Overcoming the Stratum Corneum: The Modulation of Skin Penetration

A Review

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Abstract
It is preferred that topically administered drugs act either dermally or transdermally. For that reason they have to penetrate into the deeper skin layers or permeate the skin. The outermost layer of the human skin, the stratum corneum, is responsible for its barrier function. Most topically administered drugs do not have the ability to penetrate the stratum corneum. In these cases modulations of the skin penetration profiles of these drugs and skin barrier manipulations are necessary. A skin penetration enhancement can be achieved either chemically, physically or by use of appropriate formulations. Numerous chemical compounds have been evaluated for penetration-enhancing activity, and different modes of action have been identified for skin penetration enhancement. In addition to chemical methods, skin penetration of drugs can be improved by physical options such as iontophoresis and phonophoresis, as well as by combinations of both chemical and physical methods or by combinations of several physical methods. There are cases where skin penetration of the drug used in the formulation is not the aim of the topical administration. Penetration reducers can be used to prevent chemicals entering the systemic circulation. This article concentrates on the progress made mainly over the last decade by use of chemical penetration enhancers. The different action modes of these substances are explained, including the basic principles of the physical skin penetration enhancement techniques and examples for their application.

Structure of the Stratum Corneum and Drug Options to Overcome the Barrier

The skin is the largest human organ. It ensures that harmful substances and drugs released from topically applied formulations cannot intrude into the organism off-hand [1]. The evolutionary development of the human skin as a potential protective barrier keeping water in and noxious substances out of the human body was a requirement for terrestrial life [2]. Figure 1 illustrates the complex structure of the human skin and the several layers schematically [3].

The outermost layer of the skin, the stratum corneum, is of particular interest as it determines this barrier function [4]. The qualification for this is the unique physico-chemical composition of the stratum corneum [5]. The ‘brick and mortar model’ is applied to describe the structure of the horny layer. Corneocytes are the ‘bricks’ em-
bedded in an intercellular lipid matrix of mainly fatty acids, ceramides, cholesterol and cholesterol sulfate [6]. In 1994 a more differentiated variation of this model, the domain mosaic model, was introduced by Forslind [7]. Recently, Norlen postulated two new models for stratum corneum characterization. For skin barrier formation a membrane-folding model was proposed [8] and skin barrier structure and function was simplified by a single gel phase model [9].

Three different functions may be achieved when applying drugs to the human skin. Firstly, it may be desirable to have the active remaining on the surface of the skin, e.g. for skin disinfection, dermal insect repellents and cosmetics for skin decoration. These pharmaceuticals or cosmetics are called epidermal formulations.

The second function is when formulations for topical administration are designed to allow the dermal penetration of their actives into the deeper regions of the skin such as the viable epidermis and the dermis. These are endodermal or diadermal formulations. The absorption into the systemic circulation is not the aim of these formulations. If partial absorption was to occur it could lead to adverse side effects after extensive use of these formulations [10].

Thirdly, the systemic action of drugs by transdermal application can be the aim of the topical therapy. Local reactions are undesired in this case.

All three kinds of administration are controlled by the anatomical properties of the skin (e.g. skin type and actual skin condition), drug features (e.g. lipophilicity, particle size, protein binding capacity) and the formulation features (e.g. vehicle composition, rheological properties).

There are two general options for drug substances to permeate the stratum corneum: the transepidermal route and the route via pores. Figure 2 illustrates these drug permeation options [10]. The transepidermal route can be divided into the transcellular and the intercellular route. The more direct route is the transcellular. Here the drug has to cross the skin by directly passing through both the lipid structures of the stratum corneum and the cytoplasm of the dead keratinocytes. This is the shortest route for the drug substance, but the substances encounter significant resistance to permeation because they have to cross both lipophilic and hydrophilic structures. The more common route for drugs to permeate the skin is the intercellular route [11]. Here the permeant overcomes the stratum corneum by passing between the corneocytes. Since the skin appendages (glands and hair follicles) occupy only 0.1% of the total human skin surface, the contribution to the pore route was primarily considered to be small [12]. A theoretical system validation approach has been applied for shunt route analysis [13]. Recently, it was shown that follicular penetration may also be an important pathway for the penetration of topically administered substances [14]. The follicular apparatus of hair follicles, the sweat glands and microlesions in the interfollicular horny layer were introduced as theoretical vertical pathways for percutaneous penetration [15]. The penetration of microparticles into the hair follicles and the resulting opportunities, e.g. for the transport of UV protective agents in sunscreens, have been illustrated [16].
Modulation of Skin Penetration

Penetration Enhancers

A penetration enhancement of drugs after topical application can be achieved by several compounds which are able to promote the transport of actives across the skin barrier. There are a variety of mechanisms for penetration enhancement by these substances [17]. One possibility is the interaction of the enhancers with the polar headgroups of the lipids. The lipid-lipid headgroup interactions and the packing order of the lipids are thus disturbed. The result is the facilitation of the diffusion of hydrophilic drugs [18]. By the increased flow the content of free water molecules between the bilayers is increased, which leads to an augmentation of the cross-section for polar drug diffusion. Simple hydration can be used in structure modification which results in changes to drug penetration [19]. Water is one of the most effective and safest penetration enhancers. By hydration of the stratum corneum, the penetration of most drugs can be increased. Normally in the stratum corneum the water content is 5–10%. The water content can be increased up to 50% under occlusive conditions (e.g. by use of impermeable foil or by application of occlusive vehicles) [10]. Furthermore, moisturizers such as urea can be used to increase the hydration of the stratum corneum and in consequence to improve the diffusion of hydrophilic drugs.

