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Review Article

An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action

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Abstract

Transdermal drug delivery has attracted considerable attention over the past 2-3 decades in regard of its many potential advantages. However, the role of the skin as a protective barrier renders skin absorption of most drugs problematic. Therefore, skin penetration enhancers are frequently used in the field of transdermal drug delivery in order to reversibly reduce the barrier function of the stratum corneum, the outermost layer of the skin. To date, a wide range of chemical compounds have been shown to enhance the skin penetration of therapeutic drugs. This review presents a critical account of the most commonly used chemical penetration enhancers (fatty acids and surfactants), and some newer classes of chemical enhancers (terpenes, polymers, monoolein, oxazolidinones), with emphasis on their efficacy, mechanism of action, and skin irritation potential. This review also discusses the traditional and more recently developed methods for the screening and evaluation of chemical penetration enhancers, and addresses the continuing problems in the rational selection of a chemical penetration enhancer for a specific drug to be delivered via the transdermal route.

Keywords: skin penetration enhancer, stratum corneum, mechanism of action, skin irritation

1. Introduction

Over the last 2-3 decades, the skin has become an important route for the deliver of drugs for topical, regional, or systemic action. The skin, however, has evolved as a physical and biochemical protective barrier, which prevents the loss of water from the body, and guards against entry into the body of external toxic chemicals and infectious agents, thereby maintaining homeostasis. This role of the skin as a barrier to the external environment renders the absorption and transdermal delivery of most drugs problematic. The stratum corneum (Figure 1), which is the outermost layer of the skin and comprised of keratin-rich cells embedded in multiple lipid bilayers, has been considered the rate-limiting structure governing percutaneous absorption of many kinds of permeants (Barry, 1983). For most permeants, except for those that are highly lipophilic, the intercellular lipids, which account for 5-30% of the total tissue volume (Elias and

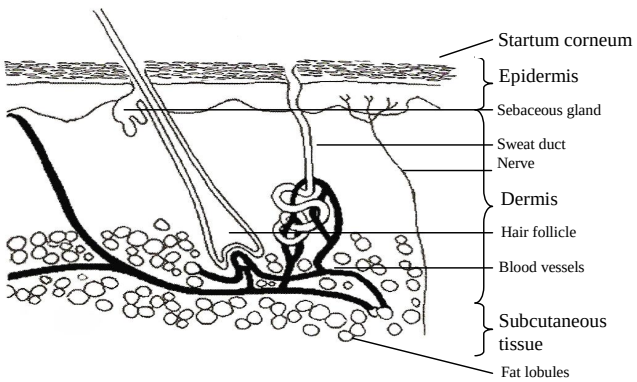

Figure 1. Diagrammatic representation of the cross-section of human skin.

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Leventhal, 1980), provide the rate determining component in the skin barrier function (Scheuplein and Blank, 1971; Elias, 1981). Many different approaches have been established in order to overcome the barrier presented by the skin, involving chemical and physical enhancement strategies (Williams and Barry, 2004). The former strategy involves chemical methods, including penetration enhancers (Hadgraft, 1999), prodrugs (Xu and Chien, 1991), colloidal formulations such as liposomes, niosomes and microemulsions (Cevc, 2004), and supersaturated systems (Pellett *et al*., 1994; Hadgraft, 1999). The latter strategy involves physical methods, including phonophoresis (Byl, 1995), electroporation (Banga *et al*., 1999), iontophoresis (Banga *et al*., 1999), magnetophoresis (Murthy *et al*., 2001), microfabricated needle (Henry *et al*., 1998; Tao and Dasai, 2003), and laser technologies (Lee *et al*., 2008). Of these two strategies, the use of chemical penetration enhancers is technically simpler, and therefore a popular technique (Yamane *et al*., 1995a). Combinations between chemical penetration enhancers and physical methods such as iontophoresis and phonophoresis have been shown to substantially enhance the skin penetration of several permeants (Trommer and Neubert, 2006). For example, the *in vitro* permeation of luteinizing hormone releasing hormone (LHRH) through human epidermis was greatly increased when enhancers (oleic acid/propylene glycol) and iontophoresis were used together (Smyth *et al*., 2002). Delivery of drugs via the skin has numerous advantages, like non-invasiveness, better patient compliance, potential for continuous or controlled delivery, and potential for delivery of certain classes of drugs that are not amenable for delivery via other routes of drug delivery (Tanner and Marks, 2008). Penetration enhancers have therefore frequently been used in the field of transdermal drug delivery research and various types of penetration enhancers with different modes of action classified (Chattaraj and Walker, 1995).

Before discussing the mechanism of action of chemical enhancers, it is important to understand the potential routes of dermal drug permeation. Theoretically, the diffusion of drugs across the normal intact skin involves two possible macro routes, the transappendageal and the transepidermal routes (Williams and Barry, 1992). The transappendageal pathway comprises transport via sweat glands and along the hair follicles with associated sebaceous glands. Their fractional area available for the drug transport is only about 0.1% of the total skin surface area. The transepidermal pathway comprises the intercellular route, in which the drugs diffuse via the lipid domain between the corneocytes, and the transcellular route, in which the drugs diffuse across the corneocytes and the lipid matrix. The intercellular route is believed to be the major pathway for the drug permeation. Although hypotheses concerning the penetration of substances into the skin have assumed diffusion through the lipid domains of the stratum corneum as the most important pathway, recent studies (Otberg *et al*., 2008) using a novel approach involving complete blocking of hair follicles have shown that certain hydrophilic drugs can in fact be delivered rapidly via the hair follicles.

2. Definitions and ideal properties of chemical penetration enhancers

Penetration enhancers (also called accelerants or sorption promoters) are defined as substances that are capable of promoting penetration of drugs into skin, or their permeation through skin, by reversibly reducing the skin barrier resistance. An ideal penetration enhancer should have the following properties (Barry, 1983; Pfister and Hsieh, 1990a; Finnin and Morgan, 1999):

1) It should be pharmacologically and chemically inert, and chemically stable.

2) It should be non-toxic, non-irritant, non-comedogenic and non-allergenic.

3) It should have a rapid onset of action, predictable duration of activity, as well as a reproducible and reversible effect.

4) It should be chemically and physically compatible with the formulation ingredients.

5) After it is removed from the skin, the stratum corneum should rapidly and fully recover its normal barrier property.

6) It should be odorless, tasteless, colorless, and inexpensive.

7) It should be pharmaceutically and cosmetically acceptable.

8) It should have a solubility parameter similar to that of skin (e.g., 20.5 $MPa^{1/2}$ (Liron and Cohen, 1984)).

In spite of the fact that a variety of compounds have been proposed as skin penetration enhancers, to date, no substance has been found to possess all the aforementioned ideal properties. Nevertheless, many known and newly developed compounds have been assessed for their enhancing abilities and some have shown more promising characteristics.

3. Mode of action of penetration enhancers

It is generally recognized that penetration enhancers enhance the permeation of drug across the skin via several mechanisms. However, to date the exact mechanisms of action of penetration enhancers are only partially known.

Skin penetration enhancers may exert their effects through one or a combination of the following mechanisms (Barry, 1987; Guy and Hadgraft, 1987; Barry, 1991a; Ghosh and Banga, 1993). The first suggested mechanism is solvent action. The penetration enhancers may plasticize or solubilize the skin-tissue components. The second proposed mechanism is the interaction of enhancers with intercellular lipids leading to disruption of the highly ordered lamellar structure, thereby increasing the diffusivity of drugs through the lipid domain. The third proposed mechanism is the interaction of enhancers with intracellular protein to promote permeation of drugs through the corneocyte layer. The fourth proposed

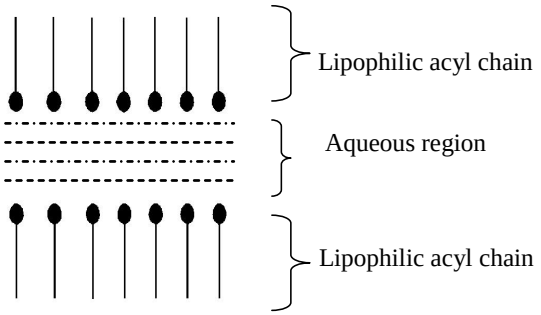

Figure 2. Diagrammatic representation of the lipid bilayers of human stratum corneum.

mechanism is an increase in the partitioning of drugs, co-enhancers or co-solvents into the stratum corneum. The latter three mechanisms have been described as the lipid-protein-partitioning (LPP) theory proposed by Barry (1991a, b). In this theory, various possible locations of enhancer action within the intercellular and intracellular regions of stratum corneum have been proposed. In the intercellular region, three active sites where a penetration enhancer may act in order to enhance the permeation of permeants have been suggested. These three active areas are the area of polar head groups of the lipids, the aqueous region between the lipid head groups, and the lipid region of the hydrophobic tails within the bilayers (Figure 2). In the case of the intracellular region, since the intercellular region of the stratum corneum is composed of keratin, penetration enhancers such as surfactants and aprotic solvents may interact with polar head groups of the keratin. These interactions result in the reduction of the binding forces between protein molecules and thereby changing the conformations of the protein helices (Barry, 1991a). In addition to the LPP theory, an alternative mechanism has been proposed by Guy and Hadgraft (1989), in which an enhancer may alter the solvent nature of viable epidermal and dermal tissue and promote the partitioning of a lipophilic drug from the stratum corneum into the deeper layer of the skin. Furthermore, a phase separation for lipophilic molecules such as oleic acid within the ordered stratum corneum lipid bilayers has been suggested (Ongpipattanakul *et al*., 1991; Walker and Hadgraft, 1991).

