Chemical Penetration Enhancers: Classification and Mode of Action

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2.1 Introduction

Skin is the largest organ in humans, which covers the whole body and protects it against water loss and against undesired penetration of exogenous substances from the environment into the skin, i.e. body. This barrier property of the skin is the main obstacle for the drug penetration into or through the skin. Various strategies are developed to overcome the impermeability of the stratum corneum (SC), being the outermost layer of the skin which provides the primary barrier to percutaneous drug penetration. Circumventing this barrier is a prerequisite for an efficient dermal/transdermal administration of drugs. One approach for

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N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_2

improving percutaneous drug penetration, which has been extensively investigated, is the use of chemical penetration enhancers (CPE), also called sorption promoters or accelerants.

2.2 Chemical Penetration Enhancers (CPEs)

Chemical penetration enhancers are agents that partition into and interact with the components of the SC, increasing skin permeability in a temporary, reversible manner.

Thus, skin penetration enhancers reversibly reduce the barrier properties, i.e. resistance of the SC to drug penetration, and allow drugs to penetrate more readily into the viable skin tissue and in some cases also into the systemic circulation (CPEs increase drug transport across the skin) (Barry 1991).

Advantages of chemical enhancers over physical enhancers (such as iontophoresis, sonophoresis, electroporation, etc.) are design flexibility, ease of application, the possibility of self-administration and prolonged drug delivery through patches, patient compliance and their incorporation into inexpensive and simple formulations (Karande and Mitragotri 2009). These advantages make chemical penetration enhancers a universally studied subject in dermal and transdermal drug delivery. However, there are a few disadvantages of this penetration enhancement method: a number of chemicals tested as penetration enhancers can cause skin irritation; some of them have low efficacy at used therapeutic levels, and chemical enhancers are not suitable for the delivery of macromolecules.

To be considered as a candidate for a CPE, a chemical compound should meet the following desirable criteria (Williams and Barry 2004):

1. It should not irritate the skin and should be non-toxic and non-allergenic.
2. It should have a rapid but reproducible and predictable effect.
3. It should not have any pharmacological activity.
4. It should work unidirectional, i.e. enhance drug penetration into the skin whilst preventing the loss of endogenous material from the body.
5. Upon removal of the enhancer, a quick and complete recovery of skin properties is expected.
6. It should be colourless and odourless.
7. It should be compatible with drugs and other excipients in the formulation.
8. It should be cosmetically acceptable when applied to the skin.

Most substances fulfil some, but not all of the above criteria. No CPE has yet been developed which possesses all the aforementioned ideal properties. Despite showing some limitations, a consistent group of chemical compounds has been used for years safely and effectively in dermal and transdermal drug delivery systems.

2.3 Classification of Chemical Penetration Enhancers

CPEs form a diverse pool of chemical compounds that can be classified into groups on the basis of how structurally related they are (Table 2.1) (Williams and Barry 2004). An extensive literature review of compounds, cited as skin penetration enhancers, can be found in Osborne and Henke (1997).

Penetration enhancers often work well when used together, i.e. they show synergistic effects in enhancing the penetration of the drug into/through the skin (when used together, they exert higher effects than when used alone). Examples of such synergistic mixtures are Azone® and fatty acids, terpenes and PG, etc. (Williams and Barry 2004).

In addition to the aforementioned classification, Asbill and Michniak (2000) classified chemical penetration enhancers according to their activity into the following groups:

- Penetration enhancers that have both dermal and transdermal activities, i.e. enhance the penetration of the drug into and through the skin, respectively
- Penetration enhancers that have transdermal activity
- Penetration enhancers that have only dermal activity

Table 2.1 Classification of chemical penetration enhancers based on their chemical structure and examples of most used enhancers

Chemical class	Enhancer
Alcohols	*Short-chain alcohols* Ethanol Isopropyl alcohol *Long-chain alcohols* Decanol Octanol *Glycols* Propylene glycol (PG)
Amides	*Cyclic amides* Azone®(1-dodecylazacycloheptan-2-one or laurocapram)
Fatty acids	Lauric acid Oleic acid Linoleic acid
Esters	*Alkyl esters* Ethyl acetate Butyl acetate Methyl acetate *Fatty acid esters* Isopropyl myristate Isopropyl palmitate
Ether alcohols	Transcutol®(diethylene glycol monoethyl ether)
Surfactants	*Anionic surfactants* Sodium lauryl sulphate (SLS) *Cationic surfactants* Benzalkonium chloride Cetylpyridinium chloride Cetyltrimethylammonium bromide; *Nonionic surfactants* Polysorbates (Tween® 20, Tween® 80, etc.) *Zwitterionic surfactants* Dodecyl betaine
Sulphoxides and analogues	Dimethyl sulphoxide (DMSO) Decylmethyl sulphoxide (DCMS)
Essential oils	Eucalyptus Ylang ylang Chenopodium
Terpenes and its derivatives	D-Limonene L-Menthol 1,8-Cineole
Pyrrolidones	N-methyl-1-2-pyrrolidone (NMP) 2-pyrrolidone (2P)
Oxazolidines	4-decyloxazolidin-2-one
Phospholipids	Phosphatidylcholine (PC)
Enzymes	Acid phosphatase, papain, phospholipase C

- Penetration retarders that deliver low concentrations of the drug into the skin and decrease drug flux

2.4 Mechanisms of Action of Chemical Penetration Enhancers

Penetration enhancers can also be classified according to their mechanism of action. Before we review the lipid-protein partitioning (LPP) theory, it would be useful to have a quick reminder of the parameters that determine the diffusion of a molecule through the skin. Factors affecting the drug permeation rate through the SC can be considered using the steady-state flux equation (Eq. 2.1) (Barry 1983):

$$\frac{dm}{dt} = \frac{DC_0K}{h} \tag{2.1}$$

dm/dt is the steady-state flux, representing the cumulative mass of the diffusant, m, passing per unit area of membrane in time t.

C_0 is the constant donor drug concentration.

K is the partition coefficient of a solute between membrane and bathing solution.

D is the diffusion coefficient.

h is the membrane thickness.

With these parameters in mind, effective penetration enhancers can increase transdermal drug delivery by (Williams and Barry 2004):

- Increasing the diffusion coefficient of the drug in the SC (e.g. by disrupting the SC lipid organisation)
- Increasing the drug concentration in the vehicle (e.g. by acting as an anti-solvent)
- Improving the partitioning between the drug formulation and the SC (e.g. by changing the solvent nature of the skin membrane in order to enhance the drug partitioning into the skin)
- Decreasing skin thickness, which is less likely

Barry and co-workers (Barry 1991; Williams and Barry 1991) postulated the lipid-protein partitioning (LPP) concept to help categorise penetration enhancers and to describe how they alter the permeability of the skin. This theory suggests three possible ways in which the permeability of the skin can be adjusted to allow easier drug transport (Fig. 2.1):