The headgroup disturbance of lipids by polar enhancer substances can also affect the hydrophobic parts of the lipids and leads to rearrangements in these bilayer areas [20]. This also explains the penetration improvement for lipophilic drugs by use of lipid headgroup-influencing hydrophilic penetration enhancers [17].

Another possibility is the interaction of lipophilic penetration enhancers with the hydrocarbon chains of the bilayer lipids. The penetration of lipophilic drugs is facilitated this way by packing order disturbance due to an increased fluidization of the hydrocarbon chains. These changes also influence the order of the polar headgroups, which explains the penetration enhancement of hydrophilic drugs by use of a lipophilic enhancer substance [21]. Figure 3 illustrates the influence of penetration enhancers on both the lipophilic pathway and the hydrophilic pathway of drug penetration.

Some of the chemical enhancer substances (structures of the frequently used substances are shown in figure 4) need suitable vehicles or cosolvents (e.g. propylene glycol) to reach the polar lipid parts and exert their functions [22]. The increase in the drug solubility and the improvement of its partition coefficient (skin/vehicle) is another mechanism explaining the action of penetration enhancers [23].

Additionally, the stratum corneum can be made more permeable for drug substances by the extraction of its lipids as the result of an interaction with chemical penetration enhancers [17].

Alcohols

Ethanol is the most intensely investigated penetration enhancer in relation to penetration behavior and its interaction with human skin. It is most commonly used in many transdermal formulations and also in patches. There are several mechanisms for the permeation-enhancing action of ethanol and higher alcohols [17]. As a solvent, ethanol is able to increase the solubility of the drug already in the formulation. Furthermore, the solu-
bility properties of the tissue can be optimized for the drug after the penetration of ethanol into the stratum corneum. Alcohols are able to extract lipids and proteins and thereby increase the porosity of the stratum corneum [19]. Thus, the penetration of hydrophilic drug substances is facilitated. The penetration enhancement of lipophilic drugs by alcohols is due to the higher solubility of the drug substances in the lipophilic areas of the stratum corneum because of the presence of alcoholic enhancers. For water soluble drugs a minimum amount of water is necessary for an optimal penetration enhancement by alcohols [17].

The length of the alkyl chain is important for the skin penetration-enhancing action of alcohols. Up to six carbon atoms the enhancement can be increased by use of alcohols with more carbon atoms. The use of alcohols with higher carbon atom numbers showed a decrease in the penetration rate during experiments with indomethacin as a model drug [24].

Recently, Krishnaiah et al. [25] tested the effects of several solvent systems on the in vitro permeability of nicardipine hydrochloride through excised rat epidermis. They found that the use of the binary solvent system, ethanol and water in the ratio of 70:30 v/v, is an effective vehicle for the development of a transdermal therapeutic system for nicardipine hydrochloride. A linear relationship between ethanol concentration and a surrogate marker of lipid removal and disorganization in the stra-
tum corneum has been found by means of corneoxenometry, a bioassay where interactions between xenobiotics and corneocytes are assessed using reflectance colorimetry [26]. The more lipophilic monoethylether of the bivalent alcohol diethylene glycol (Transcutol®) has been a frequently used skin penetration enhancer in recent years. Transcutol was shown to effectively enhance the transport of the broad-spectrum antiparasitic agent ivermectin through porcine skin [27]. Harrison et al. [28] investigated the synergism of Transcutol and Azone® on permeant diffusivity and solubility in human stratum corneum. It was shown that Azone reduced diffusional resistance of the stratum corneum and Transcutol increased the solubility of a model penetrant in this barrier. Mura et al. [29] evaluated Transcutol as a clonazepam transdermal penetration enhancer and showed in the experiments an increase in drug permeation as a function of Transcutol content in the formulation. Furthermore, a synergistic enhancement of the clonazepam flux was shown after the use of a combination of Transcutol and propylene glycol. The percutaneous penetration of nimesulide formulated in carbopole 934 gels was significantly increased compared with a control formulation after the application of a combination of oleic acid (3%) and Transcutol (30%) [30]. The incorporation of diethylene glycol monoethyl ether into a microemulsion resulted in an optimized formulation for the topical administration of the poorly water soluble drug vinpocetine [31]. This ether was also used to increase the skin accumulation of the UV absorbers oxybenzone and cinnamate [32].

Sulphoxides

Among the sulphoxides, dimethylsulphoxide (DMSO) and decymethylsulphoxide (DCMS) are frequently used as skin penetration enhancers. There are several mechanisms accountable for the enhancing effects of sulphoxides. DMSO is a powerful aprotic solvent with a high dielectricity constant because of the S-O-bond polarity. The dissolving power of DMSO for salts and polar compounds is very high and may lead in some cases to a complete ionization of salts at concentrations below 1 mM [19]. Because of its outstanding dissolving properties DMSO is able to generate solvent-filled spaces in the stratum corneum where the solubility of the drug substances is increased. Furthermore, the organization of the barrier lipids of the stratum corneum is disturbed by DMSO when administered in concentrations above 60%. A denaturation of intercellular structural proteins of the stratum corneum by DMSO and DCMS has been postulated as an additional reason for the promotion of skin penetration when using sulphoxides as enhancers. DMSO is able to change the intercellular keratin confirmation from an alpha helix to a beta sheet [19].

There are many studies described in the literature illustrating the penetration-enhancing effects of DMSO for both hydrophilic and lipophilic substances. The flux of azathioprine studied using a Franz diffusion cell and rat skin was increased by DMSO by 20.7% [33]. Significant enhancement of the permeation of prazosin hydrochloride through full-thickness skin of Swiss albino mice was measured by use of 5% DMSO [34]. DCMS showed the highest enhancing effect on the flux of isosorbide dinitrate from acrylic adhesives [35].