The mechanism of action can be assessed further by considering Fick's law of diffusion

$$J = \frac{DK\Delta C}{h}$$

where J is flux per unit area, D is diffusion coefficient of the drug in the skin, K is skin/vehicle partition coefficient, ΔC is the concentration difference across the skin, and h is the thickness of the skin (stratum corneum) or the diffusion path length in the skin (Hadgraft, 2001).

According to Fick's law, enhancement in the permeability of the drug can be achieved by altering any or all of the three parameters D, K, or h. The improvement of the drug permeation could be due to an increased diffusion within the skin, an increased partitioning or a decrease in diffusion path length. In an ideal system, diffusion path length or skin thickness is constant as the exact thickness is difficult to measure. An increase of diffusion coefficient results in an increased speed of transport through the stratum corneum. An increase of partition coefficient results in an increased amount of drug into the stratum corneum. Some enhancers might affect the diffusion coefficient, whereas others might affect the partition coefficient or the diffusion path length. Furthermore, some enhancers might affect both diffusion coefficient and partition coefficient. Many chemical enhancers including Azone, dimethyl sulfoxide (DMSO, at concentration above 50%), and terpenes have been shown to increase the diffusion coefficient by disordering the lipids in the stratum corneum (Harrison *et al*., 1996; Hadgraft, 2001; Moser *et al*., 2001). Some enhancers, such as DMSO at concentration above 40%, propylene glycol and Transcutol, have been found to increase the partitioning of drugs into the skin (Anigbogu *et al*., 1995; Harrison *et al*., 1996; Hadgraft, 2001). Oleic acid, which is a fatty acid enhancer, has been shown to improve both diffusion and partition parameters of a model drug 6-mercaptopurine for the nonpolar route of the stratum corneum in an *in vivo* skin penetration study using Wistar rat (Yamashita *et al*., 1995). However, several studies indicate that oleic acid's mechanism of action involves a change in the diffusion parameters (Mak *et al*., 1990; Hadgraft, 2001).

4. Research methodologies for studying penetration enhancers

The procedures that have been used in studying chemical enhancers can be divided into three areas: (a) screening for new chemical enhancers, (b) methods for understanding mechanism of action, and (c) methods for toxicity testing.

4.1 Screening for new chemical enhancers

Traditionally, the search for potential chemical enhancers can be carried out using Franz diffusion cell, which is basically composed of donor and receptor compartments with a piece of animal or human skin clamed between these two phases. The drug preparation containing the penetration enhancer at different concentrations is placed in the donor cell, and at suitable time intervals aliquots from the receptor fluid are withdrawn to measure the amount of drug that permeated across the skin using suitable analytical techniques. These include high performance liquid chromatography (HPLC), liquid scintillation counting (if radiolabelled drug is available) and ultraviolet (UV) or fluorescence spectroscopy. Such measurements and skin permeation studies can be exceptionally labor intensive, time consuming, and costly. As a result, novel techniques have been developed and proved to be valuable in identifying new potential skin penetration enhancers. These techniques include high-throughput

methods (Karande and Mitragotri, 2002; Karande *et al*., 2004) and electrical resistance-based methods (Rachakonda *et al*., 2008). A high-throughput method was developed by Karande and Mitagotri (2002), whereas the electrical resistance-based method was developed later by Rachakonda *et al*. (2008). These two techniques can identify the potential chemical enhancers by determining the changes in electrical conductance of the skin or the changes in electrical resistance of the skin, respectively. Both electrical conductivity and electrical resistance have long been used to assess the integrity of the skin prior to *in vitro* permeation studies. Likewise, the effects of chemical enhancers on the barrier properties of the skin can be elucidated by measuring these electrical changes in the presence of potential penetration enhancers. It was concluded that the high-throughput technique allowed rapid screening of penetration enhancers for transdermal drug delivery (Karande and Mitragotri, 2002). The authors also suggested the feasibility of using this technique to discover new and effective enhancer mixtures. Karande *et al*. (2004) have subsequently demonstrated that the high-throughput technique was over 100-fold more efficient than the more commonly used Franz diffusion cell method in the discovery of penetration enhancer mixtures. These newer faster methodologies have enabled the discovery of synergistic behavior of mixtures of known penetration enhancers, and led to the development of such mixtures for transdermal delivery of macromolecules (e.g. peptides and proteins).

4.2 Methods for understanding mechanism of action

Effects and mechanisms of action of chemical enhancers have been investigated using a variety of techniques. These include permeation studies, vasoconstrictor assay differential scanning calorimetry (DSC), infrared spectroscopy, X-ray diffractometry, and electron spin resonance spectroscopy.

4.2.1 Permeation studies

The effect of chemical enhancers on the skin can be assessed by *in vitro* and *in vivo* permeation studies. The former involve excised skins in diffusion chambers, whereas the latter employ live animals or human subjects *in situ*. In the case of *in vitro* permeation study, several types of diffusion cells made out of glass, stainless steel or Teflon® have been designed. These include vertical diffusion cells (side-by-side), Franz diffusion cells and flow-through diffusion cells. Among these three types, the Franz cell is the most popular model for studying the diffusion of permeant across the skin. Unlike *in vitro* studies, *in vivo* permeation studies with chemical penetration enhancers are seldom performed, particularly in human subjects. This is because of the difficulty in regulating experimental factors, such as drug delivery and analysis, experimental design and skin temperature. Nevertheless, some *in vivo* experiments have been performed to investigate the effects of particular chemical enhancers on animal models (Huang *et al*., 1999; Ogiso *et al*., 2001).

4.2.2 DSC

The DSC technique has been used to investigate how enhancers interact with the stratum corneum. It has provided useful information regarding the structure of the stratum corneum. Typically, a DSC thermal profile of hydrated human stratum corneum is composed of four major endothermic transitions, namely T1, T2, T3, and T4. The endotherm T1 (35-42^{o}C) is attributed to the melting of lipid/fat contamination of the samples and is not important in elucidating the mode of action of chemical enhancers. The endotherms T2 (60-77^{o}C) and T3 (70-90^{o}C) are attributed to the melting of bilayer lipids, whereas the endotherm T4 (95-120^{o}C) is associated with protein conformation (Golden *et al*., 1986; Goodman and Barry, 1986; Al-Saidan *et al*., 1998). Differences between lipid transition temperatures of humans and animals have been reported (Al-Saidan *et al*., 1998). The interaction between the chemical enhancers and the skin can be examined by measuring the thermal transitions in the presence of the enhancers. The mode of action of a variety of chemical enhancers (e. g. surfactants and terpenes) has been evaluated by the DSC technique (Barry and Williams, 1993; Kim *et al*., 2008).

4.2.3 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy can be useful for studying the interaction between chemical enhancers and the stratum corneum (Clancy *et al*., 1994). Many of the infrared (IR) spectral bands of the stratum corneum can be attributed to the lipid or protein molecular vibrations. Some spectral regions of interest are the IR peaks near 2850 cm^{-1} and 2920 cm^{-1} owing to symmetric and asymmetric methylene group (H-C-H) stretching, respectively. Golden *et al*. (1986) suggested that the main contribution to the C-H stretching peaks of the stratum corneum was the absorbance of the hydrocarbon chains of the stratum corneum lipids (Golden *et al*., 1986).

The FTIR spectral parameters that can be used as indicators of relative lipid acyl chain disorder are a blue shift of C-H stretching absorbances, a bandwidth at 70% height of the C-H stretching absorbances (Knutson *et al*., 1985), and a ratio of the intensities of the C-H asymmetric and symmetric absorbances (Clancy *et al*., 1994). Furthermore, the heights and areas of these C-H asymmetric and symmetric stretching absorbance peaks correspond to the amount of the lipids present in the stratum corneum. Accordingly, any extraction of the stratum corneum lipids by chemical enhancers results in a decreased peak height and area of these absorbances (Levang *et al*., 1999). Changes in the IR spectrum provide information at the molecular level whereas transitions in the DSC thermal profile provide information at the macroscopic level. When these two techniques have been used together,

they can provide independent and complementary information about the structure of the stratum corneum (Golden *et al*., 1986; Kim *et al*., 2008). FTIR spectroscopy has been used to evaluate mechanisms of action of several chemical enhancers including propylene glycol, ethanol, terpenes (Bounoure, *et al*., 2008; Kim *et al*., 2008). The FTIR technique can also be used to evaluate the effects of chemical enhancers in human volunteers.

4.2.4 Electron spin resonance spectroscopy

Electron spin resonance spectroscopy (ESR) is a form of absorption spectroscopy used for studying a variety of biological membranes. Like most biological membranes, the stratum corneum has no paramagnetic components. For this reason, a molecule with a stable paramagnetic group, known as spin-labeling agent, has to be specifically incorporated with the lipid or the lipid part of the biological membrane. Generally, 5, 7, 12, and 16-doxyl stearic acids, which are fatty acids with a nitroxide free radical group, have been used as lipid spin-labeled reagents (Quan and Maibach, 1995; Quan *et al*., 1995). ESR technique can provide information about phase transitions and polarity of microenvironments surrounding the spin labels. In addition, molecular interactions within the stratum corneum can be obtained by using this technique.

