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European Journal of Pharmaceutics and Biopharmaceutics 46 (1998) 1–13

European Journal of Pharmaceutics and Biopharmaceutics

Review article

Percutaneous penetration enhancement and its quantification

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Received 20 March 1997; accepted 15 August 1997

Abstract

True penetration enhancing effects resulting from structural alterations of the barrier stratum corneum manifest themselves in an increase of the drug diffusion coefficient D_B and/or of the drug solubility in the barrier c_{sB}. The quantification of enhancing effects on drug penetration is possible either by the direct determination of the drug fluxes or by an indirect determination through the measurement of the pharmacodynamic response. In both cases the thermodynamic drug activity has to be considered. In the case of pharmacodynamic measurements, enhancing effects may be determined from the horizontal distance of activity-response lines obtained without and with enhancer, respectively, i.e. the quotient of the drug concentrations that induce the same effect. The activity-standardized bioavailability factors f_a obtained from the horizontal distances correspond to the enhancer-induced relative changes in the permeabilities P_B, or more exactly in the product $D_B \times c_{sB}$. On the other hand, the vertical distance between the activity-response lines, i.e. the differences in the drug response after application of preparations with equal (even maximum) thermodynamic drug activities may be used to quantify penetration enhancing effects. © 1998 Elsevier Science B.V. All rights reserved

Keywords: Transdermal drug delivery; Penetration enhancement; Thermodynamic activity; In vivo; Activity-response curves

1. Introduction

The efficacy of percutaneously applied drug preparations for local or regional therapy still leaves a lot to be desired. Even the transdermal delivery of systemically acting drugs may cause problems. The number of drugs, that lead to plasma levels in the therapeutic range after transdermal application, is small. Even for readily penetrating drugs a daily dose of not more than a few milligrams is required [1]. The penetration rate of drugs from transdermal systems, i.e. nitroglycerin, does not really seem to be regulated by the drug release rate from the transdermal system [2,3]: the drug penetration of the stratum corneum is the penetration rate limiting step. The reason for these problems is the distinct barrier function of the stratum corneum. The drug penetration rate and the drug permeability of the barrier P_B increase primarily with increasing partition coefficients $PC_{\text{stratum corneum/vehicle}}$ or $PC_{\text{octanol/water}}$ and with decreasing relative molecular weight [4–7]. High permeabilities can be expected, in particular, for small molecules with sufficient affinity to the stratum corneum. For the maximum drug penetration rate per skin area unit J_{max}, i.e. the flux from saturated systems, among other parameters the solubility in the vehicle, c_{sV} has to be considered [6,7].

The drug penetration rate may be increased by the use of penetration enhancers [8–10].

The penetration rate or the bioavailability rate of cutaneously applied drugs is dependent only on the thermodynamic activity of the drug in the vehicle if specific penetration enhancements are absent [11–14]. If potential penetration enhancers are investigated in solution type preparations, it must be considered that both, penetration enhancement and thermodynamic effects may influence the drug flux. Only suspension type preparations lead to equal, or in this case, maximum, thermodynamic activities

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PII S0939-6411(97)00149-5

[13,15]. A statement concerning the presence or the extent of a penetration enhancing effect cannot be made without knowledge of the thermodynamic drug activity.

The efficacy of penetration enhancers can be quantified not only by the determination of their influence on the drug flux in vitro [16–22] or in vivo [23–26], but also on their effect on the resulting drug response in vivo [14,27–29]. Data that are obtained by the determination of the influence of the enhancers on the drug response, are of greater clinical relevance.

The term 'penetration enhancement' will be defined precisely in Section 2. Furthermore, an overview of the mechanisms of penetration enhancement and the possibilities of its quantification will be given with the main focus on the indirect flux determination by measurement of the pharmacodynamic response.

2. Penetration enhancement: definition and mechanisms

2.1. Substance transport through the stratum corneum

2.1.1. Influence in accordance with Fick's First Law

The stratum corneum represents the main barrier for skin permeation of drugs and other compounds [30,31].

Since the stratum corneum can be considered as a partition membrane, the penetration rate of drugs through the stratum corneum may be described by Fick's First Law (Eq. (1)) [31–33]. The drug flux, J, (Eq. (2)) represents the penetrating drug mass per time and area unit. Penetration rate and flux are directly proportional to the drug permeability of the barrier P_B. If the partition coefficient $PC_{B/V}$ in Eq. (2) is replaced by Eq. (3), Eq. (4) is obtained.

$$-\frac{dc_V}{dt} = k_p \times c_V = \frac{D_B \times PC_{B/V}}{l} \times \frac{A}{V_V} \times c_V \quad (1)$$

$$J = P_B \times c_V = \frac{D_B \times PC_{B/V}}{l} \times c_V \quad (2)$$

$$PC_{B/V} = \frac{c_{sB}}{c_{sV}} \quad (3)$$

$$J = \frac{D_B \times c_{sB}}{l} \times \frac{c_V}{c_{sV}} \quad (4)$$

where c_V is the drug concentration in the vehicle, $-dc_V/dt$ is the rate of the drug concentration decrease in the vehicle, k_p is the penetration rate constant, D_B is the effective drug diffusion coefficient in the stratum corneum, $PC_{B/V}$ is the partition coefficient of the drug between the barrier stratum corneum and the vehicle, l is the thickness of the stratum corneum, A is the area of application, V_V is the vehicle volume, P_B is the drug permeability of the barrier stratum corneum, c_{sB} is the drug solubility in the barrier stratum corneum and c_{sV} is the drug solubility in the vehicle.