The problem with DMSO, as with many potent chemical skin penetration enhancers, is the skin irritating effects the substance may cause when used in the concentration required for skin penetration enhancement. Erythema, scaling, contact urticaria, stinging, burning and systemic symptoms are described after DMSO application [36]. Due to its potential toxicity and the possible side effects, other enhancers with similar effects could be a better choice [37].

Azone and Derivatives

Azone (laurocapram) and its derivatives are the first molecules which were specifically designed as penetration enhancers. Azone is a highly lipophilic liquid with an octanol/water partition coefficient of 6.21 [17]. It is a chemically stable compound and an excellent solvent for many drugs. The substance has a low irritating potential, a very low toxicity and nearly no pharmacological activity. Azone and its derivatives were heavily investigated in the 1980s. They can be used as penetration enhancers for hydrophilic and lipophilic substances and for peptide molecules as well, e.g. insulin and vasopressin. Interestingly, Azone and derivatives are effective penetration enhancers when used in low concentrations (1–5%). Their activity can be increased by the addition of co-solvents like propylene glycol or ethanol. Azone derivatives are soluble in these liquids and compatible with most organic solvents, and these are other advantages of this type of artificial enhancers [19].

Although Azone and its derivatives have now been in use as penetration enhancers for over 20 years, their mechanism of action is under ongoing research. The penetration-enhancing effects of these compounds are probably due to an intercalation into the structured lipids of the stratum corneum and the disturbance of the lipid packing order. The carbon chain of the Azone molecule consists of 12 carbon atoms. The dimensions of this chain
are comparable with the dimensions of the cholesterol skeleton. A decrease in the cholesterol-cholesterol interferences and the cholesterol-ceramide interactions can be assumed in the presence of Azone derivatives. The fluidity of the hydrophobic stratum corneum regions is increased and the permeation resistance of the horny layer against drug substances is reduced by Azone [17].

Among the large number of investigated Azone derivatives, the compounds with a chain length of 12 carbon atoms were the most effective ones independent of the ring dimensions.

The penetration behavior of sodium naproxen was determined recently by formulating the active in Pluronic F-127 gels containing Azone and the powerful solubilizing agent Transcutol. It was found that the combination of Azone and Transcutol enhanced sodium naproxen penetration, with enhancement ratios of up to 2-fold compared with the formulation containing only Transcutol [38].

Pyrrolidones

Pyrrolidones and related compounds have been investigated as penetration enhancers. As with many other penetration enhancers, pyrrolidones are able to promote both the penetration of hydrophilic drugs and the penetration of lipophilic drug substances. N-Methyl-2-pyrrolidone (NMP) and 2-pyrrolidone (2P) as well as 2-pyrrolidone-5-carboxylic acid are the most widely used enhancers of this group. NMP was shown to increase the skin permeation of estradiol in Yucatan micropig epidermis using a modified Franz-type diffusion cell when added at a concentration of 10% to an oily gel formulation consisting of isocetyl stearate and hydrogenated phospholipids [39]. More recently, the role of NMP as an enhancer for permeants delivered from an aqueous phase was investigated in the transdermal delivery of the local anesthetics lidocaine free base, lidocaine hydrochloride and prilocaine hydrochloride [40]. A flux increase of all compounds tested in the study was measured, demonstrating the capability of NMP to enhance hydrophilic and lipophilic drugs from an aqueous phase. An improvement in the skin permeation of griseofulvin, a drug with poor solubility in both water and oil, was shown by use of NMP as a penetration enhancer as well [41].

Hydrophilic pyrrolidones primarily enhance penetration via the polar route, whereas more lipophilic pyrrolidone derivatives like NMP are able to penetrate into the hydrophobic regions of the stratum corneum and reduce the barrier function in these areas. The more lipophilic derivative N-dodecyl-2-pyrrolidone has been evaluated as a transdermal penetration enhancer using a novel skin alternative for the test and showed a statistically significant higher skin content of the model drug hydrocortisone in comparison with the control [42]. The effects of NMP and 2P on the release and skin permeation of bupropranolol from reservoir-type transdermal delivery systems have recently been investigated. A 3-fold higher penetration rate was shown for 2P and a 1.5-fold higher penetration rate was measured in the case of NMP when using the pyrrolidones at a concentration of 5% in bupropranolol polymer gels [43]. 1-octyl-2-pyrrolidone was used as a penetration enhancer for model analysis of corticosterone flux enhancement across hairless mouse skin using a one-layer and a two-layer model [44].

However, the clinical use of pyrrolidones as skin penetration enhancers is limited because of adverse reactions to these compounds. Erythema and other irritant cutaneous reactions were observed after pyrrolidone use on human skin [45].

Urea and Derivatives

Urea is an odorless and colorless crystalline solid. It is a slightly hygroscopic substance with good water solubility and weak alkaline properties. It is prone to hydrolysis.

Urea is used in dermatology as a hydrating agent for the treatment of psoriasis, neurodermatitis and other hyperkeratotic skin conditions. As a moderate keratolytic substance, it influences the stratum corneum keratinocytes with species-specific percutaneous absorption rates [17].

The keratolytic properties of urea and its derivatives are one reason for the modest penetration enhancement achieved by use of these compounds. The other reason for the enhancing effects of urea derivatives is the increase in the stratum corneum water content by these moisturizing agents. This may lead to hydrophilic diffusion channels within the barrier [19].