The action of chemical enhancers on the stratum corneum can be examined by measuring changes in the ESR spectra of membrane-incorporated spin labels. ESR has been used to investigate the mechanism of action of several penetration enhancers in human stratum corneum such as Azone (Quan and Maibach, 1995) and surfactants (Kawasaki *et al*., 1997; Mizushima *et al.*, 2000).

4.2.5 X-ray diffractometry

Small and wide angle X-ray diffraction is valuable as a tool for studying molecular interactions and packing of molecules within the stratum corneum. It provides information about the structure and organization of biological lipid assemblies. X-ray techniques have been used to elucidate the mode of action of a variety of chemical enhancers. These include terpenes, Azone, and its derivatives, (Cornwell *et al*., 1994; Bouwstra *et al*., 1996Cornwell *et al*., 1996).

4.2.6 Vasoconstrictor assay

Previously used to evaluate the activity and bioavailability of corticosteroid formulations, the vasoconstrictor or blanching test has been applied for study the action of chemical enhancers. The test has been used for drugs that can elicit a local vasoconstriction effect, and therefore only a limited number of drugs (e. g. corticosteroids) can be assessed by this technique. The effect of several chemical enhancers (e. g. Azone, oleic acid) on the bioavailability of betamethasone-17-benzoate was demonstrated (Bennett *et al*., 1985). In another study, the skin blanching measurement was used to elucidate the effect of propylene glycol enhancement on the bioavailability of topical betamethasone 17-valerate under occluded and non-occluded conditions (Haigh and Smith, 1995). As with FTIR spectroscopy, the blanching test can be applied to *in vivo* evaluation in human volunteers.

4.3 Methods for toxicity testing

In addition to the efficacy, the safety of chemical enhancers is a vital issue. It is generally recognized that the potential toxicity of some chemical enhancers limits their uses in dermatological or cosmetic preparations. Due to the defensive function of the skin, it can interact with several compounds including chemical enhancers. Since chemical enhancers are not selective towards the dead cells of the stratum corneum, they may induce several skin responses, such as irritation, rashes, and inflammation when penetrating through the viable epidermal layers of the skin. The skin irritation potential, and possible damage produced by the application of the chemical enhancers can be assessed by several techniques, including *in vivo* Draize test method (Bashir and Maibach, 2001), *in vivo* histopathological examination (Phillips and Michniak, 1995), *in vivo* laser doppler velocimetry (Tanojo, *et al*., 1998), *in vivo* bioengineering methods such as transepidermal water loss (TEWL) and electrical capacitance (Bashir and Maibach, 2001; Panchagnula *et al*., 2005), and *in vitro* cell culture techniques (Robinson *et al*., 2001).

The *in vivo* methods can be carried out in human or animal models. The main problem of human testing is the high cost. In the past decade, animal testing has been criticized by animal-rights activists for being inhumane. In recent years, animal testing for toxic effects in cosmetic products has been banned in several countries, such as countries in the European Union. In view of this, *in vitro* cell culture techniques have been developed as alternative procedures for assessing the skin irritation responses. Several high quality culture systems (i. e. EpiDerm and Episkin) have been constructed and evaluated through meticulous validation studies (Robinson *et al*., 2001).

5. Classification of penetration enhancers

A large number of compounds have been reported to increase the penetration of drugs through the skin, and therefore a simple, relevant system for classification of compounds is essential. Several classification systems have been used in the literature. Lambert *et al*. (1993) divided most penetration enhancers into three classes, namely simple fatty acids and alcohols, weak surfactants containing a moderately sized polar group (e.g. Azone), and those enhancers that function mainly as solvents and hydrogen bond acceptors (e.g. dimethylsulfoxide, dimethylacetamide, and dimethylformamide). Hori *et al*. (1989) classified penetration enhancers into three distinct areas, Area I, Area II, and Area III,

according to a conceptual diagram. The construction of this diagram was based on the "organic" and "inorganic" characters of compounds, with the organic character depending on carbon atoms and the inorganic character depending on substituted groups. With respect to this diagram, Area I consists of solvent-type enhancers such as dimethylsulfoxide, ethanol, propylene glycol, and *N*-methyl pyrrolidone. Area II comprises enhancers for hydrophilic drugs such as Azone, oleic acid, and lauryl alcohols. As suggested by Barry and Williams (1995), alcohol and ketone terpenes could be categorized in Area II. Area III is composed of enhancers for lipophilic drugs including hydrocarbon terpenes. For the other terpenes such as oxides, the use of the conceptual diagram to predict their skin penetration enhancing activity may be misleading (Barry and Williams, 1995). Pfister and Hsieh (1990a, b) categorized penetration enhancers as either polar or nonpolar based on the Hildebrand solubility parameter. Chattaraj and Walker (1995) categorized penetration enhancers into 10 classes according to their chemical structures; sulfoxides, alcohols, polyols, fatty acids, fatty acid esters, amides, surfactants, terpenes, alkanols and organic acids. Asbill and Michniak (2000) have suggested that chemical enhancers may be placed into several groups depending on their activity.

Classification of chemical enhancers based on their chemical structures can be considered as the most promising system in comparison with the other categorizations. Overall, it is the simplest and easiest system that allows rapid identification. For the systems recommended by Hori *et al*. (1989), or Asbill and Michniak (2000), experimental data concerning their enhancing action is required prior to the classification. The system suggested by Lambert *et al*. (1993) is not appropriate as many new chemical enhancers, both natural and synthetic, have been discovered. Some enhancers with sophisticated structures cannot be easily categorized into one of the three classes. In this overview, the skin penetration enhancers are categorized according to their chemical groups, similar to the classification of Chattaraj and Walker (1995).

6. Types of penetration enhancers

One of the main reasons for the current limited use of the skin as a portal for systemic delivery of drugs is that very few of the chemical penetration enhancers to date have ideal properties (see Table 1). In some cases the concentrations of enhancers required for effective flux of therapeutic substances are very high (i.e. dimethyl sulfoxide > 60%), many of these chemical penetration enhancers cause too much disruption of the lipid-bilayer of the stratum corneum, this resulting in skin irritation and other side effects. Additionally, most of the earlier chemical penetration enhancers did not effectively enhance the absorption of higher molecular weight compounds (peptides and proteins). In the last decade, newer chemical penetration enhancers have been developed that appear to be considerably less toxic, and that promote the flux of higher molecular weight compounds. Since there are

Table 1. Types of chemical penetration enhancers classified by functional groups and chemical structures, complied using data in Chattaraj and Walker (1995); Osborne and Henke (1997); Asbill and Michniak (2000); Williams and Barry (2004).

Types	Examples
Water	water
Sulfoxides and similar compounds	dimethylsulfoxide, dimethylacetamide, dimethylformamide
Pyrrolidones	2-pyrrolidone, *N*-methyl-2-pyrrolidone, 1-lauryl-2- pyrrolidone
Alcohols	ethanol, 1-octanol, 1-hexanol, 1-decanol, lauryl alcohol, linolenyl alcohol
Glycols	propylene glycol, butane-1,2-diol, polyethylene glycol 400
Urea and derivatives	urea, 1-dodecylurea, 1-dodecyl-3-methylurea,1-dodecyl-3-methylthiourea
Azone and derivatives	Azone (laurocapram; 1-dodecylazacycloheptan-2-one), 1-alkyl- or 1-alkenylazacycloalkanones
Enzymes	Acid phosphatase, calonase, papain
Iminosulfuranes	*S, S*-dimethyl-*N*-(5-nitro-2-pyridyl) iminosulfurane, *S, S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane
Cyclodextrins	2-hydroxypropyl-β-cyclodextrin, methylated-β-cyclodextrin
Fatty acid esters	cetyl lactate, butylacetate, isopropyl myristate
Fatty acids	alkanoic acids, oleic acid, lauric acid, capric acid
Surfactants	sorbitan monopalmitate, sorbitan trioleate, cetyl trimethyl ammonium bromide, sodium lauryl sulfate
Terpenes	limonene, nerolidol, farnesol, carvone, menthone
Polymers	β-*D* -glucopyranosyl-terminated oligodimethylsiloxanes, 1-alkyl-3-β-*D* -glucopyranosyl-1,1,3,3-tetramethyldisiloxanes
Monoolein	monoolein
Oxazolidinones	4-decyloxazolidin-2-one, 3-acetyl-4-decyloxazolidin-2-one

so many classes of chemical enhancers available, only six groups of chemical penetration enhancers are selected to be reviewed in the current paper. These selected chemical enhancers are fatty acids, surfactants, terpenes, polymers, monoolein, and oxazolidinones. Fatty acids and surfactants are representative of commonly used chemical enhancers that potentially cause skin irritation, whereas the rest are representative of a newer class of penetration enhancers that exhibit low toxicity to the skin. These newer classes of chemical penetration enhancers have a much better chance of getting regulatory approval for use as 'excepients' in transdermal delivery systems. Their application in skin delivery, skin irritation potential, and proposed mechanism of action are discussed.