1. Disruption of the intercellular lipid bilayer (lipid modification)

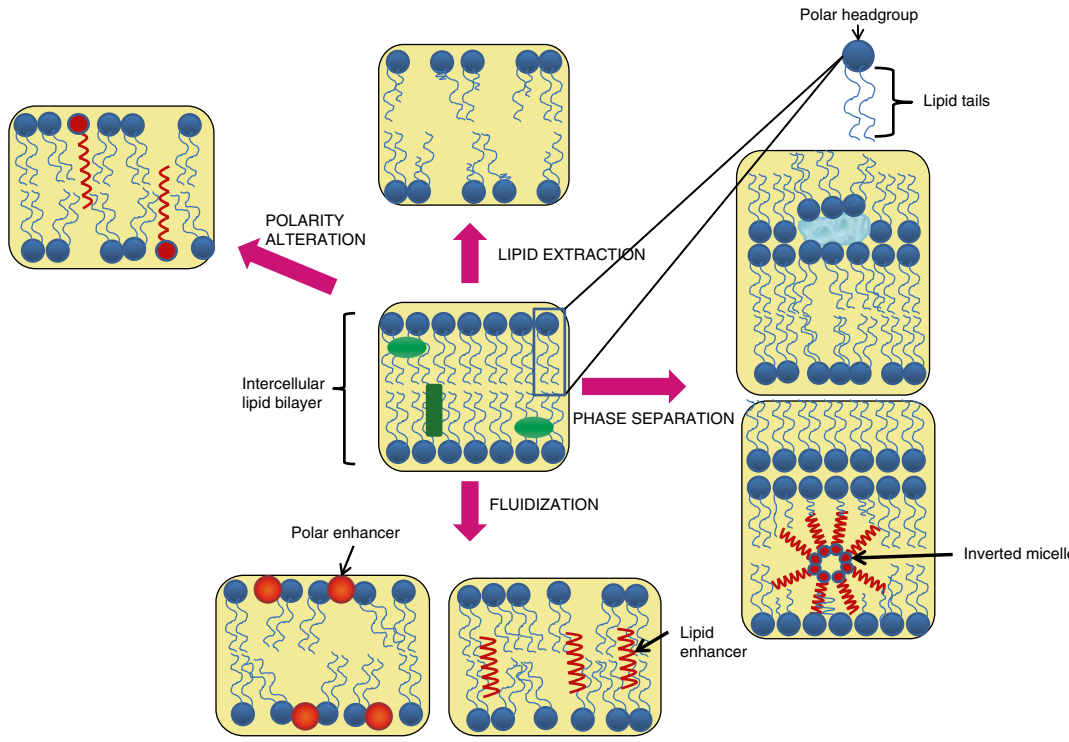

Fig. 2.1 Possible effects of chemical penetration enhancers on the structure of the lipid bilayer (Modified from Barry 2004)

2. Interaction with the intracellular proteins of the SC (protein modification)
3. Increasing partitioning of a drug, co-enhancer or co-solvent into the SC (partitioning promotion)

CPEs act by one or usually by more of the aforementioned modes of action.

2.4.1 Lipid Modification

In this mode of action, the enhancers will change the structural organisation of the lipid bilayers of the SC (Fig. 2.1). As a result, the SC barrier will become more permeable and the diffusion coefficient of the drug would increase (Eq. 2.1). Enhancer molecules will form microcavities within the lipid bilayer and in this way increase the free volume available for drug diffusion. This disturbance of the lipid bilayer can be homogeneous when the enhancer molecules are dispersed evenly throughout the lipids. Azone®, terpenes, fatty acids, alcohols and DMSO favour the homogeneous type of behaviour (Barry 2001; Benson 2005). Penetration enhancers (e.g. oleic acid and terpenes at high concentration) can also concentrate heterogeneously (i.e. they phase separate – the "pooling" phenomenon) within the domains of the lipid bilayers forming permeable "pores" that provide less resistance for penetration of polar molecules (Ongpipattanakul et al. 1991; Cornwell et al. 1996). Solvents such as DMSO, ethanol and chloroform/methanol mixture may extract lipids and form aqueous channels in the SC, which results in a more permeable SC (Menczel 1995; Hatta et al. 2010).

CPEs are believed to interact with lipid bilayers of the SC at three main sites (Lane et al. 2012):

1. Interaction with the polar head groups of the lipids (e.g. Azone®); these enhancers due to forming H bonds and/or by ionic forces disturb the hydration spheres of the lipid bilayers and thereby disrupt their packing order within

the polar plane, which leads to the fluidization of the intercellular lipids as well as to the increase of the water volume between the lipid layers; the consequence is a decreased resistance to drug diffusion.
2. Interaction in the aqueous domain of the lipid bilayers which increases the solubility of this site for the drug (e.g. propylene glycol, ethanol, Transcutol®, N-methyl pyrrolidone, etc.); these solvents change the solubility parameter of the skin in order to match the solubility parameter of the drug/permeant, which leads to an enhanced partitioning of the drug from the vehicle into the SC.
3. Interaction with the lipid alkyl chain – CPEs insert directly between the hydrophobic lipid tails (e.g. long-chained and less polar enhancers, such as oleic acid, isopropyl myristate, etc.); these enhancers disrupt the packing of lipids and thereby increase the fluidization of lipids, which increases drug diffusion through the SC.

The main penetration pathway for drugs is the intercellular route. Consequently penetration enhancers need to modify the intercellular lipids of the SC to be effective. They may interact at the level of polar head groups and/or between the hydrophobic tails of the intercellular lipids arranged in bilayers (Barry 1991). Lipophilic enhancers (i.e. oleic acid and limonene) can directly fluidize ceramide acyl chains, thus increasing the spacing of the lipid packing. In addition, they can modify the acyl chain packing indirectly by decreasing the strength of the intermolecular hydrogen bonds between the polar head groups (Guillard et al. 2009). The same study showed that hydrophilic enhancers (i.e. ethanol and DMSO) have no influence on the acyl chain organisation, but show a complex action on the polar head groups, weakening the hydrogen bonds within the polar head groups of ceramides (Guillard et al. 2009). According to du Plessis et al. (2001), these interactions compete with water-mediated intermolecular hydrogen bonding and ionic forces, thus disturbing the hydrogen spheres of the lipids resulting in alterations in the head group domain. This effect would consequently also alter the packing of the lipid acyl chains, and hence, ethanol has an indirect impact on acyl chains. On the other hand, the action of ethanol on acyl chains would be indirect, and moreover, the main mode of action of this compound is to reduce the barrier function by extraction of the SC lipids.

Moghadam et al. (2013) also showed that all three classes of investigated CPEs (solvents, terpenes and surfactants) with the exception of two solvents, propylene glycol and ethanol, affected SC lipids, i.e. they caused increased disordering of lamellar and lateral packing of lipids. The highest degree of SC lipid disordering was caused by surfactants (especially sodium dodecyl sulphate, didecyldimethylammonium bromide, didecyltrimethylammonium bromide) followed by terpenes, such as nerol.

2.4.2 Protein Modification

Another mode of action of CPEs is by interacting and binding with the keratin filaments. DMSO, ionic surfactants, urea and decylmethyl sulphoxide can interact with the keratin in the corneocytes. This may cause a conformational change in the proteins, disrupting the order in the corneocytes; keratin may be denatured or its conformation may be modified, and vacuoles can be formed. The diffusion of the drug through the SC is then increased (Eq. 2.1), and therefore, the permeation of the drug is increased, too. However, the intracellular penetration pathway is usually not important for drug permeation, and most studies suggest that lipid fluidization is the primary way of modifying the SC, which leads to an enhanced percutaneous drug penetration (Barry 2001). It was shown that solvents used at high concentrations,when enhancing drug permeation, may cause fissuring of the intercellular lipids and splitting of SC squames, by affecting the desmosomes that maintain the cohesion between corneocytes. These phenomena would cause severe skin irritation which is not acceptable, and hence, these enhancers at high concentrations do not present candidates for topical and transdermal formulations (Barry 1991; Benson 2005).

2.4.3 Partitioning Promotion

As seen from Eq. (2.1), partition coefficient (K), diffusion coefficient (D) and drug concentration in the vehicle (C_0) are the parameters that change the rate of drug penetration. Some solvents (ethanol, propylene glycol, Transcutol®, N-methyl pyrrolidone) can penetrate into the SC and change its chemical properties and thereby its solvent properties. This allows for the increase of the partitioning (K in Eq. 2.1) of a drug, co-enhancer and co-solvent into the SC and their increased solubility within the SC (Barry 2001; Benson 2005). An example is the increased penetration of nitroglycerin and estradiol by the use of ethanol as a co-solvent. Further, propylene glycol is used synergistically with Azone®, oleic acid and terpenes in order to enhance their penetration and thereby their concentration in the SC (Barry 2001).

2.4.4 Indirect Penetration Enhancement

Apart from directly affecting the skin by modes of action described above which enhance percutaneous drug permeation, chemical enhancers can modify the drug formulation itself and work indirectly on enhancing drug permeation through the skin. They can act by (Williams and Barry 2004):

1. Changing the thermodynamic activity of the vehicle, i.e. by fast permeation of a solvent from the donor into the skin, the permeant is left in a more thermodynamically active state. This effect is known as the "push effect".
2. The "drag effect", implying that the enhancer-solvent "drags" the drug with it into the skin.
3. Solubilising the permeant in the donor (e.g. surfactants change the barrier properties of the skin directly, but in addition they form micelles which ease solubilisation of the permeant); this is important for permeants with low solubility, where the use of enhancers may decrease depletion effects and extend the drug permeation through the skin (Songkro 2009).