It follows from Eq. (4), that a flux increase may be achieved on the one hand by increasing the drug diffusion coefficient in the barrier D_B and the drug solubility in the barrier c_{sB}. On the other hand the flux can be increased by an increase of the drug concentration in the vehicle c_V or a decrease of the drug solubility in the vehicle c_{sV}.

2.1.2. Importance of the penetration routes

The stratum corneum can be considered as a partition membrane for substances that penetrate mainly via the lipid route, i.e. intercellularly through the lipid lamellae.

It was postulated for a long time that non-polar substances only penetrate intercellularly and polar substances penetrate transcellularly through both the corneocytes and the lipid lamellae [32,34]. However, for the penetration not only of water but also of salicylic acid it was found that the lipid content of the barrier – not the thickness of the barrier or the number of cell layers – influence the barrier function [35,36]. Recent studies show that substances with a great polarity spectrum all penetrate via the intercellular route [37,38]. The skin appendages only represent a volume fraction of 0.01–0.1% of the stratum corneum, resulting in a relatively small diffusion volume. Therefore, the penetration via the pore route should be less relevant [30,39]. However, some studies show that penetration through pores and follicles has to be considered [40].

The penetration of ions seems to follow a different mechanism than the penetration of uncharged molecules: it proceeds through so-called aqueous shunt routes [41,42]. Penetration through water-filled pores is also postulated for amino acids and peptides [43]. The contribution of the pore route to the overall route of penetration is of importance even for more lipophilic compounds such as acyclovir butyrate, an acyclovir-prodrug and various corticosteroids [44,45]. If ionic compounds are applied with direct current, iontophoresis, the pore route is the main route of transport after an initial lag phase [46]. The effective pore radii are in the range 0.4–1.6 nm for rat skin [47] and 1.5–2.5 nm for human skin [48].

With appropriately prepared skin samples penetration routes can also be visualized by using microscopic methods [49,50].

If a substance penetrates only via the hydrophilic route, Fick's First Law of diffusion is not valid as it has been presented in Eq. (1). The penetration rate no longer correlates with the lipophilicity of the substance, i.e. with its solubility in the lipid-barrier, but with the solubility in water. This penetration mechanism, which is also of importance for enteral absorption, is not considered in this review.

2.1.3. Penetration enhancement and thermodynamic activity

Definition of penetration enhancement. The quotient c_V/c_{sV} in Eq. (4) is a measure of the thermodynamic drug activity in solution type preparations as long as a certain solubility limit in the vehicle is not exceeded. Given a predefined thermodynamic drug activity in the vehicle and

thickness of the barrier, an increase of the flux can be achieved by increasing the drug diffusion coefficient in the barrier D_B and/or the drug solubility in the barrier c_{sB}. True penetration enhancement may be defined as the increase of these two parameters through the influence of penetration enhancers on the stratum corneum.

Sometimes penetration enhancement effects are called 'pull-effects' and the effects of the thermodynamic drug activity in the vehicle on drug penetration 'push-effects' [51–53].

Consideration of the thermodynamic activity. The consideration of the thermodynamic drug activity in the test vehicle, T, from which the drug penetration shall be enhanced in comparison to a standard vehicle, St, is possible in various ways.

If the drug solubilities in the vehicles are known, the relative thermodynamic activities in the test vehicle a_{relT} and the standard vehicle a_{relSt} ($0 \leq a_{rel} \leq 1$) can be determined from the ratio c_V/c_{sV}.

The relative thermodynamic activity coefficient $\gamma_{T/St}$ represents the ratio of the drug concentration in the standard and a test preparation that lead to the same drug activities. It represents the quotient of a_{relT} and a_{relSt}, if the same drug concentration in the test and standard vehicle is given. The relative thermodynamic activity coefficient $\gamma_{T/St}$ can be defined as the ratio of the drug solubilities in the standard and a test vehicle (Eq. (5)).

$$\gamma_{T/St} = \frac{c_{sSt}}{c_{sT}} \tag{5}$$

It may be deduced from data by Reimann for methyl nicotinate [54,55] that the calculation of the relative thermodynamic activity coefficients $\gamma_{T/St}$ from the corresponding solubilities in the vehicles is allowed up to solubilities of about 10 g × 100 ml^{-1}. The relative thermodynamic activity coefficients $\gamma_{T/St}$ calculated from solubilities exceeding this limit are too small in comparison to the values calculated from partition coefficients. The reason is the decrease of the drug activity at higher concentrations. The determination of these coefficients is only possible from the partition coefficients between the standard and a test vehicle $PC_{St/T}$. Since the partition coefficients are not directly accessible if the two phases are miscible, they are determined according to Eq. (6). X represents a phase which is not miscible with either vehicle [26,54].

$$PC_{St/T} = \gamma_{T/St} = \frac{PC_{St/X}}{PC_{T/X}} = \frac{PC_{X/T}}{PC_{X/St}} \tag{6}$$