In a trial involving over 200 patients suffering from various skin disorders, urea was suggested as an effective moisturizer and an enhancer of hydrocortisone penetration into the skin [46]. A nonirritating chemical enhancer system containing ethanol, menthol, camphor, methyl salicylate and urea in a hydrogel was shown to strongly enhance the skin penetration of the nonapeptide leuprolide [47]. In a study on the percutaneous absorption of progesterone, the most efficient skin penetration enhancer besides Azone was urea in polyethylene glycol bases. The diffusion was enhanced 2.5-fold compared with the pure base [48].
However, the application of urea and its derivatives as penetration enhancers is limited by their inadequate chemical stability, the proteolytic properties and the skin irritating effects connected with these properties [17].

To reduce skin irritation and increase skin penetration enhancement, cyclic urea analogues were synthesized. These was then tested using snake skin and hairless mouse skin with indomethacin as a model drug. The idea was to have more effective penetration enhancers which are decomposed by skin esterases after drug penetration promotion. Wong et al. [49] synthesized urea derivatives with enhancing effects comparable to Azone.

Alkyl-N,N-Disubstituted Aminoacetates

As long chained alcohols, fatty acids and esters were used for skin penetration enhancement, disubstituted aminoacetates were introduced as skin penetration enhancer substances. Representatives of this group of penetration enhancers are dodecyl-N,N-dimethylaminoacetate and dodecyl-2-methyl-2-(N,N-dimethylaminoacetate) (DDAIP). These substances are not soluble in water, but soluble in most of the organic solvents and in water and alcohol mixtures. The skin penetration promotion potential of these substances is in the same dimension as Azone or even higher. The penetration enhancing activity is decreased by the increase in the N,N-dialkyl carbon chain. The skin-irritating potential of the aminoacetates is very low [17]. This is due to the biological decomposition of these enhancers by the skin enzymes to N,N-dialkyl carbonates and the corresponding alcohols. They enhance skin penetration by the interaction with stratum corneum keratin and the increase in the hydration efficiency resulting from these interactions.

In 1989 Wong et al. [50] introduced the newly synthesized alcohol derivatives of N,N-disubstituted amino acids with low toxicity, and evaluated the derivatives for their transdermal penetration-enhancing effects on the transport of indomethacin from petrolatum ointments across the shed skin of black rat snake (Elaphe obsoleta). The penetration fluxes of indomethacin increased linearly as the concentration of DDAIP increased from 2.5 to 15%. Snake skin pretreatment experiments indicated that the application of DDAIP significantly increased skin permeability. Electron micrograph studies showed clearly that the enhancer was able to interact with both lipid-rich layers and keratin-rich layers. Later, the effectiveness of DDAIP was tested using the above-mentioned snake skin, rabbit pinna skin and human skin for penetration experiments with the drugs indomethacin, 5-fluorouracil and propranolol-HCl [51]. With all skins and all model drugs, DDAIP increased drug permeability at least as well as Azone. In most cases it was the more effective penetration enhancer. The electrochemical investigation of human cadaver skin by impedance spectroscopy with and without penetration enhancers (DDAIP and Azone were used) revealed new insights into the mechanism of action of the enhancers [52]. The enhancers appeared to open new penetration routes and increased the ohmic resistance, capacity properties and fractal dimension of the skin. By fluorescence spectroscopic studies dodecyl-N,N-dimethylaminoacetate was shown to alter molecular movement on the surface of the bilayers, resulting in a decrease in anisotropy of 19% [53]. By use of DDAIP as an enhancer in penetration studies of miconazole through shed snake skin, the permeation increased 11-fold compared with that of the suspension without DDAIP pretreatment [54]. The concentration of the azole in the skin increased 8-fold, indicating that the enhancement effect is connected with high partition of miconazole into the skin. Recently, Wolka et al. [55] studied the interaction of DDAIP with a phospholipid model membrane by differential scanning calorimetry for further clarification of the mechanism of action of this skin penetration enhancer. The results suggested that drug transport is enhanced by DDAIP by interaction with the polar regions of the phospholipid bilayers and also by increasing the motion-al freedom of lipid hydrocarbon chains.

Propylene Glycol

Among the polyvalent alcohols, propylene glycol is the most frequently used co-solvent in dermatology. The action as a real penetration enhancer is debated controversially in the literature. Penetration and permeation enhancement was shown as well as the opposite effects when using propylene glycol in formulations for topical administration. Thus, the action as a co-solvent seems to be in the foreground [17]. The activity of propylene glycol as a co-solvent is restricted to the formulation. However, it is able to penetrate and thereby can transport lipophilic substances or other enhancers via solvent drag. This may account for the synergistic action of propylene glycol and Azone and propylene glycol and oleic acid. The action of propylene glycol as a penetration enhancer seems to be the mechanism of action only for the skin penetration of drugs which are better soluble in alcohol than in water. The solvation of keratin within the stratum corneum by competition with water for the hydrogen bond binding sites and the intercalation in the polar headgroups of the lipid bilayers by propylene glycol are postulated as mechanisms of action for the penetration-
enhancing effects of propylene glycol in the literature as well [19].

Recently the in vitro percutaneous penetration of acyclovir from solvent systems and from carpopol 971-P hydrogels was studied regarding the influence of propylene glycol [56]. It was shown quantitatively that the enhancer effect of propylene glycol in the permeation of acyclovir across human epidermis depends on the concentration of the alcohol. The concentrations of propylene glycol used in this study varied between 0 and 70% w/w. In the solvent systems a maximum enhancement ratio was determined for the system containing 70% propylene glycol. On the other hand, carpopol 971-P gels containing 50% of propylene glycol provided the highest enhancer effect for acyclovir skin penetration.