2.5 Different Classes of Chemical Penetration Enhancers

As mentioned in Sect. 2.3, CPEs are typically classified into structurally related chemical compound groups. CPEs are able to use different mechanisms of action, and it is difficult to assign a primary mode of action for many of the chemical enhancers. For these reasons, the classification of CPEs is based on their chemical structure and not on their mechanism of action.

In the following section, some groups of CPEs will be described briefly with their known mechanisms of action. For detailed reviews, please refer to the separate chapters in this volume.

2.5.1 Terpenes

Terpenes are derived from plant essential oils, and both are used as potent penetration enhancers (Herman and Herman 2015). Terpenes are more often used, and they represent not toxic substances, which cause only mild limited irritation of the skin, and they interact reversibly with the SC lipids (Obata et al. 1991; Okabe et al. 1990). They have been classified as being generally regarded as safe (GRAS) substances (Akimoto et al. 2001). Chemical structure of terpenes is based on repeated isoprene units (C_5H_8) (with the exception of lavandulol). Depending on the number of isoprene units, they can be classified into monoterpenes (C_{10}), sesquiterpenes (C_{15}) or diterpenes (C_{20}). In addition to this, terpenes include different chemical groups (hydrocarbons, alcohols, oxides, ketones, esters) (Aqil et al. 2007). Terpenes have been studied as skin penetration enhancers for both hydrophilic (such as propranolol, bupranolol, etc.) and lipophilic drugs (such as ibuprofen, hydrocortisone, etc.) (Zhao and Singh 1999; Babu and Pandit 2005; Brain et al. 2006; Vaddi et al. 2002).

The most extensively studied terpenes are D-limonene, 1.8-cineole and L-menthol, which have been used for penetration enhancement of both hydrophilic and lipophilic drugs (Aqil et al. 2007; Song et al. 2009).

How effective terpenes are as penetration enhancers is determined by their lipophilicity, size and chirality, boiling point and energy of vaporisation and degree of unsaturation (Aqil et al. 2007).

Williams and Barry (1991) evaluated different monoterpenes as enhancers for 5-fluorouracil and found a *structure-activity relationship*. Hori et al. (1991) and Moghimi et al. (1997) confirmed that amongst terpenes, hydrophilic terpenes (alcohols, ketones and oxide terpenes, like fenchone and thymol) are more effective in enhancing the permeation of hydrophilic drugs (propranolol), whereas hydrocarbon terpenes (like limonene and cymene) are more active towards lipophilic drugs (diazepam). Moreover, Tas et al. (2007) observed that anethole, a hydrophobic terpene, enhanced significantly the absorption of the highly lipophilic etodolac, whilst the hydrophilic terpenes, menthol and carvacrol, did not enhance the absorption of etodolac. Hence, high lipophilicity of terpenes is important for enhancing the permeation of lipophilic drugs (Ghafourian et al. 2004).

The *mechanism of drug penetration enhancement* induced by terpenes depends on the applied drug. In the case of hydrophilic drugs (such as 5-fluorouracil), the primary mechanism of action of terpenes is to increase drug diffusivity in the SC by disrupting the lipid structure of the SC (Williams and Barry 1991; Cornwell and Barry 1994). As to partitioning, terpenes do not increase the partitioning of the hydrophilic drug into the human SC, since the drug (e.g. 5-fluorouracil) is less soluble in all terpenes than in water. For more lipophilic drugs (such as estradiol), terpenes increase drug diffusivity, but also increase drug partitioning into the SC (Williams and Barry 1991). The increase in partitioning is proposed to be due to solvent effects, since estradiol is moderately soluble in many terpenes. The permeation of lipophilic drugs increases proportionally to their solubility in the enhancer (Williams and Barry 1991).

The mechanism by which terpenes *increase drug diffusivity* in the SC, being the main mode of terpenes' penetration enhancing action, has been investigated by different techniques. It has been shown by differential scanning calorimetry (DSC) measurements that terpenes reduce lipid phase transition temperatures, indicating that they may increase SC permeability by disrupting the intercellular lipid bilayers (Williams and Barry 1989; Cornwell and Barry 1994), which has been confirmed for 1.8-cineole, menthone and nerolidol (in propylene glycol/water systems), being lipid disruptive, whereas no clear proof was found for the lipid disruptive effect of D-limonene (Yamane et al. 1995; Cornwell et al. 1996). Obtained DSC results revealed that D-Limonene produced a freezing point depression effect on SC lipids, implying little interaction with lipids at skin temperature, whilst its small enhancement effect is believed to be due to phase separation of the oil in SC lipid bilayers (Yamane et al. 1995; Cornwell et al. 1996). In contrast, small angle X-ray diffraction (SAXD) studies have indicated that besides 1,8-cineole, also D-limonene disrupts SC bilayer lipids (i.e. induces reductions in bilayer periodicity), whereas nerolidol (a long-chain sesquiterpene) reinforces the bilayers, possibly by orienting alongside the SC lipids (Cornwell et al. 1996). According to Cornwell and Barry (1994), nerolidol disrupted the organised packing of the SC lipids due to its amphiphilic structure and was able to increase the diffusivity of 5-fluorouracil.

Recent SAXD and wide angle X-ray diffraction (WAXD) measurements have confirmed that D-limonene caused a slight disruption of the organised lipid bilayers' structures (the hexagonal hydrocarbon-chain packing structure was much strongly disrupted than the orthorhombic one), an increase of the repeat distance of the long lamellar structure by incorporating D-limonene molecules and formation of "pools" of D-limonene in the hydrophobic region of the intercellular lipid matrix in the SC (Hatta et al. 2010). As to L-menthol, SAXD and WAXD studies revealed that it increased the repeat distance of the lamellar structure in lipid bilayers and inhibited the formation of hexagonal hydrocarbon chain packing. In addition, DSC studies showed that it decreased the phase transition temperature of ceramide 2/cholesterol mixtures. Thus, L-menthol changed the lamellar structure and the intermolecular interaction between ceramide 2 and

cholesterol, indicating fluidization of lipid structures (ceramide 2/cholesterol mixtures), which promotes the percutaneous drug permeation (Watanabe et al. 2009). Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) study revealed that administration of L-menthol causes disorder of the intercellular lipids in the SC similar to that of heat application (Obata et al. 2010), which was also proposed by Watanabe et al. (2009). DSC and ATR-FTIR studies with 1,8-cineole and L-menthol revealed that both terpenes exerted effects on both lipid acyl tails and polar head groups, as demonstrated by a reduction in the main transition temperature (T_m) and in the non-hydrogen bonded amide I stretching frequency, respectively (Narishetty and Panchagnula 2005). However, at physiological temperature, terpenes mainly act at polar head groups and break the inter- and intralamellar hydrogen bonding network, which decreases integrity in the SC barrier. Terpenes also increased the hydration levels of the lipid system probably by forming new aqueous channels. These results indicated that 1.8-cineole and L-menthol enhanced transdermal permeation of the investigated drug zidovudine by transforming SC lipids from a highly ordered orthorhombic perpendicular subcellular packing to a less ordered hexagonal subcellular packing (Narishetty and Panchagnula 2004).

Jain et al. (2002) showed using FTIR that terpenes with weak self-association or which have the ability of donating or accepting H bonds (like menthol, cineole, menthone) increase the permeation of a water-soluble, polar drug, imipramine hydrochloride, through the breaking of hydrogen bonds between ceramides' polar head groups.

Linalool, carvacrol and terpineol were suggested to interact with SC lipids to enhance the transdermal delivery of haloperidol across human skin (Vaddi et al. 2002).

Terpenes, when applied in high quantities, can increase drug diffusivity also by making "pools" inside the SC. In this case they are phase separated within the SC from undisrupted lipid bilayers, i.e. they exist within separate domains in the SC. In this way permeable "pores" are formed, being important for the permeation of polar drugs (Cornwell and Barry 1994; Cornwell et al. 1996).