6.1 Fatty acids

6.1.1 Effects of fatty acids on skin penetration of drugs

Fatty acids consist of an aliphatic hydrocarbon chain and a terminal carboxylic acid group. Fatty acids differ in their aliphatic chain length, which is either saturated or unsaturated, in the number, position, and configuration of double bonds and may have branching and other substituents. Examples of fatty acids are given in Table 2.

A wide variety of long chain fatty acids have a potential utility as skin permeation enhancers. Most studies on fatty acid penetration enhancers have focused on oleic acid, a monounsaturated fatty acid with a characteristic lard-like odor. Its enhancing activity was found to be dependent on the use of a co-solvent, such as propylene glycol, which usually acted synergistically (Bennett *et al*., 1985; Seki *et al*., 1989; Larrucea *et al*., 2001). A recent investigation has revealed that oleic acid remarkably enhances the permeation of indapamide, an anti-hypertensive drug across rat skin *in vitro* (Ren *et al*., 2008). Examples of other studies in which fatty acids have been investigated as skin penetration enhancers are given in Table 3. The enhancing effects of fatty acids depend on several factors, including the physicochemical nature of the permeants (Oh *et al*., 2001), the vehicle used to deliver permeants, the fatty acid selected, as well as the chemical structures of the fatty acid (Aungst, 1995). By using excised rat skin, it was demonstrated that the alkyl chain length of fatty acids affected their enhancing activities (Morimoto *et al*., 1996). Based on the results of several studies (Aungst *et al*., 1986; Ogiso and Shintani 1990; Komata *et al*., 1992), the enhancing effects of saturated fatty acids were greatest for C_{10} and C_{12} fatty acids. Also, the enhancer activity was influenced by the bond saturation. It was found that the unsaturated long-chain fatty acids ($^3C_{18}$) showed a greater enhancement than the analogous saturated fatty acids (Aungst, 1995). Moreover, the branching of fatty acids appeared to affect their enhancing activity (Aungst *et al*., 1986). Concentrations of fatty acids also seem to influence their enhancing activities. The skin permeation of meloxicam through human cadaver skin was found to increase as the concentration of oleic acid increased from 0.4 to 1%. However, a decreased permeation was observed when a higher concentration (5%) was used (Jantharaprapap and Stagni, 2007).

6.1.2 Mechanism of action

Evidences from DSC studies have revealed that fatty acids (especially *cis*-unsaturated fatty acids such as oleic acid) reduce the ordered intercellular lipid domains of the stratum corneum (Barry, 1987). Oleic acid may act by disrupting of the intercellular lipid domains, an effect that has been characterized by infrared spectroscopic studies (Francoeur *et al*., 1990; Mak *et al*., 1990). Additionally, it has been suggested that oleic acid may exist as heterogeneously dispersed fluid domains in the ordered stratum corneum lipid bilayers (Ongpipattanakul *et al*., 1991), thereby providing a pathway of lower resistance for the drug transport.

Table 2. Terminology of some fatty acids.

Carbon chain length	Chemical name	Common name	Chemical structure/Type of fatty acid
10	decanoic	capric	$CH_3(CH_2)_8COOH$/saturated
12	dodecanoic	lauric	$CH_3(CH_2)_{10}COOH$/saturated
14	tetradecanoic	myristic	$CH_3(CH_2)_{12}COOH$/saturated
14	*cis*-9-tetradecenoic	myristoleic	$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$/unsaturated
16	hexadecanoic	palmitic	$CH_3(CH_2)_{14}COOH$/saturated
16	*cis*-9-hexadecenoic	palmitoleic	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$/unsaturated
18	octadecanoic	stearic	$CH_3(CH_2)_{16}COOH$/saturated
18	*cis*-9-octadecenoic	oleic	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$/unsaturated
18	*cis*,*cis*-9,12-octadecenoic	linoleic	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$/unsaturated
20	eicosanoic	arachidic	$CH_3(CH_2)_{18}COOH$/saturated
20	all *cis*-5,8,11,14-eicosatetraenoic	arachidonic	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$/unsaturated

Table 3. Studies of fatty acids as skin penetration enhancers.

Permeant	Fatty acid	Skin[a]	Reference
Dihydroergotamine	oleic acid, lauric acid(6% in propylene glycol)	Rabbit	Niazy, 1991
Leuprolide	lauric acid, capric acid(2% in ethanol: water (4:1))	Nude mouse	Lu *et al*., 1992
Piroxicam	e.g. lauric acid, myristic acid(5% in fatty alcohol-propylene glycol base)	Rat	Hsu *et al*., 1994
Pyrene butyric acid	oleic acid, 10-methylpalmitic acid, 10-methylhexadec-9-enoic acid (5% in propylene glycol)	Human	Schneider *et al*., 1996
LHRH[b]	e.g. lauric acid, palmitic acid, oleic acid, linoleic acid (10% in ethanol)	Porcine	Bhatia and Singh, 1998
Melatonin	oleic acid(5% in propylene glycol)	Hairless mouse	Oh *et al*., 2001
Flurbiprofen	unsaturated fatty acids: oleic acid, linoleic acid, linolenic acid (5% in carboxymethyl cellulose hydrogel)	Wistar rat *in vitro* and *in vivo*	Fang *et al*., 2003a
Lidocaine	conjugates of unsaturated fatty acids (e.g. oleic acid, linoleic acid, linolenic acid) with propylene glycol	Porcine	Ben-Shabat *et al*., 2007
Ketotifen	lauric acid, oleic acid (5% in stick-type formulation)	Hairless mouse	Kimura *et al*., 2007

[a] *In vitro* studies
[b] Pretreatment of skin with fatty acids

6.1.3 Skin toxicity

Fatty acids have the potential to cause skin irritation, which has lead to limitation in their use. The extent of skin irritation depends on concentration and type of fatty acids used. For example, it has been shown that an application of 5% oleic acid in propylene glycol to the skin of six human volunteers for 6 hours resulted in minor irritation, whereas severe irritation occurred when 20% oleic acid in propylene glycol was applied (Loftsson *et al*., 1987). Fang *et al*. (2003a) investigated the skin irritation potential of unsaturated fatty acids (oleic acid, linoleic acid, and linolenic acid) and other skin penetration enhancers using several techniques, such as *in vitro* cell culture, *in vivo* TEWL and *in vivo* colorimetry. The *in vivo* assessment was performed in rats. Generally, the most irritating substance was found to be the fatty acids. Interestingly, the reduction of skin irritation was observed as unsaturated fatty acids (e.g., oleic and linoleic acids) were conjugated with propylene glycol (Ben-Shabat *et al*., 2007). A recent study (Touitou *et al*., 2008) performed in mice, has revealed that 10% oleic acid in ethanolic solution affects the morphology of epidermal Langerhans cells and causes a decrease in the density of these cells.

It must be pointed out that long term efficacy and safety studies remain to be carried out before it can finally be concluded that the incorporation of fatty acids into transdermal products is beneficial and safe for human use. The serious undesirable effect of oleic acid on Langerhans cells, the antigen presenting cells of the skin, must be taken into account. Furthermore, efficacy and safety information of fatty acids from human volunteer studies is necessary for the decision-making process. Nevertheless, fatty acids have previously been used as stiffening agents in several commercial cosmetics and dermatological preparations (i.e. lipsticks, creams, lotions); products that are not strictly regulated in any country.

6.2 Surfactants

6.2.1 Effects of surfactants on skin penetration of drugs

Surfactants play an important role in many products, including, pharmaceuticals, cosmetics, toiletries, and food formulations. They have long been used as solubilizers, detergents, wetting agents, adhesives, personal products, emulsifiers and suspending agents (Falbe, 1987). Surfactants generally consist of a lipophilic alkyl or aryl chain together with a hydrophilic head group. They can be classified into four main categories according to the presence of formally charged groups in the head; anionic (e.g. sodium lauryl sulfate), cationic (e.g. cetyltrimethyl ammonium bromide), nonionic (e.g. polyoxyethylene sorbitan monopalmitate) and amphoteric (e.g. *N*-dodecyl-*N*, *N*-dimethylbetaine) (Attwood and Florence, 1983). It is generally recognized that nonionic surfactants possess the least toxicity and skin irritation potential (Walters, 1990), and therefore they have been widely investigated as skin penetration enhancers. Generally, the investigation of enhancing abilities of nonionic surfactants has been focused on five principal series of surfactants, which are polysorbates, sorbitan esters, polyoxyethylene alkyl

ethers, polyoxyethylene alkylphenols and poloxamers (Attwood and Florence, 1983), (see Table 4). For example, pretreatment of skin with Span 20 (1 and 5% w/v in ethanolic solution) significantly increased the penetration of 5-fluorouracil, antipyrine and 2-phenyl ethanol through Wistar rat epidermis *in vitro* (Lo′pez *et al*., 2000). Tween 20 has been shown to increase the permeation of hydrocortisone and lidocaine across hairless mouse skin *in vitro* (Sarpotdar and Zatz, 1986a, b), but did not enhance the permeation of naloxone (Aungst *et al*., 1986). In another study, the flux of diazepam was found to increase at low concentrations of surfactant enhancers (see Table 5), but reduced drug transport was observed when the enhancer concentration was higher than 1% w/w (Shokri *et al*., 2001). These conflicting results of surfactant effects on skin permeation can be rationalized by a consideration of the self-association properties of the surfactant molecules (Walters, 1990). As skin penetration enhancers, surfactants have direct effects on the skin barrier properties, and indirect effects on the thermodynamic activity of the permeant in the vehicle. The thermodynamic activity of a permeant in the vehicle is the driving force for permeant diffusion into the skin. The monomers of the surfactant can penetrate and interact with the skin to modify its barrier properties, thereby permitting easier penetration of permeant into the skin. On the other hand, the micelles, which have a strong solubilizing capacity, produce a markedly reduced thermodynamic activity of the permeant within the vehicle, thereby reducing the transport rate of the permeant.