Funke et al. [57] investigated the transdermal delivery of highly lipophilic antiestrogens by in vitro permeation studies through excised skin of hairless mice. Several penetration enhancers were tested in this study. It was shown that an outstanding permeation enhancement can be achieved for the highly lipophilic drugs by the combination of propylene glycol and lauric acid. Furthermore, the group demonstrated that the mechanism of the observed effects is the mutual permeation enhancement of these two penetration enhancers and their synergistic lipid-fluidizing action in the stratum corneum.

Increased drug skin penetration after the use of propylene glycol and fatty acid combinations was also observed by Larrucea et al. [58]. The combination of oleic acid with propylene glycol led to a greater absorption of tenoxicam formulated in carpopol 940 gels. The flux values studied using Franz-type diffusion cells gradually increased with increasing concentrations of both compounds, showing the synergistic effects again.

Surfactants

Surfactants are frequently used as emulsifiers in formulations for dermal application. They are added in order to solubilize lipophilic actives within the formulations. The improvement of the drug solubility can be achieved, for example, by the formation of micelles by the surfactant molecules in the formulation. Surfactants have the potential for the solubilization of the stratum corneum lipids and thus act as penetration enhancers. Keratin interactions are also thought to explain the penetration-enhancing effects of surfactants. Normally, cationic surfactants are more effective as penetration enhancers than anionic or nonionic compounds. The potential for skin irritation is connected with the penetration-enhancing effects of the surfactants. Therefore, in formulations for dermal application, mostly nonionic surfactants are used, which tend to be widely regarded as safe. Surfactants with an analogue structure to the stratum corneum bilayer lipids have low skin-irritating potentials, but also low skin penetration-enhancing effects. This is due to surfactant monomer integration into the bilayers instead of micelle formation of the lipids.

Among the nonionic surfactants, sucrose fatty acid esters have been shown to temporarily alter membrane barrier properties. These substances show many advantages as penetration enhancers, e.g. biodegradability and lack of toxicity. Sucrose oleate and sucrose laureate were shown to promote the in vivo percutaneous penetration of the model permeant 4-hydroxy-benzonitril. The effects were monitored by attenuated total reflectance Fourier transform infrared spectroscopy in conjunction with tape stripping [59]. In another study, the effects of sucrose esters on the permeation of lidocaine were investigated as a function of vehicle pH. The results suggested that sucrose laureate enhanced the penetration of the ionized form of the drug (12-fold greater flux than control), whereas sucrose oleate was more effective in promoting the nonionic species. Transcutol was used additionally as a solubilizer in both experiments [60].

Besides the sugar-based surfactants, the fatty alcohol ethers of polyoxyethylene are frequently used as nonionic surfactants for the promotion of the skin penetration of drug substances. Shin et al. [61] showed the best enhancing effect of polyoxyethylene-2-oleyl ether for enhanced transdermal delivery from bioadhesive carpopol gels containing tretinoin, among various enhancers tested in the study. The ether was also able to increase the transdermal delivery of the antihistaminic tripolidine from an ethylene vinyl acetate matrix system in rabbits and rats [62]. In a former study of the same research group, polyoxyethylene-2-oleyl ether also showed the best enhancement among several enhancers tested for the atenolol transdermal drug delivery from an ethylene vinyl acetate matrix [63]. Another group of nonionic surfactants which can be used for skin penetration enhancement are the partial fatty acid esters of sorbitan. Sorbitan monolaurate 20 (Span 20) has been tested as a potential skin penetration enhancer in transdermal matrix type patches using diclofenac diethylamine as the active agent [64]. With Span 20, an enhancement of skin permeation of the drug of up to 30% was measured with rat skin using a modified Keshary-Chien diffusion cell.
Terpenes and Terpenoids

Terpenes and related substances are highly lipophilic compounds and have high octanol/water permeation coefficients. Terpenes are nonaromatic ingredients of essential oils and consist of carbon, hydrogen and oxygen atoms only. As penetration enhancers, they interact with intercellular lipids and influence the nonpolar penetration route. Co-solvents like propylene glycol or ethanol have synergistic effects when added to the terpenoids.

Godwin and Michniak [65] studied the influences of drug lipophilicity on terpenes as transdermal penetration enhancers. The drugs caffeine, hydrocortisone and triamcinolone acetonide in propylene glycol were investigated. Their results showed that the percutaneous penetration of hydrocortisone and caffeine can be significantly enhanced using a combination of terpenes and propylene glycol. Geraniol was the most effective compound for caffeine penetration enhancement, and alphaterpineol the most effective for hydrocortisone penetration enhancement. For triamcinolone acetonide no significant enhancement effects by terpene derivatives were observed.

Terpenes extracted from plants are good candidates for permeation enhancers because of their relatively low irritation potential. They are designated as ‘generally recognized as safe’ by the FDA. The alcohol-type terpene p-menthane-3,6-diol, originating in the leaf of Eucalyptus citriodora, was tested for its enhancement effect on in vivo permeation of the hydrophilic drug antiypirine and the lipophilic drug indomethacin through Yucatan micropig skin [66]. The permeation of antipyrine was increased 3-fold by the terpene. Skin concentration of indomethacin was increased about 11-fold.

Unjacketed Franz diffusion cells were used by Ota et al. [67] to study the enhancing effects of terpenes on percutaneous absorption of the highly lipophilic drug midazolam. Among the terpenes tested (geraniol, limonene, menthol and citronellol) only limonene was able to enhance midazolam penetration through rat skin, and provided useful information to develop a new dosage formulation for midazolam. Limonene also showed the greatest ability to enhance the flux of sumatriptan succinate in a study where several chemical penetration enhancers were tested. A 22-fold higher flux than the control was measured [68].