Terpenes can also enhance drug diffusivity by extraction of SC lipids. FTIR studies revealed the permeation enhancement of nicorandil by nerolidol and carvone due to partial lipid extraction (Krishnaiah et al. 2006); propranolol hydrochloride by menthone and D-limonene (in combination with ethanol) due to lipid extraction, macroscopic barrier perturbations and increased partitioning of the drug to the SC (Zhao and Singh 1999); and tamoxifen by eugenol and D-limonene (in combination with ethanol) due to lipid extraction and increased partitioning and by menthone only due to lipid extraction (Zhao and Singh 1998). Enhanced permeability of the SC to tamoxifen due to lipid extraction and macroscopic barrier perturbation was also found after application of menthone, eugenol and limonene in combination with propylene glycol. Moreover, the effective diffusion coefficient of tamoxifen through the epidermis was enhanced following treatment with either eugenol or limonene (Zhao and Singh 2000). FTIR and DSC studies revealed that the diterpene forskolin enhanced skin permeation of valsartan by disruption and extraction of lipid bilayers of the SC (Rizwan et al. 2008).

Besides lipid modification, also protein modification can influence the drug diffusivity after application of terpenes, as a proportion of terpenes may distribute into the corneocytes. In the corneocytes, terpenes interact with keratin, opening up the dense protein structure making it more permeable. Despite higher partitioning of terpenes into the lipid domains, their uptake into the protein domains may be significant due to the fact that intracellular protein domains make up 70–95 % of the SC volume (Cornwell et al. 1996). However, the transcellular penetration route has not been recognised as an important route.

As to indirect action of terpenes on the percutaneous drug penetration, it was found that 1,8-cineole increased the permeation of mefenamic acid by the "drag" or "pull" effect (Heard et al. 2006). Terpenes may also increase the drug permeation by increasing its thermodynamic activity in the vehicle (Kanikkannan et al. 2000).

Terpenes have mostly been used together with propylene glycol as they provide in that combination a higher enhancer efficacy, because of their

synergistic effect. The combination of menthol/propylene glycol enhanced the permeation of propofol (Yamato et al. 2009) and imipramine hydrochloride (Shah et al. 2008). DSC and SAXD investigations provided fragmented evidence that terpene/propylene glycol synergy may produce enhanced lipid bilayer disruption (Cornwell et al. 1996). It has also been proposed that propylene glycol increases the partitioning of the terpene into the SC, where it can exert its enhancer effect (Barry 2001). Terpenes have also been used with ethanol. 1,8-cineole and L-menthol applied at 5 % w/v in 66.6 % ethanol as a vehicle significantly enhanced the pseudo-steady-state flux of zidovudine (Narishetty and Panchagnula 2005). Menthone (1, 2, 3 and 5 %) in combination with 50 % ethanol (Zhao et al. 2001) and 5 % w/v of other terpenes (carvone, 1.8-cineole, menthol and thymol) in 50 % ethanol (Gao and Singh 1998) significantly enhanced the flux of tamoxifen compared to the control (50 % ethanol).

2.5.2 Azone

Azone® (1-dodecylazacycloheptan-2-one or laurocapram) was the first synthetic penetration enhancer. It shows very low toxicity and only mild skin irritancy. Structurally it has a polar head group attached to a C12 chain; therefore, it is very lipophilic. It can enhance the penetration of both hydrophilic and lipophilic drugs (Jampilek and Brychtova 2012). Azone® is one of the most studied chemical penetration enhancers, but despite this, it is not available in commercial topical or transdermal products. Azone® is a very effective chemical penetration enhancer at concentrations of 0.1–5 % (Jampilek and Brychtova 2012). Its mechanism of action is still not clear. Different hypotheses were suggested to explain the enhancing activity of Azone®. On the basis of DSC, FTIR and drug permeation studies, it was proposed that Azone® acts on the lipid bilayers of the SC, fluidizing them which leads to an increase of the drug diffusion (Harrison et al. 1996a, b). These authors assumed a homogeneous distribution of Azone® through the SC lipid array.

Due to the structure of Azone®, Hadgraft et al. (1996) suggested that hydrogen bonds are formed between the polar head group of Azone® and ceramides, being important for its penetration-enhancing activity. According to Hadgraft (1999), Azone® due to its long chain easily integrates into the bilayer and then the polar head group oxygen competes for the H bonds in the ceramide head groups, weakening them and thus forming the so-called channels, i.e. areas of increased fluidity in the lamellae that facilitate drug penetration.

Jampilek and Brychtova (2012) suggest that Azone® disrupts the packing of the lipid bilayer of the SC by heterogeneously partitioning into it and spreading across separate domains of the bilayer. This assumption is supported by Azone's angled conformation that forms a shape of a bent spoon which eases its intercalation between ceramides of the SC. Electron diffraction studies provided evidence that Azone® exists or partly exists as a distinct phase (i.e. in separate domains) within the SC lipids (Pilgram et al. 2001).

2.5.3 Fatty Acids

Fatty acids have been approved by the FDA as GRAS and have been used as penetration enhancers in some commercially available products (Ibrahim and Li 2010). Their effectiveness as penetration enhancers depends on their degree of saturation (and site of saturation), chain length and their structure. It was found for saturated fatty acids that a chain length of C10 to C12 would be optimal for an effective penetration enhancer, whilst in unsaturated fatty acids, C18 was optimal (Aungst et al. 1986; Aungst 1989). The higher the degree of unsaturation, the better enhancer the fatty acid would be. In the case of unsaturated fatty acids, the cis conformation allows for a higher disruption of the lipids in the SC than the trans conformation and a higher drug flux was obtained when the distance of the double bond from the carboxylic group was larger (Golden et al. 1987). Oleic acid presents the most investigated fatty acid as it was shown to be a potent penetration enhancer for various drugs

(Choi et al. 2012; Baek et al. 2013; Patel et al. 2014). It has been used in commercial transdermal products (Lane 2013). Oleic acid in its cis conformation was shown to increase lipid fluidity in porcine skin (Golden et al. 1987). From its structure, it is expected that oleic acid interacts with lipid chains in the SC, thus disturbing the lipid packing which would increase the bilayer fluidity. FTIR studies proposed a penetration-enhancing mechanism in which oleic acid exists as a separate phase, i.e. forms a "pool" in the SC lipids (Ongpipattanakul et al. 1991), which was confirmed by freeze fracture electron microscopy studies (Tanojo et al. 1997). Thus, oleic acid enhances drug penetration by forming permeable defects within the SC lipids, which results from the cis double bond of oleic acid favouring it to condense with itself rather than distribute homogeneously in the skin lipids (Lane 2013).

Because of their lipophilic nature, fatty acids are often used with co-solvents (such as propylene glycol, benzyl alcohol, isopropyl myristate). Studies showed that fatty acids and co-solvents act in a synergistic way to enhance the penetration of a drug. The mechanism of action proposed is the increased partitioning of fatty acids into the SC by the application of co-solvents (Barry 2001; Ibrahim and Li 2010).

2.5.4 Alcohols

Transdermal and dermal delivery systems often utilise alcohols to improve penetration enhancement of drugs. Alcohols used can be within the short-chain (ethanol, isopropyl alcohol) or the long-chain group, i.e. fatty alcohols (1-butanol, 1-propanol, 1-octanol, decanol, etc.) (Williams and Barry 2004; Lane 2013). Other alcohols, such as glycols (especially propylene glycol), have also been used in dermal and transdermal products.

Alcohols can exert their permeation-enhancing activity through various mechanisms: extraction of lipids and proteins, fluidization of lipids, increasing drug partitioning and drug solubility and changing the thermodynamic activity of the drug (Lane 2013; Williams and Barry 2004).

Studies with ethanol showed that it was effective in enhancing the flux of levonorgestrel, hydrocortisone, estradiol and 5-fluorouracil through rat skin in vitro (Williams and Barry 2004), estradiol through human skin in vivo (Pershing et al. 1990) and hinokitiol through hairless mouse skin in vitro (Joo et al. 2008). There are reports that suggested that the effect of ethanol on SC is concentration dependent, i.e. with the increase of ethanol concentration, the drug diffusion is enhanced up to an ethanol concentration whereas higher concentrations decrease drug penetration (Berner et al. 1989; Megrab et al. 1995; Thomas and Panchagnula 2003; Watkinson et al. 2009a). Ethanol can cause lipid and protein extraction from the SC when used at high concentrations (75 %v/v), and it will cause dehydration when used as absolute (Goates and Knutson 1994; Megrab et al. 1995). It is believed that skin dehydration which occurs at higher ethanol concentrations reduces percutaneous drug permeation (Williams and Barry 2004).