The enhancing activity of anionic surfactants on the percutaneous absorption of drugs has been demonstrated. For example, sodium decyl and dodecyl sulfates increased the *in vitro* permeation rates of several drugs including naproxen (Chowhan and Pritchard, 1978) and naloxone (Aungst *et al*., 1986). Sodium lauryl sulfate at 5% showed a remarkable enhancing activity on the skin permeation of lorazepam across rat skin *in vitro*. A marked increase in the drug flux was attributed to the skin damage caused by this anionic surfactant at 5% concentration, the highest concentration used in the study (Nokhodchi *et al*., 2003).

Cationic surfactants have been shown to promote the permeation of lidocaine from saturated systems in propylene glycol-water mixtures through excised human skin (Kushla *et al*., 1993). Cetyltrimethylammonium bromide was found to be an effective enhancer for the permeation of lorazepam across rat skin *in vitro* (Nokhodchi *et al*., 2003). Interestingly, ionic surfactants have been shown to have greater skin penetration enhancement effect than the nonionic surfactants (Ashton *et al*., 1992). This is possibly due to the fact that ionic surfactants cause more skin damage than the nonionic ones. Other investigations of surfactants as skin penetration enhancers are summarized in Table 5.

The synergistic effects of certain binary mixtures of surfactants have been reported. The combination of an anionic surfactant, sodium lauroylsarcosinate, and a nonionic surfactant, sorbitan monolaurate, more markedly increased the transdermal flux of drugs than the individual components used alone. Moreover, the formulation exhibited a reduction in skin irritation (Karande *et al*., 2004). Apart from the synergistic action between surfactants, the synergistic effects between surfactants and polar solvents (e.g. propylene glycol and ethanol) have been described (Naik *et al*., 2000; Nokhodchi *et al*., 2003; Kim *et al*., 2008).

Table 4. Examples of nonionic surfactants in each group.

Groups	Chemical name	Trade name
Polysorbates	polyoxyethylene (20) sorbitant monolaurate	Tween 20
	polyoxyethylene (20) sorbitant monopalmitate	Tween 40
	polyoxyethylene (20) sorbitant monostearate	Tween 60
	polyoxyethylene (20) sorbitant monooleate	Tween 80
Sorbitan esters	sorbitan monolaurate	Span 20
	sorbitan monopalmitate	Span 40
	sorbitan monostearate	Span 60
	sorbitan monooleate	Span 80
Polyoxyethylene alkyl ethers	polyoxyethylene (4) lauryl ether	Brij 30
	polyoxyethylene (23) lauryl ether	Brij 35
	polyoxyethylene (2) cetyl ether	Brij 52
	polyoxyethylene (10) cetyl ether	Brij 56
	polyoxyethylene (2) stearyl ether	Brij 72
	polyoxyethylene (20) oleyl ether	Brij 98
	polyoxyethylene (6) cetyl ether	Texafor A 6
Polyoxyethylene alkylphenols	polyoxyethylene (10) octyl phenol	Triton X-100
	polyoxyethylene (10) nonyl phenol	Rewopal HV 10
Poloxamers	Polyoxyethylene (140) polyoxypropylene (26-31)	Pluronic F68
	polyoxyethylene (15) polyoxypropylene (26-31)	Pluronic L62
	polyoxyethylene (25) polyoxypropylene (26-31)	Pluronic L64

Table 5. Studies of surfactants as skin penetration enhancers.

Permeant	Surfactant	Skin[a]	Reference
Flufenamic acid	nonionic surfactants e.g. polyoxyethylene (20) sorbitant monopalmitate, sorbitan monopalmitate, sorbitan trioleate, poloxamer 184, 231(10% in white petrolatum)	Rabbit	Hwang *et al*., 1983.
Naloxone	nonionic surfactants e.g.sorbitan laurate, polysorbate 20 polyoxyethylene (4) lauryl ether, sorbitan oleate, poloxamer 188anionic surfactants e.g. sodium lauryl sulfate, sodium laurate, sodium oleate(10% in propylene glycol)	Human	Aungst *et al*., 1986.
Methyl nicotinate	cetyl trimethyl ammonium bromide, sodium lauryl sulphate, Brij 36T(1.5% in aqueous solution)	Human	Ashton *et al*., 1992
Nitroglycerin[b]	oleyl surfactants containing ethylene oxide (EO) e.g. EO-2-oleyl ether, EO-10-oleyl ether(0.14 M in propylene glycol)	Human	Kadir *et al*., 1993
Diazepam	sodium lauryl sulfate, cetyltrimethylammonium bromide, benzalkonium chloride, polyoxyethylene (20) sorbitant monooleate (0-5% in water-propylene glycol system (1:1 v/v))	Rat	Shokri *et al*., 2001
Lidocaine hydrochloride	nonionic surfactants: sucrose laureate, sucrose oleate (2% in Transcutol)	Porcine ear	Cázares-Delgadillo *et al*., 2005
Ketotifen	polyoxyethylene lauryl ether, polyoxyethylene lauryl ether, sodium dodecyl sulfate(5% in stick-type formulation)	Hairless mouse	Kimura *et al*., 2007

[a] *In vitro* studies
[b] Pretreatment of skin with the enhancer

The enhancing ability of surfactants is governed by several factors, including their functional groups, hydrocarbon chain length, degree and position of unsaturation, physicochemical properties of permeants, nature of the vehicles, and whether the surfactants are used alone or in combination (Cázares-Delgadillo *et al*., 2005). The influence of the polar head group on the enhancing activity of surfactants has been demonstrated. Walters *et al*. (1982) and Walters (1989) state that the polar head group of surfactants affects their enhancing activities. Similarly, Lo′pez *et al*. (2000), who studied the influence of a polar functional group on the enhancing activity of Span 20 and its ethoxylate derivatives (Tween 20 and Azone), found that the nature of the enhancer head group greatly influenced the impairment of the cutaneous barrier.

6.2.2 Mechanism of action

It is apparent that the mechanism of action of surfactants on the skin is related to their ability to bind to the stratum corneum proteins (Breuer, 1979). However, the results from DSC have revealed that their ability to impair the barrier function cannot be attributed to surfactant interaction with proteins alone (Golden *et al*., 1986). An additional mechanism for anionic surfactants may involve disruption of the intercellular lipid matrix (Imokawa *et al*., 1989), selective loss of intercellular lipids (Imokawa *et al*., 1989), and an increase in the hydration levels of the tissues as a result of more exposure of water-binding sites (Rhein *et al*., 1986). Nonionic surfactants may alter the partitioning potential of permeant in favour of enhanced permeation (Shen *et al*., 1976).

6.2.3 Skin toxicity

Several problems (e.g. skin irritation and swelling of the stratum corneum) have been reported when anionic and cationic surfactants have been utilized (Bodde *et al*., 1989). Using TEWL as an index for skin irritation, TEWL in human volunteers was found to increase after an anionic surfactant, sodium lauryl sulfate, was applied onto the skin (Tupker *et al*., 1990). The skin irritation caused by cationic surfactants is more severe than anionic surfactants, leading to constraints in their use as skin penetration enhancers. An attempt to reduce the skin irritation of these surfactants, and other chemical enhancers, has led to the development of several new types of penetration enhancers and, one of these is a polymer type enhancer. In a study by Aoyagi *et al*. (1990), a surfactant (benzalkonium chloride) was polymerized and turned to a macromolecule, which permeated with difficulty

through the skin, thereby preventing irritation to the skin. Details of this polymeric enhancer are discussed later in section 6.4.

Similarly to fatty acids, safety and efficacy of surfactants (in particular, ionic surfactants) is a vital issue. Long term studies in animal models, and testing in human volunteers, are essential before they are used to increase the systemic delivery of transdermally applied drugs.