The adjustment of the test and standard preparations to equal relative thermodynamic activities can be achieved by division of the drug concentration in the standard c_{St} by the relative thermodynamic activity coefficient $\gamma_{T/St}$ (Eqs. (7), (8)).

$$a_{relT} = c_T \times \gamma_{T/St} \tag{7}$$

$$a_{relT} = a_{relSt} = c_T \times \gamma_{T/St} = c_{St} \times 1.00 \quad \Rightarrow \quad c_T = \frac{c_{St}}{\gamma_{T/St}} \tag{8}$$

If the determination of the relative thermodynamic activity coefficient $\gamma_{T/St}$ from the solubilities (Eq. (5)) is allowed, the adjustment to equal drug activities may easily be done by using the fractions of the respective solubilities.

It has to be considered that any change in the composition of the vehicle usually alters the drug solubility and therefore its thermodynamic activity. If drug concentrations above the solubility, i.e. suspensions are used, the dissolved amount of drug and therefore the thermodynamic activity remains constant and reaches its maximum [11,15,56]. The addition of penetration enhancers must not increase the drug solubility to such a degree, that the drug concentration falls below the solubility.

The penetration of the enhancers themselves into the stratum corneum is a basic requirement for their efficacy. It is possible to facilitate the penetration of the enhancers by appropriate pretreatment of the skin.

After pretreatment, drug preparations free of enhancer with no influence of the thermodynamic drug activity are applied to the skin. If this method is used, the determination of the thermodynamic drug activity in the vehicle is not necessary.

Examinations that consider the thermodynamic activity of the substance to be enhanced, mainly use either the suspension method [19,21,57–60] or the pretreatment method [16,18,61–64]. Unfortunately, studies with drug solutions that contain different amounts of potentially enhancing compounds have been carried out lately without considering their influence on drug activity [65–68].

Since the penetration of the enhancers into the stratum corneum is essential for their efficacy, it is important for them also to show high relative thermodynamic activities. This fact is considered in only a few studies [20,57,61,69, 70].

3. Influence of penetration enhancers on the structure of the stratum corneum

Since the enhancers themselves usually penetrate into the stratum corneum, they can influence its structure. Several methods exist to examine the structure of the stratum corneum (see Section 4). It is important to be aware of the fact that structural changes of the stratum corneum not necessarily have to lead to penetration enhancement or penetration inhibition. The diffusion of drugs or their solubility in the barrier may be unaffected by structural changes of the stratum corneum. Therefore, such investigations can only contribute to the clarification of the causes of penetration enhancement.

The lipid-protein-partitioning (LPP) theory by Barry [22,71,72] offers the most comprehensive survey of the possible interactions between penetration enhancers and

the stratum corneum. According to this theory, the three main mechanisms of enhancement are: (1) interactions with the intercellular lipids; (2) interactions with the intracellular keratin; and (3) the penetration of high amounts of enhancers or so-called cosolvents into the stratum corneum with a resulting improved dissolving capacity of the barrier for drugs and/or co-enhancers. The latter is postulated especially for small polar enhancer molecules such as dimethyl sulfoxide, ethanol and propylene glycol.

Interactions between penetration enhancers and intercellular lipids, which cause a so-called lipid fluidization, can be found most often [64,73–77] and may take place in different regions of the lipid domain. Even transepidermal water loss TEWL correlates with the fluidity of the lipid chains [78].

Polar molecules are supposed to interact with the polar head groups of the lipids via hydrogen bonds and ionic forces, which influence the degree of hydration and also the arrangement of the lipids. Lipophilic compounds are supposed to be inserted into the cholesterol-enforced side chain area and thus to increase the diffusion of penetrating substances by an altered arrangement of the lipid lamellae with increased fluidity [72]. This theory assumes that the rigidity of the lipid lamellae is the primary obstacle for drug penetration of the stratum corneum and the reason for the small diffusion coefficient. The effective diffusion coefficient D_B in well-hydrated stratum corneum is within a range of 10^{-10}–10^{-9} $cm^2\ s^{-1}$ whereas, in the viable epidermis and dermis it amounts to about 10^{-6} $cm^2\ s^{-1}$ [79].

Several research groups do not consider the rigidity of the lipid lamellae to be responsible for the low value of D_B and the diffusional resistance of the skin, but regard the extended diffusion pathway of the intercellular transport and the resulting smaller sectional area of diffusion or the smaller diffusion volume as crucial. In particular Hadgraft and Albery et al. [80–82], Lange-Liekfeldt and Liekfeldt et al. [83,84] as well as Potts et al. [85,86] support this theory. The group of Potts considers the extended diffusion pathway as the only reason for the diffusional resistance of the stratum corneum, which seems to be not correct.

If in the case of intercellular penetration the geometry of the stratum corneum alone is responsible for the low values of the effective diffusion coefficient D_B, the true diffusion coefficient D_0 can be calculated from D_B (Eq. (9)):

$$D_0 = D_B \times \frac{l_D}{l} \times \frac{A_{tot}}{A_i} \quad (9)$$

where l_D is the length of the diffusion pathway, l the thickness of the stratum corneum, A_{tot} the entire diffusion area of the stratum corneum and A_i the resulting diffusion area assuming intercellular penetration [83]. (In [83], Eq. (6), $D_{eff} = D_B$ and $D = D_0$ have been exchanged by mistake.)

