) are listed in italics in the table.

$$\text{Penetration modifier ratio (PMR)} = \frac{\text{flux of agent in presence of penetration modifier}}{\text{flux of agent in absence of penetration modifier}}.$$

$$^{\ddagger}\text{(PMR)} = \frac{\text{Kp of agent in presence of penetration modifier}}{\text{Kp of agent in absence of penetration modifier}}.$$

Kp = permeability coefficient.

$$^{\S}\text{(PMR)} = \frac{\text{Amount of penetrated agent in presence of penetration modifier}}{\text{Amount of penetrated agent in absence of penetration modifier}}.$$

Table 2. Selected penetration modifiers with their mechanisms of action and penetration modifier ratios (PMR) (continued).

Penetration modifiers used (category and examples)	Suggested mechanism of action	Drugs tested (enhancer/retardant, *PMR**)
Zwitterionic (dodecyl botanies)		
Nonionic (Polysorbates 20, 60, 80), Tween (20, 40, 60, 80), Polyxamer (231, 182), Brij (93, 96)	Solubilize and extract membrane components Increase membrane fluidity and reduce diffusional resistance Emulsify sebum in the skin, enhancing the thermodynamic activity of drugs	Hydrocortisone (Polysorbate 60, *2.52*) [62] Lorazepam (Tween 80 0.5%, *2.33*) [61]
Terpenes		
1,8-Cineole, carvone, menthol, limonene	Modify the solvent nature of the SC, improving drug partitioning into tissue Disrupt SC bilayer lipids, increasing diffusivity Open polar pathways in and across SC Act as solvents, altering the thermodynamic activity of the drug Act on the polar head groups of lipids leading to subsequent disruption of the interlamellar and intralamellar hydrogen bonding networks	Tamoxifen (1,8-Cineole 20%, *7.03*) [24] Caffeine (carvone 10%, *12.4*) [63,64]
Amides		
Urea, dimethylformamide (DMF), dimethylacetamide (DMA), dodecylisobutyramide, dodecyl(2-methoxyethyl)acetamide	Urea functions as a hydrating agent and has keratolytic properties thereby creating diffusion channels DMA and DMF disrupt the lipid packing and increase the fluidity of lipids	Progesterone (Urea 5%, *2.7*) [65] Hydrocortisone (dodecyl (2-methoxyethyl)acetamide, *35.2*) [66]
Cyclic amides		
Azone (1-dodecylazacycloheptane-2-one) and analogs	Azone partitions into the lipid bilayers, disrupts their packing and increases membrane fluidity Azone has been shown to be an enhancer for both hydrophilic and hydrophobic drugs	Hydrocortisone (Azone *19.5*) [66] 5-Fluorouracil (Azone *24.21*) [67]
Pyrrolidone and derivatives		
N-methyl-2-pyrrolidone (NMP), 2-pyrrolidone (2P), *N*-dodecyl-2-pyrrolidone	Increase permeation by interacting with keratins and lipids in the skin Pyrrolidones also form reservoirs within the skin membranes leading to a potential sustained release effect on drug release	Hydrocortisone (*N*-dodecyl-2-pyrrolidone, *42*‡) [68]
Miscellaneous enhancers		
DDAA (Dodecyl dimethylaminoacetate) DDAIP (Dodecyl-2-(*N,N*-dimethylamino) propionate)	DDAIP interacts with the polar region of the phospholipid bilayer Increases the motional freedom of lipid hydrocarbon chains	Indometacin (DDAIP) [69]

*Penetration modifier ratios (PMR) are listed in italics in the table.

$$\text{Penetration modifier ratio (PMR)} = \frac{\text{flux of agent in presence of penetration modifier}}{\text{flux of agent in absence of penetration modifier}}.$$

$$^{\ddagger}\text{(PMR)} = \frac{\text{Kp of agent in presence of penetration modifier}}{\text{Kp of agent in absence of penetration modifier}}.$$

Kp = permeability coefficient.

$$^{\S}\text{(PMR)} = \frac{\text{Amount of penetrated agent in presence of penetration modifier}}{\text{Amount of penetrated agent in absence of penetration modifier}}.$$

Table 2. Selected penetration modifiers with their mechanisms of action and penetration modifier ratios (PMR) (continued).

Penetration modifiers used (category and examples)	Suggested mechanism of action	Drugs tested (enhancer/retardant, *PMR**)
Retardants		
Iminosulfurane analogs: *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane (R-1) *S,S*-dimethyl-*N*-benzenesulfonylimino sulfurane (R-2) *S,S*-dimethyl-*N*-(4-chlorobenzenesulfonyl) iminosulfurane (R-3)	Postulated to decrease drug partitioning into the skin by imparting order to skin lipids	Hydrocortisone (R-1, 0.12, R-2, *0.5*, R-3, *0.5*) [35]
Laurocapram analog: N0915	Modification of hydrogen bonding within the SC lipids producing condensation of the lipid lamellae	Metronidazole (0.2)[§][30]

*Penetration modifier ratios (PMR) are listed in italics in the table.

$$\text{Penetration modifier ratio (PMR)} = \frac{\text{flux of agent in presence of penetration modifier}}{\text{flux of agent in absence of penetration modifier}}.$$

$$^{\ddagger}(\text{PMR}) = \frac{\text{Kp of agent in presence of penetration modifier}}{\text{Kp of agent in absence of penetration modifier}}.$$

Kp = permeability coefficient.

$$^{\S}(\text{PMR}) = \frac{\text{Amount of penetrated agent in presence of penetration modifier}}{\text{Amount of penetrated agent in absence of penetration modifier}}.$$

epidermal keratinocytes (isolated from rat) indicated that enzymatic activity in isolated epidermal keratinocytes attained similar levels as whole skin after culturing them for 10 – 14 days. This suggests the use of 10 – 14-day-old primary keratinocytes culture as a better experimental model for the investigation of P450 catalyzed metabolism of xenobiotics in skin [46]. Unlike therapeutic agents, there have been no reports about cutaneous biotransformation of permeation modifiers. However, there are studies being performed in Michniak's group to investigate the activity of percutaneous modifiers on cutaneous bioconversion that may further increase or decrease the percutaneous permeability of the modifier, as well as that of the active.

Although advancement in the field of analytical and microscopic techniques has resulted in improved understanding of the barrier properties of the skin, the reason why less resistance is offered by SC to certain agents, especially agrochemicals, cosmetics, chemical warfare agents, certain pharmaceuticals and household cleaning agents, is still unclear. Efforts should be made to design a formulation that would minimize the risks associated with these compounds. In order to achieve these goals, there is a need for exploration of novel penetration modifiers that are not only limited to enhancers that compromise the barrier function of SC, but also extend to the agents that can strengthen the barrier properties of skin. Since the skin is the protective organ of body against xenobiotics, research should focus on the aspects of reversibility of action of penetration modifiers on termination of application of formulation containing these agents. The thorough and absolute understanding of the barrier properties of the skin, along with its metabolism, would aid in designing pharmaceutical, cosmetic and other consumer products that would not only be more effective, but also perhaps less irritating to the skin membrane.

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