As to the mechanism of the permeation-enhancing effect of ethanol, ethanol may exert it through various mechanisms.

Being a solvent, ethanol can increase the solubility of the drug in the donor phase, which is important for poorly soluble drugs that may deplete within in the donor. Kadir et al. (1987) suggested that in patch formulations, ethanol can use the "push effect" mechanism to promote a greater driving force for the drug permeation. This effect happens due to the fast permeation of ethanol or ethanol evaporation from the donor phase, which makes the drug concentration higher reaching a supersaturated state, possessing higher driving force for drug permeation. Another mechanism which arises also from the rapid ethanol permeation is the "pull" or "drag" effect, where ethanol carries the permeant with it (Heard et al. 2006). However, that mechanism has been rejected in the case of morphine hydrochloride permeation from formulations where ethanol was used as a solvent (Morimoto et al. 2002).

As to the effect on the skin barrier function by acting on intercellular lipids, many enhancers and also ethanol act mainly in this way. According to Barry (2001), these enhancers may disrupt the SC

lipid organisation by penetrating into the lipid bilayers of the SC, where they can rotate, vibrate and form microcavities, thereby increasing the free volume for drug diffusion. Ethanol as a hydrophilic enhancer exerts a direct effect on the polar head groups of the ceramides in the SC by weakening their H bonds, which results in alterations in the head group domain (Guillard et al. 2009). Due to this effect, ethanol exerts an indirect effect on the organisation of acyl chains in the SC lipid bilayers (du Plessis et al. 2001), i.e. this kind of perturbations leads to fluidization of the lipid domain and increase of the water volume between layers, resulting in decreased resistance to drug diffusion and increased drug permeation (Lane et al. 2012). Hatta et al. (2010) used small- and wide-angle X-ray diffraction to follow the changes in the SC lipids and proteins after applying ethanol. This study showed that ethanol caused a slight disruption of the lipid structure, more strongly at the orthorhombic hydrocarbon chain packaging, and partial disruption of the soft keratin structure in the corneocytes. In addition to this, the authors suggest that ethanol formed "pools" in the hydrophilic region of the intercellular lipid matrix of the SC. The results of this study indicate that ethanol generates routes through which hydrophilic molecules penetrate the skin. When used at high concentrations for a prolonged time, ethanol can extract some of the lipid fraction from within the SC, as aforementioned (Williams and Barry 2004). Thermogravimetric analysis (TGA) and FTIR spectroscopy revealed that the application of ethanol, due to extraction of SC lipids, dehydrated the skin as it decreased the bound water content. This decreased the permeation of the solute, whilst increasing TEWL, and partitioning was predominant for the permeation of the solute (Shah et al. 2008).

As to partitioning promotion, ethanol as a solvent can influence the solubility of the skin tissue which results in improved partitioning of the drug into the skin membrane (Megrab et al. 1995). This effect of ethanol occurs due to its effect on the aqueous domain of the lipid bilayers, which increases the solubility of this site for the drug, i.e. it alters the solubility parameter of the skin by shifting it to a value close to that of the drug (Lane et al. 2012).

Ethanol has been due to its advantages used as a penetration enhancer in various commercial topical and transdermal preparations. For an overview of topical and transdermal preparations containing penetration enhancers available on the market, refer to Lane (2013).

2.5.5 Glycols

Propylene glycol is the most commonly used glycol in dermal and transdermal formulations (for commercial formulations, refer to Lane (2013)). It has been used as a co-solvent and has been recognised for its penetration-enhancing properties. Propylene glycol can be applied alone or as a vehicle for other penetration enhancers (such as oleic acid) and shows synergistic action when used in combination. The exact mechanism of its drug penetration-enhancing ability is not clearly understood; however, it is believed to be similar to that of ethanol. As a co-solvent, propylene glycol permeates rapidly through the skin which may alter the thermodynamic activity of the drug and modify/enhance the driving force for drug diffusion ("push effect") (Williams and Barry 2004). The "pull" or "drag effect" was also proposed for propylene glycol (Hoelgaard and Mollgaard 1985). It has been observed that the permeant penetration depth is highly correlated with the depth of penetration of PG (Pudney et al. 2007; Bonnist et al. 2011). Further, PG affects also the lipid domains in the SC in different ways. One of them is due to the fast partition of propylene glycol into the tissue where it interacts with the aqueous domain of lipid bilayers, changing the solubility properties of this site of skin for the drug, thereby increasing drug solubility and hence drug partitioning into the skin (Lane et al. 2012). The significant permeation enhancement of triprolidine base from PG was explained by this mode of action of PG (Kasting et al. 1993). Watkinson et al. (2009b) also reported that the main penetration-enhancing mechanism of PG in increasing drug (ibuprofen) permeation was by increasing drug solubility and partitioning and not diffusion. Bouwstra et al. (1991) proposed also that PG does not intercalate within the lipid

bilayers but may be incorporated in the polar head group domain of the packed lipids. This is in accordance with a recent study (Brinkmann and Müller-Goymann 2005) which revealed that PG integrates into the hydrophilic regions of the lipid bilayers between the polar head groups in the perpendicular direction to the bilayer, thereby increasing the distance in the lamellar phase. Further, findings from a study suggested that PG caused skin dehydration (Bouwstra et al. 1989). Shah et al. (2008) showed that PG and ethanol decreased the bound water content through protein alterations and extraction of lipids, respectively, causing dehydration and increased partitioning of the drug through dehydrated skin. Several research studies showed a correlation between the PG concentration used and the degree of drug penetration (Trottet et al. 2004; Watkinson et al. 2009b).

Propylene glycol shows synergistic action with terpenes (Cornwell et al. 1996; Yamato et al. 2009; Furuishi et al. 2013), oleic acid (Brinkmann and Müller-Goymann 2005; Choi et al. 2012), Azone® (Brinkmann and Müller-Goymann 2005) and other penetration enhancers.

2.5.6 Surfactants

Many studies that used surfactants in transdermal delivery systems have reported that they are effective as chemical penetration enhancers (Som et al. 2012). The major drawback when using surfactants is their skin irritancy and toxicity, which defines their limited use in vivo (for topical and transdermal penetration enhancement). Surfactants are amphiphatic molecules that have a hydrophobic part (lipophilic alkyl or aryl fatty chain) and a hydrophilic part (polar head group). They can be classified according to the nature of their polar group into: anionic, cationic, zwitterionic and nonionic surfactants. It should be kept in mind when applying surfactants that they are able to form micelles, solubilise the active and lower its thermodynamic activity and ultimately its skin permeation (Lane 2013). Effectiveness as chemical penetration enhancers will depend on the structure, chain length and polarity of the surfactant. Ionic surfactants interact with both keratin in the corneocytes and the lipid matrix of the SC (Som et al. 2012). Anionic surfactants such as sodium lauryl sulphate (SLS) interact with the lipids and keratin in the skin, and after increased exposure to them, they show greater penetration enhancement of the drug but also greater damage to the skin than other surfactants. Cationic surfactants (benzalkonium chloride, cetylpyridinium chloride) are even more potent in penetration enhancement of drugs and unfortunately in damaging the skin (Som et al. 2012). They interact with the SC proteins through polar and hydrophobic interactions. As they are strong skin irritants, they are generally not used as CPE for dermal and transdermal penetration enhancement. Nonionic surfactants (polyoxyethylene sorbitan fatty acid esters (polysorbates) – such as Tween 20, Tween 80, polyoxyethylene alkyl ethers (Brij), etc.) are better tolerated than the surfactants with the charged head group, but are less efficient as CPE (Williams and Barry 2004). The mechanism of action of nonionic surfactants includes the following effects: (1) on the lipid level: increasing the fluidity of the lipid bilayer, solubilisation and extraction of the lipids; (2) on the level of proteins: interactions with the keratin filaments in corneocytes; and (3) on the level of the drug: changing the thermodynamic activity of the drug, allowing more effective penetration into the skin. Zwitterionic surfactants (dodecyl betaine, hexadecyl betaine, hexadecylsulfobetaine, N, N-dimethyl-N-dodecyl amine oxide, dodecyltrimethylammonium bromide) also use fluidization of the lipids as their mechanism of action (Ridout et al. 1991).