The combination of terpenes (5%) and ethanol was able to increase significantly the flux of the luteinizing hormone-releasing hormone through terpenes/ethanol-treated epidermis [69]. Limo-

In search of more effective and safer compounds for skin penetration enhancement, Takanashi et al. [72] synthesized thomenthol derivatives and introduced them as novel percutaneous absorption enhancers providing both enhancement factors and skin irritation factors.

Fatty Acids

The penetration-enhancing effects of fatty acids have been described many times in the literature. The measured effects are strongly influenced by the fatty acid structure and the vehicles used for the formulations. The most frequently used compound in this field and the most investigated substance is oleic acid. Generally, saturated fatty acids are less effective than their unsaturated derivatives. The more double bonds there are in the molecules, the more effective are unsaturated fatty acids. Furthermore, fatty acids with cis configurations are more effective penetration enhancers than fatty acids with trans configured double bonds. The cis unsaturated compounds have more potential for disturbing the lipid packing order within the bilayers. Spectroscopic investigations with deuterated oleic acid have revealed that oleic acid molecules at higher concentrations are able to form separate phases within the bilayer lipids. This would lead to permeability defects within the bilayers and facilitate the permeation of hydrophilic compounds through the stratum corneum.

The transdermal delivery of the nonsteroidal anti-infl ammatory agent ketorolac tromethamine was investigated studying the effects of vehicles and penetration enhancers [73]. For the study, five fatty acids (caprylic, capric, lauric, oleic and linoleic acid) were added to propylene glycol at concentrations of 1, 3, 5 and 10%. The highest enhancing effect was attained with 10% caprylic acid in propylene glycol.

The effects of oleic acid on the ultrastructure of the stratum corneum lipids of rat skin were examined by electron microscopy using osmium or rhutenium tetroxide postfixation and lanthanum tracer studies. The results showed that oleic acid might increase the epidermal per-
meability via a mechanism involving perturbation of stratum corneum lipid bilayers and lacunae formation as penetration enhancing effects [74]. Butyl paraben, methyl paraben and caffeine were used as model penetrants to test the effects of unsaturated fatty acids in benzyl alcohol on percutaneous drug permeation. The permeation of butyl paraben was enhanced to a similar extent by all three fatty acids tested (oleic acid, palmitoleic acid and linoleic acid), whereas palmitoleic acid caused a significant greater enhancement in the flux of both methyl paraben and caffeine compared with the oleic and linoleic acid effects and with the control. It was proposed that the synergistic mechanisms of fatty acids and benzyl alcohol were augmenting the polar penetration route by interactions with both polar and nonpolar stratum corneum lipids [75].

Even the in vitro permeability of the peptide drug insulin was increased by the use of fatty acids as skin penetration enhancers. The 3-fold unsaturated linolenic acid produced greater permeability through porcine epidermis than the other fatty acids tested using Franz diffusion cells. The flux of the derivative lispro insulin was also significantly higher by use of linolenic acid compared with the control [76].

Again, the skin-irritating potential of fatty acids when used at higher concentrations is the disadvantage of their application.

Esters

Alkyl esters and fatty acid esters are also frequently used skin penetration enhancers. Ethylacetate, methylacetate, butylacetate and methylpropionate are used as well as isopropyl-n-butyrate, isopropyl-n-decanoate, isopropylmyristate and isopropylnalmitate for example. Ethyl acetate was found to be the best penetration enhancer for the transdermal delivery of the contraceptive levonorgestrel [77]. The ester was able to speed up absorption 17-fold in rat skin. Using excised hairless rat skin, the skin permeation enhancement of papaverine hydrochloride by monoglycerides and caprylic acid esters was evaluated and compared with the enhancement effects of free fatty acids [78]. It was shown that free fatty acids mainly affected the diffusion of the drug, and the monoglycerides affected the partition. Enhancement was marked in the case of glyceryl monocaprylate. A linear relationship between the flux of papaverine hydrochloride and the amount of enhancer in skin was established.

Glycerol monooctate in the presence of propylene glycol was able to enhance the 5-aminolevulinic acid in vitro skin penetration and the in vivo protophorphyrin IX accumulations in hairless mouse skin. These are important factors for the success of the photodynamic therapy in skin cancer [79]. Porcine ear skin mounted in a Franz-type diffusion cell was used to investigate the topical delivery of cyclosporin A. The drug is of great interest for the treatment of autoimmune skin disorders, but is frequently ineffective due to poor drug penetration in the skin. Glycerol monooleate at concentrations of up to 10% in propylene glycol formulations enhanced both the topical and the transdermal delivery [80].

By synthesis of esters with more optimized penetration-enhancing action, several research groups tried to find novel ester penetration enhancers. The facilitated transport of the two model steroids hydrocortisone-21-acetate and betamethasone-17-valerate by several synthesized esters and amides of clofibric acid was shown by Michniak et al. [81]. The amide analogues were more effective in this study than the equivalent ester compounds of the same carbon chain length. Lactam-N-acetic acid esters were synthesized and showed significantly higher enhancement ratios for hydrocortisone-21-acetate in hairless mouse skin than the ratio found when using Azone as an enhancer [82]. The addition of an N-acetylproline ester with an alkyl length of 16 carbon atoms significantly increased the fluxes of benzepril and hydrocortisone compared with the control [83]. Franz diffusion cells were used for the experiments, and hairless mouse skin was used as the penetration barrier. Differential scanning calorimetric studies suggested that the synthesized enhancer may be acting on the stratum corneum lipids with a similar effect to that of Azone. The corresponding membrane/vehicle partition coefficient studies indicated an increase in the benzepril partition coefficient with enhancer treatment compared to the control.