Lieckfeldt et al. obtained a diffusion coefficient of 10^{-8} $cm^2\ s^{-1}$ for a lamellar gel phase consisting of cholesterol, ceramides and fatty acids [87], which should correspond to the true diffusion coefficient in the stratum corneum D_0. With regard to the order of magnitude D_0 thus seems comparable to the diffusion coefficients in lipophilic vehicles such as petrolatum or Plastibase® ($D = 2.10^{-8}$ $cm^2\ s^{-1}$ after a long storage period and 2.10^{-7} $cm^2\ s^{-1}$ [88], respectively). As long as the length of the diffusion pathway is not influenced, lipid fluidization cannot lead to an increase of the diffusion coefficient in the range of several orders of magnitude, since the diffusion coefficient in lipids will always be smaller than that in water ($D = 10^{-5}$ $cm^2\ s^{-1}$).

Under consideration of the different thermodynamic drug activity various in vitro penetration studies with lipophilic enhancers such as azone, azone-analogues, oleic acid and other fatty acids, show that not the increase of the diffusion coefficient but the improvement of the partitioning into the stratum corneum and thus the increase of the solubility in the barrier is the enhancing mechanism [16,18,19]. It is postulated for azone that the penetration enhancing effect is caused by the insertion of azone into the intercellular lipid lamellae which leads to fluidization [73,89]. Therefore, lipid fluidization must also lead to an increase of the drug solubility in the barrier.

While at the end of the 1980s it was postulated that oleic acid also leads to lipid fluidization by insertion into the lipid lamellae [75], today it is assumed that oleic acid exists in the stratum corneum as a separate phase and forms small channels [90–92]. Below the phase-transition-temperature of the endogenous lipids and thus at physiological temperatures, oleic acid does not influence their fluidity [92]. In non-treated stratum corneum ‘solid’ and ‘liquid’ gel phases probably already exist at physiological temperatures [93,94]. While sphingolipids and free sterols may be arranged in cohesive lamellar gel structures, less polar lipids may be confined within separate, relatively loosely packed compartments in the stratum corneums interstices [95]. Defects associated with water at the interfaces between the solid and the liquid areas could be responsible for the basal permeability of the skin for polar molecules. Such unusually high permeabilities for ions such as Na^+ or K^+ are also found with liposomes consisting of phospholipids, if the gel phase and the liquid crystalline phase are in equilibrium [96]. The enhancement of polar molecules especially by oleic acid could be a consequence of the increase of the water associated defects [90].

Also for terpenes such as (+)-limonene, which can be regarded as penetration enhancers, there is no evidence for lipid fluidization according to X-ray-diffraction studies. Hence it may be concluded that they also exist as a separate phase in the stratum corneum [97]. In particular the pretreatment of the skin with penetration enhancers may lead to lipid extraction [77,98–101]. However, the subsequent penetration of substances is not dependent on the extracted amount of lipids, but on the type of lipid [102]. The drug flux correlates with the ethanol flux for high ethanol concentrations [103]. Lipid extraction is supposed to lead to the formation of solvent filled pores [104].

Polar penetration enhancers do not only interact with intercellular lipids, but also with the intracellular keratin,

which leads to changes of the protein conformation [72]. Dimethyl-, hexylmethyl- and decylmethyl sulfoxide convert the α-helix structure of keratin into a β-sheet structure [105]. Lauric acid and various derivatives enhance the penetration of verapamil when using native and even lipid-extracted mouse skin. This finding can also be explained by interactions with proteins [21]. As it has been shown in the past that the intercellular route is the main route of penetration, these protein-enhancer interactions should be of secondary importance for penetration enhancement. However, interactions only with the keratin portion were found for the enhancer dithiothreitol which increases the flux of mannit and sucrose without influencing their solubility in the stratum corneum or in the vehicle [106].

The results of a recent study show that the lipophilic penetration enhancers oleic acid and isopropyl myristate, but not hexadecane, increase the permeability of the corneocytes for polar substances after pretreatment of the skin or the stratum corneum with the respective enhancer. However, it is not clear whether this effect is caused by interaction with the intracellular lipids or proteins [107].

The combination of hydrophilic and lipophilic enhancers often leads to synergistic effects. Examples are the combination of propylene glycol, transcutol (diethylene glycol monoethyl ether) or ethanol with azone, free fatty acids or terpenes such as (+)-limonene [23,108–112]. Therefore, enhancers are occasionally classified in two groups: polar compounds and lipophilic compounds. The combination of one enhancer of each group is supposed to act synergistically [113,114]. Barry postulates with his LPP-theory that at first the co-solvent penetrates into the stratum corneum and thereby increases the partitioning of the co-enhancer into the stratum corneum. However, Wotton et al. observe penetration enhancement for propylene glycol by azone [108]. The mechanisms of synergistic effects should better be looked at in detail from case to case.

4. Methods for the quantification of true penetration enhancement

4.1. In-vitro-methods

The quantification of enhancing effects on drug penetration is possible either by direct determination of the drug flux or by indirect determination taking advantage of the pharmacodynamic response. In both cases the thermodynamic drug activity has to be taken into consideration.