2.5.7 Sulphoxides

DMSO has initially been used as a co-solvent in transdermal formulations to help improve drug partitioning into the skin (Karande and Mitragotri 2009). It is colourless, odourless and hygroscopic. Many studies reported its use as a penetration enhancer for both hydrophilic and lipophilic compounds. It has been shown to enhance the percutaneous penetration of antiviral agents, steroids and

antibiotics (Williams and Barry 2004). DMSO can enhance the drug penetration via a number of suggested mechanisms: extraction of skin lipids; interactions with keratin, such as changing the intercellular keratin conformation, from α helical to a β sheet, as well as displacement of bound water from keratin; and interactions with the lipid alkyl chains in the SC (Williams and Barry 2004; Lane 2013). Barry (1987) proposed that DMSO can exert its enhancer activity indirectly forming solvent-filled "pools" where the solubility of the drug is increased and directly by displacing water molecules initially from the proteins and then from the lipid polar head groups. These actions of DMSO would enable the formation of micro-channels between the lipid bilayers. In addition to this, it was proposed that DMSO could interact with the polar head groups of lipids, thus destabilising the regular lipid structure, making it more fluid and less resistant to drug diffusion (Williams and Barry 2004). DMSO may also change the solubility properties of SC for the permeant, by affecting the aqueous domain in the lipid bilayers, and hence facilitate drug partitioning from the vehicle into the SC (Lane et al. 2012; Williams and Barry 2004).

However, for an effective penetration enhancement, high concentrations of DMSO are required (Notman et al. 2008). This in turn can cause skin irritation and the production of a malodourous metabolite in the breath. These factors limit the commercial application of DMSO in transdermal products. Other compounds in the sulphoxide group have been tested for enhancer efficacy, dimethylformamide (DMF), dimethylacetamide (DMAC) and decylmethyl sulphoxide (DCMS), but they too either have adverse effects on the skin (DMF, DMAC) or are less effective enhancers for lipophilic drugs (DCMS) (Williams and Barry 2004).

2.5.8 Esters

The most common ester investigated as a penetration enhancer is isopropyl myristate, which has been used in commercial products (Lane 2013). Different research results were reported regarding the enhancing mechanism of this fatty acid ester. DSC studies indicated that isopropyl myristate integrates itself within the lipid bilayers and causes a more fluid bilayer structure, facilitating the drug flux (Leopold and Lippold 1995). Other data implied that isopropyl myristate pretreatment resulted in a more densely packed lipid bilayer (Brinkmann and Müller-Goymann 2003). In addition, studies from Santos et al. (2012) suggested that isopropyl myristate can increase drug solubility in the SC.

Octyl salicylate (OSAL), commonly used as a chemical sunscreen and regarded as safe (up to concentrations of 5 %), has also been used as a penetration enhancer. It has been used in commercial transdermal products (Lane 2013). Again, various results have been published regarding the mechanism of action for OSAL (Lane 2013). One hypothesis is that OSAL creates solvent "pools", which help to enhance drug diffusion (Santos et al. 2012).

Also other fatty acid esters are used in commercial products, such as sorbitan monooleate, glyceryl monooleate, glyceryl monolaurate, etc. (Lane 2013).

2.5.9 Transcutol® (Diethylene Glycol Monoethyl Ether)

Transcutol® represents a potent, non-toxic, biodegradable solubiliser able to significantly enhance percutaneous penetration of different drugs, such as lidocaine (Cázares-Delgadillo et al. 2005), clonazepam (Mura et al. 2000), etc. Thus, Transcutol® has been used in a number of dermal and transdermal commercial products (Lane 2013). It has been usually used in combination with a co-solvent, such as propylene glycol (Mura et al. 2000). It has also been used together with Azone® as it shows in that combination a higher enhancing effect than when used alone (Escobar-Chávez et al. 2005).

According to Harrison et al. (1996a), Transcutol® enhanced the permeation of the model permeant 4-cyanophenol by increasing its solubility in the SC. However, further studies are needed to explain its mechanism of enhancing the percutaneous drug penetration.

2.5.10 Water

Water is the most commonly used compound in transdermal formulations and the most natural penetration enhancer. In the SC, 15–20 % of the tissue dry weight is made of water that either is bound to some structural elements of the SC (25–35 % of water in the SC) or is in a free form, able to act as a solvent for polar molecules (Williams and Barry 2004).

Mechanism of action of water in penetration is not clear. It is believed that increased hydration generally will enhance drug flux across the SC for a range of drugs. However, some authors found that occlusion does not always increase drug permeation (Bucks and Maibach 1999). For hydrophilic drugs, the proposed mode of action of water is that water increases the solubility of the polar drug and then its partitioning into the SC (Williams and Barry 2004). Results about water's ability to modify the lipid membrane are contradictory. Bouwstra et al. (2003) showed that water does not affect the packing of lipids in the SC. Van Hal et al. (1996) showed the existence of water "pools" and occasionally vesicle-like structures within the intercellular lipid bilayers, but without significant disruption of the lipid domains. The existence of water "pools" suggests that a lipid-water phase separation occurred. Elias et al. (2002) proposed the existence of an "aqueous pore pathway" in the SC. In this model, lacunar domains that are heterogeneously distributed in the lipid bilayer under physiological conditions would under special conditions (extensive hydration, occlusion, etc.) interconnect to form a continuous "aqueous pore pathway" that would allow enhanced drug permeation.

Conclusion

Despite the extensive research carried out in the field of chemical penetration enhancers for dermal and transdermal drug delivery, very few of these compounds have found their application in commercial dermal and transdermal products. Limitations of their use stem from low efficacy in enhancing dermal/transdermal drug delivery when used at low concentrations and skin irritation (Karande and Mitragotri 2009). In addition to this, the experimental design used in studies with CPEs represents a problem, i.e. a number of studies have been carried out, but mostly in vitro and in non-human skin. Further, infinite doses are mostly used instead of finite doses which simulate the in vivo application of creams, gels, lotions and sprays in patients. Experiments that simulate clinical situations would improve the current knowledge about the possible use of CPEs and contribute to a better understanding of their mode of action and their therapeutic effectiveness. The potential of chemical enhancers lies in their combined use with other enhancers – due to their possible synergistic penetration-enhancing effect – either with other chemicals (which enables higher effectiveness concomitant with a reduction of their applied concentration and thus lower skin irritation) or with physical methods for dermal and transdermal drug delivery enhancement.

References

Akimoto T, Kawahara K, Nagase Y, Aoyagi T (2001) Polymeric transdermal drug penetration enhancer. The enhancing effect of oligodimethylsiloxane containing a glucopyranosyl end group. J Control Release 77: 49–57

Aqil M, Ahad A, Sultana Y, Ali A (2007) Status of terpenes as skin penetration enhancers. Drug Discov Today 12(23–24):1061–1067

Asbill CS, Michniak BB (2000) Percutaneous penetration enhancers: local versus transdermal activity. Pharm Sci Technol Today 3(1):36–41

Aungst BJ (1989) Structure/effect studies of fatty acid isomers as skin penetration enhancers and skin irritants. Pharm Res 6(3):244–247

Aungst BJ, Rogers N, Shefter E (1986) Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 33(1–3):225–234

Babu RJ, Pandit JK (2005) Effect of penetration enhancers on the transdermal delivery of bupranolol through rat skin. Drug Deliv 12:165–169

Baek JS, Lim JH, Kang JS, Shin SC, Jung SH, Cho CW (2013) Enhanced transdermal drug delivery of zaltoprofen using a novel formulation. Int J Pharm 453(2):358–362

Barry BW (1983) Dermatological formulations: percutaneous absorption. Marcel Dekker, New York