Cyclodextrins

Cyclodextrins are cyclic nonreducing maltooligosaccharides. They are able to form inclusion complexes with lipophilic drugs and increase their solubility, particularly in aqueous solutions. Cyclodextrins are not comparable with the other penetration enhancers concerning their enhancing effects because they are not able to penetrate the skin under normal conditions. In combination with lipophilic enhancers (fatty acids, Azone) a synergistic effect can be achieved.

Uekama et al. [84] observed an improved transdermal delivery of prostaglandin E1 through hairless mouse skin from an ointment by use of carboxymethyl-ethyl-beta-cyclodextrin and Azone as a penetration enhancer. Furthermore the cyclodextrin was able to markedly improve the
stability of the prostaglandin in the fatty acid/propylene glycol ointment. An in vivo transdermal absorption rat model was used to study the percutaneous absorption enhancing effects of 2-hydroxypropyl-beta-cyclodextrin and 2,6-dimethyl-beta-cyclodextrin [85]. The transdermal absorption of the cytochrome P450 inhibitor liarozole was increased significantly. The results from differential scanning calorimetry and those from permeability experiments revealed that 2,6-dimethyl-beta-cyclodextrin acted by modifying the stratum corneum barrier, whereas 2-hydroxypropyl-beta-cyclodextrin influenced the partition behavior of the drug in the skin. Williams et al. [86] investigated the transdermal permeation modulation by cyclodextrins in a mechanistic study. The test drugs were 5-fluorouracil and estradiol. Permeation modulation by beta- and 2-hydroxypropyl-beta-cyclodextrins alone and in combination with terpenes was tested. The cyclodextrins did not enhance the flux of the test drugs. Complexation of the terpenes resulted in reduced enhancer efficacy. The incorporation into a barrier cream retarded toluene permeation through the skin, and it was concluded that cyclodextrins themselves are not penetration enhancers for the test substances but can be useful to reduce percutaneous absorption of toxic materials on occupational exposure. Ventura et al. [87] studied the percutaneous absorption of papaverine through rat skin. A 10% aqueous solution of hydroxypropyl-beta-cyclodextrin was suggested to be the most suitable transdermal penetration enhancer for papaverine. Recently Babu and Pandit [88] showed the effects of cyclodextrins on the complexation and transdermal delivery of bupranolol through rat skin. They used side-by-side diffusion cells and pH 7.4 phosphate buffered saline. Both cyclodextrins investigated, hydroxypropyl-beta-cyclodextrin and partially methylated cyclodextrin, were found to be suitable for improving the solubility, and showed penetration enhancement of the beta-blocking agent bupranolol when used in certain concentrations.

Novel Penetration Enhancers and Novel Discovery Techniques

The research work to find novel skin penetration enhancers with higher enhancement action and less irritation potency is still going on. Iminosulfuranes are synthetically designed DMSO-related compounds which can be synthesized by DMSO treatment with trifluoroacetic anhydride. An enhancement activity has been achieved for some of the compounds without any toxicity [89]. Ascorbic acid has been tested for its penetration enhancement properties using haloperidol as a model drug and amber glass Franz-type diffusion cells for the permeation studies. Ascorbic acid did not increase the permeation of the drug but increased the haloperidol solubility in the vehicle, which leads to a concentration-dependent increase in the haloperidol flux [90]. The primary capsacinoid capsaicin was tested to compare its skin penetration-enhancing effects with those of Azone. It was found that capsaicin caused stratum corneum alterations and that the capsacinoid was able to enhance the penetration of the model drug naproxen studied by use of the isolated perfused rabbit ear model and full-thickness human skin [91].

As the topical therapy with peptides would be useful for the treatment of cutaneous diseases, a comparative study was carried out to test the skin penetration of protein transduction domains and a conjugated peptide. The two protein transduction domains tested in this study were able to penetrate the porcine ear skin and carried a conjugated model peptide with them. The normally used chemical penetration enhancer oleic acid had no additional penetration enhancing effect in this study [92]. It was able to increase the topical delivery of a nontransducible peptide investigated as a control substance in this study.

To evaluate the effects of penetration enhancers on drug delivery through skin and to be able to predict the enhancing power, the quantitative structure-activity relationship (QSAR) technique is used more frequently in this field. For 5-fluorouracil and diclofenac sodium the resulting QSARs indicated that less hydrophobic enhancers were the most active. In contrast, for skin permeation promotion of hydrocortisone, benazepril and estradiol, a linear relationship between enhancement activities and octanol/water partition coefficients of enhancers were evident [93]. Santos-Filho et al. [94] used molecular similarity and QSAR analyses to develop compact, robust and definite models for skin penetration of organic compounds. It was found that a combination of nonmembrane interaction QSAR descriptors and membrane-interaction QSAR descriptors yielded the optimum models regarding both the statistical measures of fit and model predictivity.

An experimental tool, in vitro impedance-guided high-throughput screening (INSIGHT), was used by Karande et al. [95] for the discovery of transdermal penetration enhancers. The group reported an over 100-fold greater efficiency compared with current tools. In another study by the same group, more than 300 potential skin penetration enhancers were designed by reengineering the knowledge on these compounds back into the molecular struc-
ture. The molecules found in his study were screened in silico and subsequently tested in vitro for molecular delivery [96].