In vitro flux measurements are often carried out in diffusion cells that consist of a donor and a receptor compartment separated by the skin sample. The donor compartment contains the vehicle with a defined drug concentration. The receptor compartment simulates the sink-conditions that are given in vivo in the viable epidermis and dermis. In the case of infinite-dose studies the flux ($\mu g\ cm^{-2}\ h^{-1}$) can be calculated from the increasing amount of drug in the receptor medium. If the cumulative amount of drug in the receptor medium (μg) is plotted versus time t (h), the flux is obtained by division of the slope of the linear part of the curve (steady-state) ($\mu g\ h^{-1}$) by that area, A, of treated skin, that is accessible to drug diffusion (cm^2). The flux from solution type preparations can be determined by measuring the decrease of the drug concentration in the donor. The effective diffusion coefficient in the barrier stratum corneum D_B may be calculated from the lag-time t_{lag} according to Eq. (10), if the thickness of the stratum corneum l is known.

$$D_B = \frac{l^2}{6 \times t_{lag}} \tag{10}$$

However, t_{lag} values calculated with this formula are usually underestimated, since drug adsorption to skin components [6] is not considered [115].

In Eq. (1) the partition coefficient $PC_{B/V}$ is the only unknown parameter and can therefore easily be calculated. If drug suspensions are used in the donor, the changes of the partition coefficient $PC_{B/V}$ correspond to changes of the drug solubility in the barrier (see Eq. (4)). If the skin is pretreated with penetration enhancers or if the enhancers are not co-administered with the drug, they should not influence the drug solubility in the vehicle. Under these circumstances the partition coefficient is only dependent on the drug solubility in the barrier. Thus, penetration enhancing effects are quantifiable with regard to D_B and $PC_{B/V}$, respectively.

Various types of diffusion cells, that fulfil different requirements, have been developed. A review about in vitro skin permeation techniques has been published by Friend [116].

Excised human skin, available from autopsies or from breast reduction surgery is often used for in vitro studies. Since these resources are limited, animal skin and artificial membranes are used as models for human skin [117]. Especially mouse and rat skin, but also shed snake skin and less often guinea pig [16,18], rabbit [110], porcine [75] and monkey skin [118] are used as skin models. The keratinized mucosal membrane of the hamster cheek pouch has also been investigated [58,119,120]. When preparing sheets of isolated stratum corneum holes can arise in the area of the skin appendages, which may lead to a substantial increase in membrane permeability. Since the keratinized mucosal membrane of the hamster cheek pouch has no appendages, the risk of a formation of holes is reduced [120]. However, this membrane is suitable only if the contribution of the pore route to the overall diffusion pathway is negligible.

In addition to isolated stratum corneum sheets the epidermis and full thickness skin may serve as model membrane. They have the advantage of greater mechanical stability, but their enzyme systems lead to a high metabolic activity in the viable skin layers [121,122]. If the penetrating substance is metabolized during diffusion through the viable skin layers its concentration in the receptor compartment may be underestimated.

The stratum corneum permeability is dependent on the skin source [123]. Under the influence of penetration enhancers the permeability of levonorgestrel decreased in the following order: hairless mouse skin > hairless guinea pig skin ≥ rat skin > human skin [124]. Monkey skin is more permeable for nafareline acetate than human skin is [118]. The permeability of human skin for *N*-methyl-2-pyrrolidone is comparable to that of guinea pig skin, while the permeability of mice, rat and new-born piglet skin is greater by a factor of about 5–8 [125]. It is assumed that there is a difference in the mechanism of interaction between penetration enhancers such as myristic acid and decylmethyl sulfoxide and human skin as compared to guinea pig skin [126].

For a number of substances the permeability of shed snake skin is comparable to that of human skin, even though it is slightly lower in general. It shows similar lipophilicity and is more similar to human skin than it is to mouse skin with regard to the influence of the molecular size of the penetrating drug [127,128]. As in the case of the keratinized mucosal membrane of the hamster cheek pouch, shed snake skin is devoid of hair follicles.

Mouse skin is more permeable than adult human skin but seems to be a good model for preterm infant skin [129,130].

Bond and Barry have found that there is no relationship between the enhancing effects of various substances such as azones decylmethylsulfoxide and oleic acid on mouse skin and those of human skin [131]. Furthermore, the influence of hydration on mouse skin permeability is greater [132].

Consequently, skin from different sources not only shows different permeabilities but also reacts differently to the treatment with penetration enhancers.

The permeability of the skin does not only vary from species to species, but also changes with age [133,134] and anatomical site [135,136]. This explains the search for artificial membranes that are less variable. Lipid membranes with a simple or more complex structure, as in the case of liposomes consisting of stratum corneum lipids fixed to a supporting filter [137], have the disadvantage that pores are missing. Recently, artificial membranes have been developed consisting of silicone and poly(2-hydroxyethyl methacrylate), allowing drug penetration via both, the lipid and the pore route [138,139].