6.3 Terpenes

6.3.1 Effects of terpenes on skin penetration of drugs

Terpenes are a series of naturally occurring compounds that are composed of hydrocarbons and possibly oxygenated derivatives such as alcohols, aldehydes, phenols, ketones, oxides, and esters. Terpenes are frequently found in plant essential oils. Terpenes are composed of units of isoprene, C_5H_8, in a head-to-tail orientation to form linear chains or rings (Barry and Williams, 1993). Terpenes may be categorized depending on the number of their isoprene units. For example, terpenes that possess two isoprene units are classified as monoterpenes (C_{10}), whereas terpenes that have three isoprene units are classified as sesquiterpenes (C_{15}). Furthermore, terpenes may also be subdivided depending on the number of rings present in the structure (e.g. acyclic, monocyclic, dicyclic, and so forth) (Barry and Williams, 1993, 1995), or the chemical groups (e.g. alcohol, aldehyde, ketone and so forth) (Hashida and Yamashita, 1995). Examples of chemical structures of some terpenes (*p*-menthane, menthone, and menthol) are shown in Figure 3 (A). Terpenes have received considerable attention as penetration enhancers because they appear to have high percutaneous enhancing abilities, with low skin irritancy and low systemic toxicity (Williams and Barry, 1991a). Terpenes have been shown to remarkably increase drug permeation at low concentrations (1-5 %) (Williams and Barry, 1991a).

As skin penetration enhancers, terpenes have been employed directly or in combination with propylene glycol or ethanol (Williams and Barry, 1991a; Obata *et al*., 1991; Okabe *et al*., 1992; Barry and Williams, 1993; Kobayashi *et al*., 1994; Cornwell and Barry, 1995; Moghimi *et al*., 1996; Vaddi *et al*., 2002a, b; Songkro *et al*., 2003). As with oleic acid and Azone, the co-solvent is an important factor in the enhancing activity of terpenes. Synergistic activity has been reported between terpenes and propylene glycol (Yamane *et al*., 1995b; Zhao and Singh, 2000) as well as between terpenes and ethanol (Obata *et al*., 1991; Takayama and Nagai, 1994). A variety of terpenes have been shown to increase the percutaneous absorption of both hydrophilic and lipophilic drugs. Examples of these investigations are given in Table 6. Moreover, several studies showed that essential oils extracted from plant oils (e.g. *Alpinia oxyphylla*, *Zingiber officinale*) promoted the *in vitro* skin permeation of drugs across animal skins (Huang *et al*., 1995; Fang *et al*., 2003b; Songkro *et al*., 2008). Results from gas chromatograpy - mass spectroscopy (GC-MS) have revealed that the main constituents of these essential oils are terpenes (Fang *et al*., 2003b; Songkro *et al*., 2008).

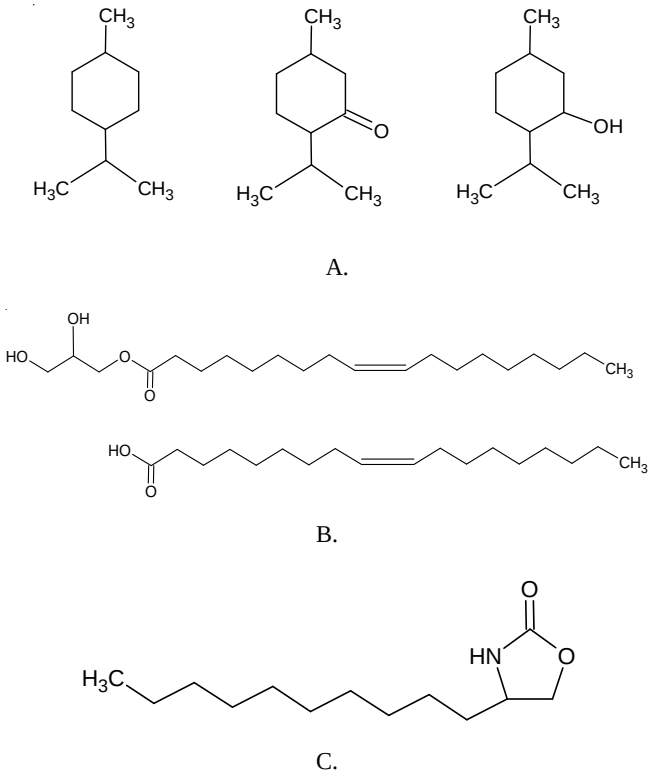

Figure 3. Chemical structures of some skin chemical penetration enhancers. A. monocyclic terpenes: *p*-menthane (left), menthone (middle), and menthol (right); B. monooelin (top) in comparison with oleic acid (bottom); C. 4-decyloxazolidin-2-one.

The chemical structures of terpenes and the physicochemical properties of the drugs play an important role in the enhancing activity of terpenes, as described by Aqil *et al*. (2007). The oxygen containing polar terpenes (e.g. carvacrol, menthol) were found to be more potent for hydrophilic drugs (e.g. propranolol hydrochloride) than the lipophilic terpenes (e.g limonene, *p*-mentnene) (Kunta *et al*., 1997; Songkro *et al*., 2003). It has been suggested by William and Barry (2004) that hydrocarbon monoterpenes should be used for lipophilic permeants. El-Katten and coworkers (2000) investigated the effect of terpene lipophilicity [log partition coefficient (K) 1.06-5.36] on the percutaneous absorption of hydrocortisone from hydroxypropyl cellulose gel formulations using hairless mouse skin *in vitro*. The terpenes studied were terpinen-4-ol, verbenone, carvone, menthone, α-terpineol, cineol, geraniol, thymol, cymene, limonene, and nerolidol. There was a linear relationship between log K of terpene and the cumulative amount of hydrocortisone in the receptor after 24 hrs. An increase in terpene lipophilicity was associated with an increase in the cumulative amount of hydrocortisone in the receptor after 24 hrs. No correlation was found between the log K of terpenes and the retention of drug in the mouse skin.

Table 6. Studies of terpenes as skin penetration enhancers.

Permeant	Surfactant	Skin[a]	Reference
Diclofenac sodium	fenchone, menthone, menthol, limonene, thymol, 1, 8-cineole(1 % in carbopol gel with propylene glycol)	Rat	Arellano *et al*., 1996
	nerolidol, farnesol, carvone, menthone and limonene oxide(0, 0.25, 0.5, 1, 1.5 and 2.5% in ethanol:glycerin:phosphate buffer solution (60:10:30))	Rat	Nokhodchi *et al*., 2007
Thyrotropin releasing hormone	cineole, carveol, menthone(3% in 47% ethanol)	Human	Magnusson *et al*., 1997
5-Fluorouracil	carvone, 1, 8 cineol, thymol(5% in 50% ethanol)	Porcine	Gao and Singh, 1997
Caffeine	11 terpenes e.g. terpinen-4-ol, α-terpineol, neomenthol, geraniol(0.4 M in propylene glycol)	Mouse	Godwin and Michniak, 1999
Tamoxifen	eugenol, limonene,menthone(5% in 50% propylene glycol)	Porcine	Zhao and Singh, 2000
Nicardipine HCl	fenchone, thymol, nerolidol(2% in hydroxypropyl cellulose gel)	Hairless mouse	El-Kattan *et al*., 2001
Ketoprofen	limonene, cineole, menthol(5% in microemulsion)	Rat	Rhee *et al*., 2001
Haloperidol	carvacrol, linalool, α-terpineol(5% in propylene glycol)	Human	Vaddi *et al*., 2002b
Haloperidol	carvacrol, linalool, α-terpineol (5% in 50% ethanol)	Human	Vaddi *et al*., 2002a
Propranolol HCl	p-menthane monoterpenes and related compounds (e.g. *p*-menthane, menthone, menthol, carvacrol, thymol)(5% in 40% ethanol)	Newborn pig	Songkro *et al*., 2003
Mefenamic acid	1,8-cineole(5, 10, 25% in polyethylene glycol 400)	Porcine ear	Heard *et al*., 2006
Ketotifen	menthol, limonene(5% in stick-type formulation)	Hairless mouse	Kimura *et al*., 2007

[a] *In vitro* studies

Furthermore, the influence of terpene concentrations on the *in vitro* percutaneous absorption of diclofenac sodium from a mixture of ethanol: glycerin: phosphate buffer solution has been investigated using Franz diffusion cell with rat skin (Nokhodchi *et al*., 2007). The results revealed that there was no direct relationship between the concentrations of terpenes and the permeation rate of the model drug.

6.3.2 Mechanism of action

DSC and X-ray diffraction studies suggest that the activity of terpenes as enhancers is a result of disrupting the intercellular lipid bilayers (Williams and Barry, 1989; Cornwell and Barry, 1993). Evidence from skin electrical conductivity measurements suggests that terpenes may create polar pathways across the stratum corneum for ions and polar drug penetration (Cornwell and Barry, 1993). In addition, results from electron paramagnetic resonance have demonstrated that terpenes fluidize the stratum corneum lipids and weaken the hydrogen-bonded network of the polar interface of the stratum corneum (dos Anjos and Alonso, 2008). Apart from their chemical structures, the mechanism of action of terpenes appears to be different, depending on the nature of permeants (e.g. hydrophilic or lipophilic). In the case of hydrophilic permeants such as 5-fluorouracil, the enhancer may function by increasing the diffusion of a drug in the stratum corneum (Williams and Barry, 1991a). For lipophilic permeants such as estradiol, the action of the enhancer is to reduce the barrier function of stratum corneum and to increase the partitioning of a drug into the stratum corneum (Williams and Barry, 1991b). It is believed that increased partition is a result of a bulk solvent effect since estradiol is only moderately soluble in terpenes (Williams and Barry, 1991b). According to the molecular modeling, it has been suggested that terpenes with structures appropriate for alignment within lipid lamellae are the most potent enhancers (Cornwell and Barry, 1994).