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6(1):85–97

Barry BW (1991) Lipid–protein-partitioning theory of skin penetration enhancement. J Control Release 15: 237–248

Barry BW (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2:101–114

Barry BW (2004) Breaching the skin's barrier to drugs. Nat Biotechnol 22(2):165–167

Benson HA (2005) Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv 2(1): 23–33

Berner B, Mazzenga GC, Otte JH, Steffens RJ, Juang R-H, Ebert CD (1989) Ethanol: water mutually enhanced transdermal therapeutic system: II. Skin permeation of ethanol and nitroglycerin. J Pharm Sci 78:402–407

Bonnist EY, Gorce JP, Mackay C, Pendlington RU, Pudney PD (2011) Measuring the penetration of a skin sensitizer and its delivery vehicles simultaneously with confocal Raman spectroscopy. Skin Pharmacol Physiol 24(5):274–283

Bouwstra JA, Peschier LJC, Brussee J, Boddé HE (1989) Effect of N-alkyl-azocycloheptan-2-ones including azone on the thermal behaviour of human stratum corneum. Int J Pharm 52:47–54

Bouwstra JA, de Vries MA, Gooris GS, Bras W, Brussee J, Ponec M (1991) Thermodynamic and structural aspects of skin barrier. J Control Release 1:209–220

Bouwstra JA, de Graaff A, Gooris GS, Nijsse J, Wiechers JW, van Aelst AC (2003) Water distribution and related morphology in human stratum corneum at different hydration levels. J Invest Dermatol 120(5):750–758

Brain KR, Green DM, Dykes PJ, Marks R, Bola TS (2006) The role of menthol in skin penetration from topical formulations of ibuprofen 5% in vivo. Skin Pharmacol Physiol 19:17–21

Brinkmann I, Müller-Goymann CC (2003) Role of isopropyl myristate, isopropyl alcohol and a combination of both in hydrocortisone permeation across the human stratum corneum. Skin Pharmacol Appl Skin Physiol 16:393–404

Brinkmann I, Müller-Goymann CC (2005) An attempt to clarify the influence of glycerol, propylene glycol, isopropyl myristate and a combination of propylene glycol and isopropyl myristate on human stratum corneum. Pharmazie 60(3):215–220

Bucks D, and Maibach HI (1999) Occlusion Does Not Uniformly Enhance Penetration *In Vivo*. p. 81–105 in Percutaneous Absorption, 3rd ed., Bronaugh RL, and Maibach HI, eds. New York: Marcel Dekker, Inc.

Cázares-Delgadillo J, Naik A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A (2005) Skin permeation enhancement by sucrose esters: a pH-dependent phenomenon. Int J Pharm 297(1–2):204–212

Choi J, Choi MK, Chong S, Chung SJ, Shim CK, Kim DD (2012) Effect of fatty acids on the transdermal delivery of donepezil: in vitro and in vivo evaluation. Int J Pharm 422(1–2):83–90

Cornwell PA, Barry BW (1994) Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. J Pharm Pharmacol 46(4):261–269

Cornwell PA, Barry BW, Bouwstra JA, Gooris GS (1996) Modes of action of terpene penetration enhancers in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. Int J Pharm 127:9–26

Du Plessis J, Pugh WJ, Judefeind A, Hadgraft J (2001) The effect of hydrogen bonding on diffusion across model membranes: consideration of the number of H-bonding groups. Eur J Pharm Sci 13:135–141

Elias PM, Tsai J, Menon GK, Holleran WM, Feingold KR (2002) The potential of metabolic interventions to enhance transdermal drug delivery. J Investig Dermatol Symp Proc 7(1):79–85

Escobar-Chávez JJ, Quintanar-Guerrero D, Ganem-Quintanar A (2005) In vivo skin permeation of sodium naproxen formulated in pluronic F-127 gels: effect of Azone and Transcutol. Drug Dev Ind Pharm 31(4–5):447–454

Furuishi T, Kato Y, Fukami T, Suzuki T, Endo T, Nagase H, Ueda H, Tomono K (2013) Effect of terpenes on the skin permeation of lomerizine dihydrochloride. J Pharm Pharm Sci 16(4):551–563

Gao S, Singh J (1998) In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J Control Release 51(2–3):193–199

Ghafourian T, Zandasrar P, Hamishekar H, Nokhodchi A (2004) The effect of penetration enhancers on drug delivery through skin: a QSAR study. J Control Release 99:113–125

Goates CY, Knutson K (1994) Enhanced permeation of polar compounds through human epidermis. I. Permeability and membrane structural changes in the presence of short chain alcohol. Biochim Biophys Acta 1195:169–179

Golden GM, McKie JE, Potts RO (1987) Role of stratum corneum lipid fluidity in transdermal drug flux. J Pharm Sci 98:4085–4099

Guillard EC, Laugel C, Baillet-Guffroy A (2009) Molecular interactions of penetration enhancers within ceramides organization: a FTIR approach. Eur J Pharm Sci 36:192–199

Hadgraft J (1999) Passive enhancement strategies in topical and transdermal drug delivery. Int J Pharm 184(1):1–6

Hadgraft J, Peck J, Williams D, Pugh J, Allan G (1996) Mechanisms of action of skin penetration enhancers/retarders: azone and analogues. Int J Pharm 141(1–2): 17–25

Harrison JE, Watkinson AC, Green DM, Hadgraft J, Brain K (1996a) The relative effect of azone and transcutol on permeant diffusivity and solubility in human stratum corneum. Pharm Res 13(4):542–546

Harrison JE, Groundwater PW, Brain K, Hadgraft J (1996b) Azone® induced fluidity in human stratum corneum. A fourier transform infrared spectroscopy investigation using the perdeuterated analogue. J Control Release 41(3):283–290

Hatta I, Nakazawa H, Obata Y, Ohta N, Inoue K, Yagi N (2010) Novel method to observe subtle structural modulation of stratum corneum on applying chemical agents. Chem Phys Lipids 163:381–389

Heard CM, Kung D, Thomas CP (2006) Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect. Int J Pharm 321:167–170

Herman A, Herman AP (2015) Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. J Pharm Pharmacol 67(4):473–485

Hoelgaard A, Mollgaard B (1985) Dermal drug delivery – improvement by choice of vehicle or drug derivative. J Control Release 2:111–120

Hori M, Satoh S, Maibach HI, Guy RH (1991) Enhancement of propranolol hydrochloride and diazepam skin absorption in vitro: effect of enhancer lipophilicity. J Pharm Sci 80:32–35

Ibrahim SA, Li SK (2010) Efficiency of fatty acids as chemical penetration enhancers: mechanisms and structure enhancement relationship. Pharm Res 27(1):115–125

Jain AK, Thomas NS, Panchagnula R (2002) Transdermal drug delivery of imipramine hydrochloride: I. Effect of terpenes. J Control Release 79:93–101

Jampilek J, Brychtova K (2012) Azone analogues: classification, design, and transdermal penetration principles. Med Res Rev 32(5):907–947

Joo HH, Kim JC, Lee HY (2008) In vitro permeation study of hinokitiol: effects of vehicles and enhancers. Drug Deliv 15:19–22

Kadir R, Stempler D, Liron Z, Cohen S (1987) Delivery of theophylline into excised human skin from alkanoic acid solutions: a 'push– pull' mechanism. J Pharm Sci 76:774–779

Kanikkannan N, Kandimalla K, Lamba SS, Singh M (2000) Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 7:593–608

Karande P, Mitragotri S (2009) Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta 1788(11):2362–2373

Kasting GB, Francis WR, Roberts GE (1993) Skin penetration enhancement of triprolidine base by propylene glycol. J Pharm Sci 82(5):551–552

Krishnaiah YS, Al-Saidan SM, Chandrasekha DV, Rama B (2006) Effect of nerodilol and carvone on in vitro permeation of nicorandil across rat epidermal membrane. Drug Dev Ind Pharm 32:423–435

Lane ME (2013) Skin penetration enhancers. Int J Pharm 447:12–21

Lane ME, Santos P, Watkinson AC, Hadgraft J (2012) Passive skin permeation enhancement. In: Benson HE, Watkinson AC (eds) Topical and transdermal drug delivery. Wiley, Hoboken, pp 23–42