In-vitro-penetration studies can simulate the transdermal drug absorption only if the penetration process is rate limiting in vivo as well as in vitro. Particularly for only moderately soluble substances the absence of the microcirculation under in vitro conditions may lead to the problem that sink conditions on the dermis side cannot be maintained [116]. In vivo, the transport of poorly water soluble drugs in the viable epidermis or the dermis may become the rate limiting step [140,141]. For instance, the viable epidermis seems to contribute to the overall diffusion barrier for the penetration of buprenorphine due to its insufficient water solubility [142]. For piroxicam it was found, that the systemic absorption in vivo is greater if the pretreated skin area is located above musculocutaneous as compared to a cutaneous vasculature. With excised skin in vitro this difference could not be found [143]. Iontophoretic delivery of lidocaine hydrochloride is not influenced by co-administration of vasoactive substances in vitro. In vivo, however, the flux is significantly increased if vasodilators such as tolazoline are added. With vasoconstrictors such as norepinephrine, the flux is decreased [144]. Not only the penetration of the stratum corneum, but also the microcirculation of the skin may be rate-limiting for certain substances.

The isolated perfused porcine skin flap (IPPSF) model [144–148] is an in vitro model that considers the above-mentioned physiological conditions. Porcine skin, perfused primarily by the caudal superficial epigastric artery and its associated veins, is surgically converted to a flap. The transdermal absorption can then be examined in perfusion cells.

Isolated perfused bovine udders are also used for penetration studies [149–151].

4.2. In-vivo-methods

The determination of the rate of drug absorption or the drug flux in vivo can be accomplished in different ways. If radiolabeled compounds are applied, the radioactivity in the plasma or in excreta may be determined as a function of time [152]. The amount of penetrated substance in different stratum corneum layers can be quantified by tape-stripping with an appropriate adhesive tape [153]. In animal models the drug amount in deeper skin layers and other tissues may be determined after sacrificing the animal. The concentration profiles obtained may give information on the respective absorption rates.

In the case of anaesthetized rats the measurement of drug absorption may be done as follows: a permanent catheter is inserted into the peripheral skin vein which is responsible for the blood supply of the treated skin area. Blood samples are collected and replaced by donor blood [154].

The skin sandwich flap (SSF) model is an in vivo model that is comparable to the IPPSF model: with microsurgical techniques a skin flap is created in vivo in rats. Subsequently, the venous blood from the independent but accessible vasculature of the flap is analyzed [155]. In the HSSF (human SSF) model human skin grafts are grafted to rat skin [25,156].

The applicability of the above-mentioned methods is restricted by law. Non-invasive in-vivo-methods, as presented below, are handled less restrictive and do not necessarily require the presence of a physician if human subjects are involved.

4.2.1. Chamber system

The non-invasive determination of a drug flux is possible by cutaneous application of glass cells [157] that can be filled with drug solutions. The cells can be fastened to the upper arms of human subjects under occlusion conditions. Glass cells have advantages compared to previously devel-

oped plastic or aluminium cells [158–160]. Sorption to glass is negligible and the transparency of the chamber material enables visual control, e.g. of cloudiness caused by sweat. In addition the area/volume ratio is optimized to improve the determination of small fluxes.

The procedure may be described as follows: drug solutions with known concentration are filled into the application cells and are replaced by the original solutions after certain intervals. The drug flux can then be calculated from the concentration difference of the solutions filled in and removed. However, it is not known whether the applied substance reaches the blood circulation in unchanged form. The substance may accumulate in the stratum corneum initially. For this reason, a lag time to reach steady state conditions has to be taken into consideration when measuring drug penetration rates [6,157].

Drug permeabilities P_B ($P_B = P/d_B$) can be calculated from the steady state fluxes. The calculation of permeability coefficients P according to $P = D_B \times PC_{B/V}$ is an approximation because in vivo the thickness of the barrier d_B can only be estimated.

The application chambers have been successfully used to determine the penetration profiles of various substances and to examine penetration enhancing effects of vehicles [6,7,26]. It is thereby possible to differentiate between thermodynamic effects, enhancing effects and drug depletion effects [161].

4.2.2. Determination of the pharmacodynamic response of suitable drugs

Drug flux and pharmacodynamic response. If the penetrant causes a physiological or pharmacological reaction when reaching the viable tissue, then this response may provide the basis for statements regarding the drug flux and the permeability of the stratum corneum. Theoretically, any stimulation of the skin and its functions may cause a quantifiable physiological reaction. Examples for stimulus-induced skin reactions are vasoconstriction, vasodilation, vascular permeability, activity of the sebaceous glands, secretion of the sweat glands and epidermal proliferation. Most often vasoconstriction or rather blanching as a result of the application of topical corticosteroids is used as response parameter [162,163]. Nicotinates are vasodilating compounds that induce an erythematous response [7,14,164,165].

The dependence of the pharmacodynamic response on the applied dose or, in the case of percutaneous application, on the drug concentration in the vehicle is described by dose-response- and concentration-response curves, respectively. In the latter case, the intensity of the response is plotted versus the logarithm of the drug concentration [29]. If the flux is response-limiting in the case of a receptor-mediated response, symmetrical curves with a sigmoidal shape cannot be expected. Curve shapes as presented in Fig. 1 are more likely. The curves level off to a plateau as soon as the drug solution is saturated (suspension-type ointments) [15].