6.3.3 Skin toxicity

Most terpenes are generally recognized as safe (GRAS), a status granted by the U.S. Food and Drug Administration (FDA). Using the Draize scoring method with rabbit, cyclic monoterpenes (*d*-limonene, terpinolene, α-terpinene, trans-*p*-methane) showed a much lower irritancy than Azone at an equivalent concentration in an ointment. Moreover, terpenes did not cause lasting erythema at all test concentrations (Okabe *et al*., 1990). In another study (Fang

et al., 2003b), *in vitro* skin toxicity of sesquiterpenes from *Alpinia oxyphylla* essential oil was evaluated using cell cultures of human skin fibroblasts and human lung epithelial cells. Two fractions of essential oils, a lower-polarity fraction and a higher-polarity fraction, were tested. Hydrocarbon sesquiterpenes were the major components in the lower-polarity fraction, whereas oxygenated sesquiterpenes were the major components in the higher-polarity fraction. The release of a local inflammatory mediator, prostaglandin E_2 (PGE_2), by fibroblasts or epithelial cells was determined. A slightly increased PGE_2 formation from skin fibroblasts was obseverd with the lower-polarity fraction. In contrast, the higher-polarity fraction greatly inhibited the release of PGE_2. Furthermore, there was no statistically significant effect of both fractions on PGE_2 release by human lung epithelial cells.

6.4 Polymers

6.4.1 Effects of polymers on skin penetration of drugs

A polymer is a large molecule consisting of the repetition of small, simple chemical units, called monomers (Billmeyer, 1984). Polymers have played a key role in a variety of areas of pharmaceutical applications, particularly in controlling the rate of drug delivery. Based on their sources, polymers can be classified into two types, synthetic or natural polymers. Examples of these polymers are summarized in Table 7. Owing to their large molecular weight, polymer enhancers are mostly retained in the stratum corneum and do not significantly penetrate deeper into the skin. Therefore, side effects such as inflammation or skin irritation are limited. Because of their safety, various types of polymers have been synthesized and investigated for their enhancing activity. These have included benzalkonium chloride and hexadecylpyridinium bromide type polymers (Aoyagi *et al*., 1990, 1991), polyethylene glycol/polydimethylsiloxane (PEG/PDMS) block copolymers with a cationic end-group (Akitomoto *et al*, 1997), β-D-glucopyranosyl-terminated oligodimethylsiloxanes (Glc-ODMS) (Akimoto *et al*., 2001), 1-alkyl-3-β-D-glucopyranosyl-1,1,3, 3-tetramethyldisiloxanes (Glc–SiCs) (Akimoto and Nagase, 2003). According to skin penetration studies using diffusion cells and excised animal skin, it was found that these aforementioned polymers were effective for enhancing the skin penetration of model permeants such as 5-fluorouracil, antipyrine, and indomethacin (Aoyagi *et al*., 1990; Akitomoto *et al*, 1997; Akimoto *et al*., 2001; Akimoto and Nagase, 2003). These polymers scarcely penetrated beyond the stratum corneum of the skin. Recently, poly (amidoamine) dendrimers, which are monodisperse hyperbranched polymers, have been shown to increase the skin penetration of 5-fluorouracil through excised porcine skin (Venuganti *et al*., 2008).

Table 7. Examples of polymers in each type, complied using data in Dunn (1991), Sugibayashi and Morimoto (1994); Gunatillake *et al.* (2006).

Polymers	
Natural type	Synthetic type
Casein	Polyethylene glycol
Gelatin	Polylactic acid
Dextran	Polyanhydrides
Starch	Polycarbonates
Collagen	Polylactones
Sodium alginate	Polyester
Cellulose	Polyesteramides
Chitin	Polyurethanes
Natural rubber	Polyvinyl alcohol
Gum arabic	Polypropylene

A number of investigations have clearly demonstrated that the skin penetration enhancing activities of polymers are governed by several factors, including molecular weight, chain length, monomer type, as well as the nature of permeants. For example, in the case of benzalkonium chloride type polymer, the monomers with hexadecyl group showed the most effective enhancement for the *in vitro* percutaneous absorption of 5-fluorouracil across rabbit skin. An increase in the molecular weight of the polymer resulted in a decrease of the enhancing activity (Aoyagi *et al*., 1990). Using a two-chamber diffusion cell with rabbit skin, PEG/ PDMS block copolymers with a cationic end-group were found to be very effective for the penetration of antipyrine, a hydrophilic drug, but not for that of indomethacin, a hydrophobic drug. The chain length of PEG and PDMS components governed the skin penetration enhancing activity of the polymer (Akitomoto *et al*, 1997). Similarly, the alkyl chain length of Glc-SiCs affected the *in vitro* skin penetration of model drugs across rat skin (Akimoto and Nagase, 2003). In another study, Akimoto *et al*. (2001) synthesized and investigated the enhancing effect of Glc-ODMS with different molecular weights on the penetration of antipyrine through rat skin *in vitro*. It was found the enhancing activity of the polymers was governed by that concentration of Glc-ODMS coexisted regardless of the chain length of the oligodimethylsiloxanes.

6.4.2 Mechanism of action

Evidences from DSC studies have revealed that polymers function by interaction with the lipid and keratin in the stratum corneum (Aoyagi *et al*., 1990). Additionally, it has been reported that polymers are capable of increasing the partition coefficient of a drug into the stratum corneum (Akitomoto *et al*, 1997). In the case of dendrimers, changes in FTIR spectrum have indicated the interaction between dendrimers and the polar head groups of skin ceramides and free fatty acids (Venuganti *et al*., 2008).

6.4.3 Skin toxicity

The skin irritation potential of the linear polymers has been tested using the Draize test in rabbit; skin irritation

was not observed (Akimoto *et al*., 2001; Akimoto and Nagase, 2003). Based on their large molecular weight, it was suggested that dendrimers were not likely to cause skin irritation (Venuganti *et al*., 2008).

6.5 Monoolein

6.5.1 Effects of monoolein on skin penetration of drugs

Monoolein is a monoglyceride, with a structure similar to oleic acid (Figure 3 (B)). Monoolein, a biodegradable polar lipid, is insoluble in water but its molecules self-associate. Monoolein is able to form a bicontinuous cubic liquid crystalline phase that can be used as a drug delivery system (Shah *et al*., 2001; Turchiello *et al*., 2003). Although it is traditionally used as an emulsifier and food additive, it has received considerable attention over the last decade as a skin penetration enhancer. Several early studies have seemed to suggest that a major use of monoolein should be for topical, rather than transdermal drug delivery. Monoolein has been reported to enhance the skin penetration of several permeants including nitredipine (Giannakou *et al*., 1995), indomethacin (Ogiso *et al*., 1995), cyclosporine A, a cyclic undecapeptide (Lopes *et al*., 2005), and doxorubicin (Herai *et al*., 2007). Furthermore, a monoolein-based liquid crystalline system has been shown to promote the penetration of vitamin K across pig ear skin *in vitro* (Lopes *et al*., 2007). As with many other penetration enhancers, concentrations of monoolein influence its skin penetration enhancing ability. Lopes *et al*. (2005), who studied effects of monoolein concentrations from 5-70% (in propylene glycol formulations) on the topical delivery of cyclosporine A using excised porcine skin, found that at a low concentration of 5%, monoolein could improve only the topical delivery of cyclosporine A, whereas a 10% concentration enhanced both topical and transdermal delivery of the model drug. Further increase in the enhancer concentrations from 20% to 70% resulted in an increase in the topical delivery of cyclosporine A, but a decrease in the transdermal delivery of drug. In another *in vitro* study, a significant increase of doxorubin in the stratum corneum of porcine skin in the first few hours was achieved in the presence of monoolein at 5% (Herai *et al*., 2007).

6.5.2 Mechanism of action

Monoolein may function by disruption of the ordered lamellar structure of the bilayers in the stratum corneum, leading to an increased lipid fluidity in the stratum corneum. Moreover, it may remove skin ceramides and soubilize lipohilic compounds in the skin (Ogiso *et al*., 1995; Pereira *et al*., 2002).

6.5.3 Skin toxicity

Although monoolein is non-toxic (Ganem-Quintanar *et al*., 2000), skin irritation caused by monoolein-based liquid crystalline systems has been reported. It was found that liquid crystalline phases of monoolein and water for topical delivery of cyclosporine A induced skin irritation in hairless mouse after a 3-day exposure (Lopes *et al*., 2006).