**Penetration Reducers**

Under specific conditions the penetration of the epidermis by xenobiotics is undesired. In these cases it has to be inhibited or retarded that compounds (e.g. pesticides or other harmful substances) will reach the systemic circulation. For these applications, skin penetration reducers or retarders are used. Another domain for these substances used to restrict the dermal and the transdermal penetration route are topically administered formulations where the actives should only act locally. The experiences on the mechanisms of skin penetration on a molecular level gathered by penetration enhancement experiments established the basis to design compounds with the opposite effect. However, the knowledge of the exact mechanisms of skin penetration retardation is quite limited up to this point, and there are only a few studies reporting experiments on this topic.

Freeman et al. [97] observed the failure of topical drugs for herpes simplex treatment formulated in ointments to penetrate human skin. They studied acyclovir and idoxuridine. The delivery of these drugs from polyethylene glycol ointments was very slow for both human and guinea pig skin. A change of the formulation to a modified aqueous cream and to DMSO resulted in an 8- and 60-fold increase in the flux of acyclovir. The retardant effect of polyethylene glycol may be due to its inability to hydrate the stratum corneum or to a relative osmotic effect which tends to dehydrate the stratum corneum.

Fatty acids have the ability to act as skin penetration retarders as well. The capability is dependent on their structure and on the vehicles used for the formulation [98]. The skin permeation of the highly lipophilic model permeant pyrene butyric acid was decreased or not affected when using unsaturated branched fatty acids in 95% propylene glycol compared with the pure vehicle. The use of the enhancer oleic acid instead of the branched fatty acids used for the retardation study led to a significant increase in skin penetration [99].

Skin barrier creams and protective gloves were developed for topical skin protection from exposure to chemical agents [100] and for the reduction of percutaneous absorption of industrial solvents [101].

**Further Possibilities to Modulate the Skin Penetration of Drugs**

Besides chemical skin penetration enhancement and altering the barrier properties by hydration of the horny layer, there are several physical skin penetration enhancement techniques which can be used to overcome some of the limitations of the chemical skin penetration enhancers.

Phonophoresis or sonophoresis uses ultrasound energy for the skin penetration enhancement of drugs [102]. Here, the ultrasound waves propagate in the skin and cause effects that increase skin penetration of various drugs, including macromolecules, via enhanced diffusion or enhanced convection [103]. For sonophoresis, ultrasound at various frequencies in the range of 20 kHz–16 MHz has been used to increase skin permeability. Low-frequency sonophoresis which is conducted at frequencies between 20 kHz and 100 kHz has been found to be more effective in transdermal transport enhancement than the techniques operating at high-frequency ultrasound [104]. As the mechanism of the enhancing effect of ultrasound, a phenomenon called acoustic cavitation is assumed. This is when gas bubbles are formed and subsequently collapse, which leads to the formation of holes in the corneocytes, an enlargement of intercellular space and the perturbation of the stratum corneum lipids (illustrated schematically in figure 5). Another effect is the temperature increase by which the fluidity of the stratum corneum lipids is increased [105]. The application of low-frequency sonophoresis in dermatocosmetology has been reported by Santioanni et al. [106]. Ultrasound waves at 25 kHz were used for the treatment of alopecia arata using a methylprednisolone ointment and a cyclosporine solution. Furthermore, melasma and solar lentigo were treated by azelain acid and kojic acid and low-frequency ultrasound.

The iontophoresis technique applies a small electric current to the skin, providing the driving force to enable the penetration of substances into the skin. Transdermal drug transport enhancement by iontophoresis is caused via direct electrophoresis, electroosmosis or enhanced diffusion [107]. When using direct electrophoresis, an active iontophoresis electrode is placed above a drug reservoir on the skin having the same charge as the penetrant. Another indifferent counter electrode is placed elsewhere on the skin (illustrated schematically in figure 6). The active electrode transports the drug into the skin by the mechanism of repulsion of equally charged carriers [108]. Electroosmosis results when the electric field of the human skin is superimposed by the artificial electric field of...
the iontophoretic device. A solvent flow across the skin is caused which is able to transport uncharged and larger molecules [109].

Like iontophoresis, electroporation enhances the transdermal drug transport through enhanced diffusion, electrophoresis and electroosmosis. In contrast to the iontophoretic techniques, electroporation uses a large voltage treatment for a short period of 10 μs to 100 ms. The short pulses of high voltage current produce temporary hydrophilic pores as aqueous pathways where drug substances, e.g. macromolecules, can pass through the skin [110].

Recently, the use of radiofrequency-driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs was introduced. Sintov et al. [111] showed penetration enhancement by radiofrequency microchanneling for the drugs granisetron hydrochloride and diclofenac sodium.

The microneedle technique uses small needles (10–200 μm height and 10–50 μm width) which are connected with the drug reservoir. The microneedle delivery device is applied to the skin surface without reaching the nerve endings of the upper dermis. The actives are able to overcome the stratum corneum without causing pain [103].
By combination of physical methods for skin penetration enhancement or by combination of physical methods with chemical enhancers described in detail in this review, synergistic effects for transdermal drug delivery can be obtained [112–115].

Conclusions

There are permanent efforts to improve dermal and transdermal drug delivery into and across the human skin. One main focus in this field of research is the evaluation of chemical substances for their skin-penetration-enhancing properties in topically administered formulations. Potential substances used for this purpose need to have both features, i.e. drug penetration-promoting effects and a low or no skin irritating potential. To obtain substances which fully meet these requirements, one approach is to synthesize penetration enhancers with the desired properties. Modern discovery techniques, e.g. QSAR and high-throughput screening, are applied for the development of novel dermal and transdermal penetration enhancers. Besides the skin penetration enhancement by chemically defined compounds, several physical methods are employed to improve transdermal drug delivery. Synergistic effects were determined by combination of the several penetration enhancing principles.

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Overcoming the Stratum Corneum: 
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