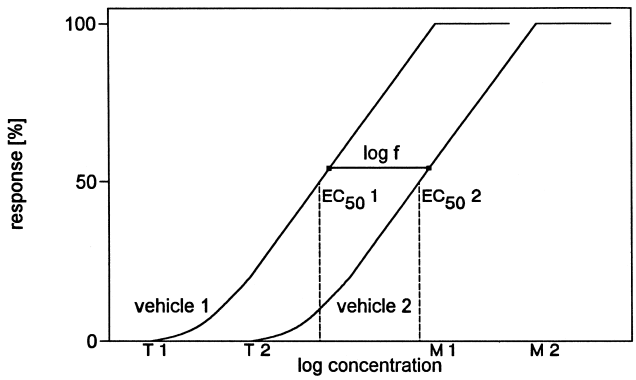

Fig. 1. Flux limited concentration-response curves as a result of the transdermal drug application in two vehicles, which show different drug affinities, but no specific effects.

They are characterized by the threshold concentration, T (minimum concentration necessary for inducing a response), a linear part, the EC_{50} (concentration that leads to 50% of the maximum effect) and the plateau of the maximum response at concentrations greater than M. If concentrations above the threshold concentration are applied, a linear correlation between the intensity of the response and the logarithm of the drug concentration up to the level of maximum response may be observed. Knowledge of the complete concentration-response curves is required for an evaluation of the investigated preparations.

A change of the penetration rate or of the penetration rate constant k_p leads to a shift of the curve along the x-axis provided that the extent of penetration remains the same. Statements with regard to changes in bioavailability are possible by vertical and horizontal comparison of the resulting concentration-response curves [29]. However, comparisons like these are only allowed within those parts of the curves, that reveal a linear correlation between the applied concentration and the resulting response and which are therefore referred to concentration-response lines.

If concentration-response lines are compared vertically a difference in the response can be determined from the response values obtained with one defined drug concentration. Differences in the response between preparations with different bioavailabilities depend on the slope of the concentration-response lines. A vertical comparison of the lines has the advantage of requiring only the determination of the response at one particular concentration.

The intensity-related bioavailability factor f may be calculated from the horizontal distance between the concentration-response lines. It represents the factor by which the drug concentration in the standard preparation has to be adjusted to achieve the same response as with the test preparation. If the extent in bioavailability is equal, f represents the difference in the rates of penetration [29].

With equal thermodynamic activities instead of equal concentrations, the resulting response remains the same as long as specific vehicle effects, e.g. penetration enhancing

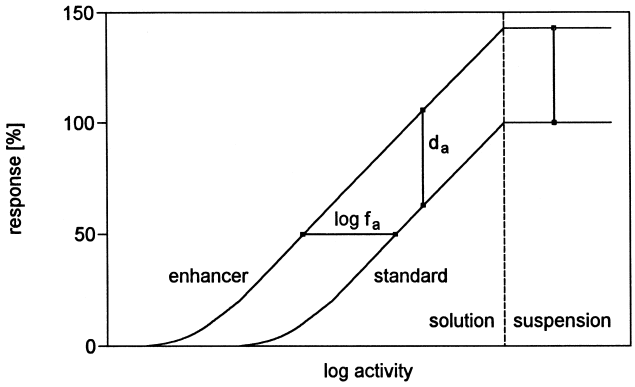

Fig. 2. Flux limited activity-response curves as a result of the transdermal drug application in a penetration-enhancing and a standard vehicle.

effects, are absent. Consequently, the respective activity-response lines are co-incident. If a test preparation exerts specific vehicle effects, the resulting activity-response lines of this test and a standard preparation are not congruent. The biovailability factor determined from the horizontal distance between the activity-response lines, which in this case is called the activity-standardized bioavailability factor f_a, will consequently differ from unity then.

Activity-response curves of a penetration-enhancing and a standard preparation are shown in Fig. 2 [166]. The curve of the penetration-enhancing formulation is shifted to the left compared to the curve of the standard formulation, i.e. despite equal activities a stronger response is observed due to penetration enhancement. Furthermore, the response plateau is elevated, i.e. even the response observed with saturated solutions and suspensions is stronger [15]. The vertical distance between activity-response lines corresponds to the difference between the plateau values of response after application of suspensions. It is called activity-standardized vertical distance d_a. In contrast to the quantification of thermodynamic effects the quantification of penetration-enhancing effects is possible even in the plateau range of the maximum response. A precondition is once more that the receptor-mediated response is only flux-limited.

Activity-standardized bioavailability factor and enhancement ratio, enhancement index. The activity-standardized bioavailability factor f_a represents the factor by which the drug activity in a standard preparation has to be adjusted to achieve the same response as with test preparation. Under infinite dose conditions it serves as direct measure for the relative change of the drug penetration rate or the penetration rate constant k_p due to enhancement effects. Since equal drug activities are applied, f_a reflects penetration enhancement but not thermodynamic effects [14,29]. The activity-standardized bioavailability factors correspond to the relative permeabilities (see Eq. (1) and Eq. (2)) at a defined thermodynamic drug activity and are therefore identical to the enhancement ratio *ER* (Eq. (11)) defined by Goodman and Barry and to the enhancement factor *EF* [26], respectively.

$$ER = \frac{P_B \text{ after pretreatment with enhancer}}{P_B \text{ before pretreatment with enhancer}} = f_a \qquad (11)$$

The enhancement ratio represents the factor by which the pretreatment with the enhancer increases the permeability of the barrier stratum corneum P_B, or more precisely, the product of the diffusion coefficient in the barrier D_B and the partition coefficient between the barrier and the vehicle $PC_{B/V}$, since the thickness of the barrier d_B may be regarded as constant. A pretreatment of the skin with the enhancers, according to Goodman and Barry, has the advantage that only one vehicle is needed for the subsequent flux measurements. There is no influence of the drug activity to consider in this case. The application of the drug preparation without pretreatment with enhancer serves as reference treatment [167].