6.6 Oxazolidinones

6.6.1 Effects of oxazolidinones on skin penetration of drugs

Oxazolidinones are a class of compounds containing 2-oxazolidone as part of the structure. Over more than a decade, several oxazolidinones have been synthesized and patented as penetration enhancing compounds (Rajadhyaksha, 1990). These have included 4-decyloxazolidin-2-one, 3-methyl-4-decyloxazolidin-2-one, 3-acetyl-4-decyloxazolidin-2-one, 4-benzyloxazolidin-2-one, 3-methyl-4-benzyloxazolidin-2-one and 5-decyloxazolidin-2-one (Rajadhyaksha, 1990). Oxazolidinones are high molecular weight compounds, and have structural features similar to sphingosine and ceramide lipids, natural components found in the upper skin layers. Oxazolidinones are capable of localizing permeants in the skin layers and thereby reducing systemic permeation. Furthermore, they are odorless and nonstaining. This makes them interesting for use in cosmetic and personal care products (Rajadhyaksha and Pfister, 1996). Of all the oxazolidinones available, 4-decyloxazolidin-2-one (Dermac SR-38) (Figure 3 (C)) is by far the most widely investigated. It is a white, crystalline, odorless solid with a low melting point close to the skin temperature (32-33^{o}C) (Rajadhyaksha *et al*., 1997). Apart from enhancing the percutaneous delivery of a variety of drugs (Davis *et al*., 2002), 4-decyloxazolidin-2-one has been reported to increase the retention of many compounds (e.g. dihydroxyacetone and retinoic acid) at local skin sites (Pfister and Rajadhyaksha, 1995). In addition to being skin penetration enhancers, oxazolidinones are also used as antibacterial agents. They are active against gram-positive pathogenic bacteria (Bozdogan and Appelbaum, 2004). This will undoubtedly be their drawback as chemical enhancers should not possess pharmacological action.

6.6.2 Mechanism of action

The mechanism of action of oxazolidinones may involve the interaction with stratum corneum lipids. It has been reported that 4-decyloxazolidin-2-one can fluidize the bilayer lipids in the stratum corneum, thereby enhancing the skin penetration of various active ingredients (Rajadhyaksha *et al*., 1997).

6.6.3 Skin toxicity

The high molecular weight and lipophilicity probably hinders significant absorption of oxazolidinones into the deeper skin layers. Therefore, the skin irritation caused by oxazolidinones is potentially minimized. It was found that 4-decyloxazolidin-2-one did not cause skin irritation in

human volunteers (Rajadhyaksha *et al*., 1997).

7. Selection of skin penetration enhancers

Skin delivery systems can be formulated to deliver an active ingredient to (a) the skin surface for localized action, or (b) the stratum corneum, or (c) skin appendages, or (d) viable epidermis and dermis, or (e) to blood vessels in dermis for systemic effects. If one considers the target site of the drugs/formulations, it may be suggested that not all types of skin products require the use of skin chemical penetration enhancers. In the case of drugs with specific surface-skin targets, such as sunscreens and pesticides, retardants rather than enhancers are preferable, since their actions are intended on the surface of the skin. Unlike skin penetration enhancers, the function of retardants is to retain the active ingredients to the skin surface (in this case) and to prevent the active ingredient penetrating into the stratum corneum and deeper layers of the skin. Potential skin penetration of such products has caused safety concerns recently (McDougal *et al*., 2007).

In the case of stratum corneum treatment (i. e. moisturizers) and skin appendage treatment (i.e. anti-acne products), skin penetration enhancers are not generally required. However, problems arise with viable epidermis - dermis treatment and systemic delivery via percutaneous absorption. To achieve therapeutic effects, skin penetration enhancers are necessary for these two types of applications.

To date, the selection of a specific enhancer for a given permeant has remained challenging. Pfister and Hsieh (1990a, b) have proposed that physicochemical properties of selected enhancers must be compared with those of the permeant. The authors have given several examples concerning this aspect, and one of these was the solubility parameter of the enhancer must approximate that of the skin. William and Barry (2004) have stated that the potency of skin penetration enhancers seem to be drug specific. It is generally recognized that structures and physiochemical properties of the chemical enhancers govern their penetration enhancement potencies. With the aid of structure - activity relationship (SAR) studies, prediction of enhancer potency may be possible for a series of permeants with similar physicochemical properties. SAR is a technique used to correlate the enhancing potency of chemical enhancer with its structure or physiochemical descriptors, such as molecular shape, size, molecular geometry, solubility parameter, electronic effect, hydrophilicity, and lipophilicity (Kanikkannan *et al*., 2006).

In many cases, a prediction of the activity of chemical enhancers based on SAR is not satisfactory. For example, the enhancing activity of monoolein has been reported to depend on its concentrations used. Low concentration (up to 10% w/w) of monoolein is found to effectively increase both topical and transdermal delivery of vitamin A, whereas high concentration (20% w/w or more) is found to enhance topical delivery. Lopes *et al*. (2005) have suggested that a high affinity between oxazolidones (at high concentration) and vitamin A may result in the retention of vitamin A in the skin.

Besides SAR, the Quantitative Structure-Activity Relationship (QSAR) technique has been employed to explore the structural requirements of chemical penetration enhancers towards different drugs (Ghafourian *et al*., 2004). In this investigation, the skin penetration enhancing activities of three classes of chemical enhancers (terpenes, pyrrolidinone, and *N*-acetylprolinate derivatives) towards five drugs with varying lipophilicities were used to construct a QSAR. These drugs included 5-fluorouracil, diclofenac sodium, hydrocortisone, estradiol and benazepril. Results from this QSAR study indicated that less hydrophobic chemical enhancers were the most active for hydrophilic drugs, 5-fluorouracil and diclofenac sodium. In the case of hydrophobic drugs, hydrocortisone, estradiol and benazepril, QSAR analyses indicated a linear relationship between penetration enhancing activity and *n*-octanol/water partition coefficients of chemical enhancers.

8. Concluding remarks

Generally, commercial skin products can be classified into two classes, topical and trandermal preparations. A topical application is intend to confine the pharmacological effects or other effects of active ingredients to the surface of the skin or within the skin. Topical formulations are available in several dosage forms, such as creams, emulsions, lotions and gels. It has been well known that topical products usually contain many components, including chemical enhancers as excipients. Normally, the intended use of these particular excipients present in the formulations is for other purposes not for increasing the availability of active ingredients in the local area of the skin. For example, several fatty acids have long been used as stiffening agents in commercial creams, lotions, and lipsticks. At present, their uses as skin chemical penetration enhancers are rarely recognized by the formulators and consumers.

A transdermal application is intended for systemic effects. To achieve therapeutically effective dose of the drug through the skin, a chemical penetration enhancer is a major tool. In the pharmaceutical science literature, a wide spectrum of chemical enhancers have been used in research to enhance skin permeability, however, only a handful are actually used in practice. This is partly due to the fact that activities of skin chemical enhancers are not specific towards stratum corneum; they generally penetrate into the deeper layers of the skin to viable epidermal cells and induce skin irritation responses. The safety of chemical enhancers is a key consideration. More than 300 chemical enhancers have been discovered, but only few enhancers (e. g. terpenes) have been classified by FDA as ‘safe’ for use in the products. It is doubtful if regulatory agencies of various countries will approve any penetration enhancer that causes such a severe disruption (albeit short-lived and reversible) of the lipid-bilayer, until long term studies demonstrate their efficacy and safety.

The nonspecific activity of chemical enhancers towards the skin layers has lead to the development of mixtures of chemical penetration enhancers, which are called synergistic combinations of penetration enhancers or SCOPE formulations (Karande *et al*., 2004). The binary mixtures of chemical enhancers not only significantly increase the skin penetration of the drug, but also increase the safety in comparison to the single enhancers.

Other reasons for the limited use of chemical enhancers in the marketplace involve the applicability of the methods that have been used to exhibit the enhancement. It should be pointed out that most data in the literature has been obtained from animal studies (mostly *in vitro* studies using excised animal skin). There are limited studies that have been conducted using human cadaver skin, and even fewer are human volunteer studies, particularly long term safety studies. In general, penetration enhancement in animal skins is found to be greater than that obtained with human skins. Since topical and transdermal products are intended for human use, the results from human studies are the most relevant.

Despite extensive research over decades in the area of trandermal drug delivery, at present only a handful of transdermal products (mostly skin patches) are available in marketplace. The current marketed patches (also known as transdermal drug delivery systems) include those for systemic delivery of scopolamine, nicotine, nitroglycerine, fentanyl, liodicaine, clonidine, estradiol and testosterone (Thong *et al*., 2007; Tanner and Marks, 2008). Although several products containing penetration enhancers have been patented (Ebert *et al*., 1992; Govil *et al*., 1993; Santus and Baker, 1993), at present only few commercial patches contain skin chemical penetration enhancers. For example, transdermal lidocaine patch (Lidoderm®, Endo Pharmaceuticals Inc., U. S. A.) contains urea and propylene glycol as chemical enhancers. Both estradiol patch (Climara®, 3M Drug Delivery Systems, U. S. A.) and nitroglycerin patch (Nitrodisc®, GD Searie, U. S. A.) contain fatty acid esters as chemical enhancers. These transdermal patches have been approved by the FDA (Thong *et al*., 2007).

Skin penetration enhancers are capable of improving the skin permeation of a variety of drugs. Some penetration enhancers are more effective for hydrophilic drugs than lipophilic drugs and *vice versa*. Fatty acids, surfactants, terpenes and polymers are more effective for transdermal drug delivery whereas monoolein and oxazolidinones are more effective for topical drug delivery, where skin is the target site. The majority of the penetration enhancers function by disruption of the highly ordered lamellar lipid domains in the stratum corneum. Nevertheless, the adverse effects caused by some of these enhancers limit their widespread use. This has led to the development of new classes of chemical enhancers with less toxicity to the skin. Although the safety issues of these chemical enhancers still require clarification, several enhancers such as terpenes and polymers could be promising in practical use.

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