Leopold CS, Lippold BC (1995) An attempt to clarify the mechanism of the penetration enhancing effects of lipophilic vehicles with differential scanning calorimetry (DSC). J Pharm Pharmacol 47(4):276–281

Megrab NA, Williams AC, Barry BW (1995) Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent system. Int J Pharm 116:101–112

Menczel EM (1995) Delipidization of the cutaneous permeability barrier and percutaneous penetration. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers. CRC Press, Boca Raton, pp 383–392

Moghadam SH, Saliaj E, Wettig SD, Dong C, Ivanova MV, Huzil JT, Foldvari M (2013) Effect of chemical permeation enhancers on stratum corneum barrier lipid organizational structure and interferon alpha permeability. Mol Pharm 10(6):2248–2260

Moghimi HR, Williams AC, Barry BW (1997) A lamellar matrix model for stratum corneum intercellular lipids. V. Effect of terpene penetration enhancers on the structure and thermal behaviour of the matrix. Int J Pharm 146:41–54

Morimoto H, Wada Y, Seki T, Sugibayashi K (2002) In vitro skin permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and L-menthol. Biol Pharm Bull 25:134–136

Mura P, Faucci MT, Bramanti G, Corti P (2000) Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. Eur J Pharm Sci 9(4):365–372

Narishetty ST, Panchagnula R (2004) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J Control Release 95(3):367–379

Narishetty ST, Panchagnula R (2005) Effect of L-menthol and 1,8-cineole on phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. J Control Release 102(1):59–70

Notman R, Anwar J, Briels WJ, Noro MG, den Otter WK (2008) Simulations of skin barrier function: free energies of hydrophobic and hydrophilic transmembrane pores in ceramide bilayers. Biophys J. 95(10): 4763–71.

Obata Y, Takayama K, Machida Y, Nagai T (1991) Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des Discov 8:137–144

Obata Y, Utsumi S, Watanabe H, Suda M, Tokudome Y, Otsuka M, Takayama K (2010) Infrared spectroscopic study of lipid interaction in stratum corneum treated with transdermal absorption enhancers. Int J Pharm 389:18–23

Okabe H, Obata Y, Takayama K, Nagai T (1990) Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. Drug Des Deliv 6:229–238

Ongpipattanakul B, Burnette RR, Potts RO, Francoeur ML (1991) Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm Res 7:350–354

Osborne DW, Henke JJ (1997) Skin penetration enhancers cited in the technical literature. Pharm Technol 21:58–66

Patel RP, Gaiakwad DR, Patel NA (2014) Formulation, optimization, and evaluation of a transdermal patch of heparin sodium. Drug Discov Ther 8(4):185–193

Pershing LK, Lambert LD, Knutson K (1990) Mechanism of ethanol-enhanced estradiol permeation across human skin in vivo. Pharm Res 7(2):170–175

Pilgram GS, van der Meulen J, Gooris GS, Koerten HK, Bouwstra JA (2001) The influence of two azones and sebaceous lipids on the lateral organization of lipids isolated from human stratum corneum. Biochim Biophys Acta 1511(2):244–254

Pudney PD, Mélot M, Caspers PJ, Van Der Pol A, Puppels GJ (2007) An in vivo confocal Raman study of the delivery of trans retinol to the skin. Appl Spectrosc 61(8):804–811

Ridout G, Hinz RS, Hostynek JJ, Reddy AK, Wiersema RJ, Hodson CD, Lorence CR, Guy RH (1991) The effects of zwitterionic surfactants on skin barrier function. Fundam Appl Toxicol 16(1):41–50

Rizwan M, Aqil M, Ahad A, Sultana Y, Ali MM (2008) Transdermal delivery of valsartan: I. Effect of various terpenes. Drug Dev Ind Pharm 34:618–626

Santos P, Watkinson AC, Hadgraft J, Lane ME (2012) Influence of penetration enhancer on drug permeation from volatile formulations. Int J Pharm 439(1–2): 260–268

Shah DK, Khandavilli S, Panchagnula R (2008) Alteration of skin hydration and its barrier function by vehicle and permeation enhancers: a study using TGA, FTIR, TEWL and drug permeation as markers. Methods Find Exp Clin Pharmacol 30:499–512

Som I, Bhatia K, Yasir M (2012) Status of surfactants as penetration enhancers in transdermal drug delivery. J Pharm Bioallied Sci 4(1):2–9

Song YH, Gwak HS, Chun IK (2009) The effects of terpenes on the permeation of lidocaine and ofloxacin from moisture-activated patches. Drug Deliv 16:75–81

Songkro S (2009) An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. Songklanakarin J Sci Technol 31(3):299–321

Tanojo H, Bosvangeest A, Bouwstra JA, Junginger HE, Bodde HE (1997) In-vitro human skin barrier perturbation by oleic-acid – thermal-analysis and freeze-fracture electron-microscopy studies. Thermochim Acta 293(1–2):77–85

Tas C, Ozkan Y, Okyar A, Savaser A (2007) In vitro and ex vivo permeation studies of etodolac from hydrophilic gels and effect of terpenes as enhancers. Drug Deliv 14:453–459

Thomas NS, Panchagnula R (2003) Transdermal delivery of zidovudine: effect of vehicles on permeation across rat skin and their mechanism of action. Eur J Pharm Sci 18:71–79

Trottet L, Merly C, Mirza M, Hadgraft J, Davis AF (2004) Effect of finite doses of propylene glycol on enhancement of in vitro percutaneous permeation of loperamide hydrochloride. Int J Pharm 274(1–2):213–219

Vaddi HK, Ho PC, Chan YW, Chan SY (2002) Terpenes in propylene glycol as skin-penetration enhancers: permeation and partition of haloperidol, fourier transform infrared spectroscopy, and differential scanning calorimetry. J Pharm Sci 91:1639–1651

Van Hal DA, Jeremiasse E, Junginger HE, Spies F, Bouwstra JA (1996) Structure of fully hydrated human stratum corneum: a freeze fracture electron microscopy study. J Invest Dermatol 106:89–95

Watanabe H, Obata Y, Ishida K, Takayama K (2009) Effect of l-menthol on the thermotropic behavior of ceramide 2/cholesterol mixtures as a model for the intercellular lipids in stratum corneum. Colloids Surf B Biointerfaces 73:116–121

Watkinson RM, Herkenne C, Guy RH, Hadgraft J, Oliveira G, Lane ME (2009a) Influence of ethanol on the solubility, ionization and permeation characteristics of ibuprofen in silicone and human skin. Skin Pharmacol Physiol 22:15–21

Watkinson RM, Guy RH, Hadgraft J, Lane ME (2009b) Optimisation of cosolvent concentration for topical drug delivery – II: influence of propylene glycol on ibuprofen permeation. Skin Pharmacol Physiol 22: 225–230

Williams AC, Barry BW (1989) Permeation, FTIR and DSC investigations of terpene penetration enhancers in human skin. J Pharm Pharmacol 41:12P

Williams AC, Barry BW (1991) The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs. Int J Pharm 74: 157–168

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56(5):603–618

Yamane MA, Williams AC, Barry BW (1995) Terpene penetration enhancers in propylene glycol/water cosolvent systems: effectiveness and mechanism of action. J Pharm Pharmacol 47:978–989

Yamato K, Takahashi Y, Akiyama H, Tsuji K, Onishi H, Machida Y (2009) Effect of penetration enhancers on transdermal delivery of propofol. Biol Pharm Bull 32:267–274

Zhao K, Singh J (1998) Mechanisms of percutaneous absorption of tamoxifen by terpenes: eugenol, D-limonene and menthone. J Control Release 55(2–3): 253–260

Zhao K, Singh J (1999) In vitro percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ethanol. J Control Release 62:359–366

Zhao K, Singh J (2000) Mechanism(s) of in vitro percutaneous absorption enhancement of tamoxifen by enhancers. J Pharm Sci 89(6):771–780

Zhao K, Singh S, Singh J (2001) Effect of menthone on the in vitro percutaneous absorption of tamoxifen and skin reversibility. Int J Pharm 219:177–181