Increases of the relative permeabilities (activity-standardized bioavailability factor f_a or enhancement ratio >1) are always caused by increases of the product $D_B \times c_{sB}$ in the case of

- pretreatment of the skin with the enhancers and subsequent treatment with the drug containing vehicle in comparison to no or less effective pretreatment,
- drug application in enhancer-containing vehicles with respect to the thermodynamic drug activity in comparison to the application in enhancer free vehicle or other standard vehicles.

Furthermore, Williams and Barry define an enhancement index *EI* (Eq. (12)).

$$EI^{\log VK_{Oct/W}AS}_{ER_{max}}(\%) = \frac{ER_{\text{after pretreatment}} - 1}{ER_{max} - 1} \times 100 \qquad (12)$$

The enhancement index represents the percentage of the maximum penetration enhancement achieved after pretreatment of the skin with enhancer. The maximum enhancement ratio ER_{max} is the quotient of the permeability of the stripped, i.e. stratum corneum-free epidermis and the permeability of the intact epidermis [168]. Since an enhancement ratio of unity means, that a penetration enhancing effect does not exist, this value is subtracted from the numerator as well as from the denominator of the quotient. The logarithm of the octanol/water partition coefficient of the drug and the maximum enhancement ratio are provided as superscript and subscript to the enhancement ratio, respectively. These two values provide information which places into context the enhancement effect. Some general conclusions may be drawn from the studies of Williams and Barry. The stratum corneum is a weaker barrier for lipophilic substances than it is for hydrophilic compounds. As a result, the maximum enhancement ratio is smaller for lipophilic molecules. Even a small enhancement ratio obtained after pretreatment with enhancer may represent a large fraction of the maximum enhancement ratio. With hydrophilic drugs large enhancement ratios are often found,

but their percentage of the maximum enhancement ratio is rather low.

Usually, parameters such as the enhancement ratio or equivalent quantities are determined to characterize penetration enhancement. A more pronounced enhancement of the penetration of hydrophilic molecules than that of lipophilic, stratum corneum-affine compounds is often found [169–172]. However, it is not known to what extent the barrier properties of the stratum corneum are compromised.

4.2.3. Influence of potential lipophilic penetration enhancers on the response of model drugs with different lipophilicities, mechanism of enhancement

The influence of various potential lipophilic penetration enhancers on the pharmacodynamic response of two model drugs has been examined with respect to the thermodynamic drug activities in the vehicles. Fatty acids, fatty alcohols and fatty acid esters were chosen as enhancers. Ethyl nicotinate ($VK_{Oct/W} = 22$) and betamethasone 17-benzoate ($VK_{Oct/W} = 13\,690$) served as hydrophilic and lipophilic model drugs, respectively. For ethyl nicotinate the reciprocal value of the relative time of onset of an erythema and for betamethasone 17-benzoate the relative blanching intensity could be used as parameters of response.

In contrast to the hydrophilic model drug ethyl nicotinate with less differentiated solubility properties and lower affinities to the lipophilic vehicles ($\gamma_{T/St}$: 0.10–1.25 referring to the standard light mineral oil), the penetration of the lipophilic model drug betamethasone 17-benzoate may be enhanced. Betamethasone 17-benzoate shows much more differentiated solubility properties ($\gamma_{T/St}$: 3.5×10^{-4}–1.00 referring to the standard light mineral oil) [166].

The penetration enhancing effect of the potential enhancers increases in a linear manner with the solubility of betamethasone 17-benzoate in the vehicle. A probable mechanism of action is the penetration of the enhancers into the barrier stratum corneum which leads to an increase of the drug solubility and the permeability of the barrier. Thus, they may act as co-solvents in the stratum corneum. Therefore, the concept that only small polar penetration enhancers can act as co-solvents in the stratum corneum, has to be reconsidered [166].

Pronounced penetration enhancing effects should rather result from an increase of the drug solubility in the barrier stratum corneum c_{sB} than from an increased drug diffusion coefficient in the barrier stratum corneum D_B. An increase in c_{sB} may be achieved with co-solvents, as shown for betamethasone 17-benzoate, or by structural changes of the stratum corneum such as lipid fluidization as a result of the penetration of even smaller amounts of vehicle components. Basic requirements for an increase in c_{sB} are differentiated solubility properties or more exactly differentiated affinities of the drug to lipophilic, stratum corneum-like vehicles. Since substances with similar solubility parameters may show totally different solubility properties, a suitable lipophilic, hydrophilic or even universal model drug for enhancement studies does not exist. The usefulness of potential penetration enhancers for a well-defined drug has always to be proven in every individual case.

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