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Review article

Passive skin penetration enhancement and its quantification in vitro

Katrin Moser[a,b], Katrin Kriwet[b], Aarti Naik[a,c], Yogeshvar N. Kalia[a,c], Richard H. Guy[a,c,*]

[a]*Laboratoire de Pharmacie Galénique, University of Geneva, Geneva, Switzerland*
[b]*Technical R&D/PHAD, Novartis Pharma AG, Basel, Switzerland*
[c]*Centre Interuniversitaire de Recherche et d'Enseignement, Archamps, France*

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Abstract

The poor penetration of drugs into the skin (and, partially, the permeation across the stratum corneum) often limits the efficacy of topical formulations. Basically, skin penetration can be enhanced by the following strategies: (i) increasing drug diffusivity in the skin; (ii) increasing drug solubility in the skin, and/or (iii) increasing the degree of saturation of the drug in the formulation. In this article, we review the literature with respect to: (i) chemical penetration enhancers, which have been shown to influence the diffusivity and/or solubility of the drug in the skin and (ii) supersaturated formulations, in which the degree of saturation of the drug is increased compared to conventional formulations. In addition, three different in vitro methods, specifically, classic diffusion cell studies, attenuated total-reflectance–Fourier transform infrared spectroscopy, and tape stripping in conjunction with an appropriate analytical technique, are considered, emphasizing their application to obtain quantitative values for skin transport parameters and to separate the kinetic or thermodynamic effects of an enhancement strategy.

Keywords: Skin penetration enhancers; Supersaturation; Diffusion coefficient; Partition coefficient; Thermodynamic activity; Attenuated total-reflectance–Fourier transform infrared spectroscopy; Tape-stripping

1. Introduction

The efficacy of topically applied drugs is often limited by their poor penetration into the skin. Methods for improving cutaneous delivery rely either on the use of chemical penetration enhancers, novel vehicle systems, e.g. microemulsions, liposomal-based delivery systems and supersaturated formulations, or more complex physical enhancement strategies, e.g. iontophoresis, sonophoresis, and electroporation [1].

This article has two principal objectives: (i) to review passive enhancement strategies, in particular, supersaturated formulations and chemical penetration enhancers and (ii) to compare the important methods used to quantify penetration enhancement in vitro.

2. Skin structure

The skin is principally composed of two parts: the outer aspect, that is to say, the epidermis and the inner dermis. The dermis contains capillaries, sebaceous and sweat glands, hair follicles and nerves; the epidermis, on the other hand, is avascular (Fig. 1). The epidermis has a multilamellar structure that represents the different stages of cell differentiation. Moving upwards from the proliferative basal layer, the cells change in an ordered fashion from metabolically active and dividing cells to dense, functionally dead, keratinized cells, i.e. the corneocytes. These latter cells are surrounded by multilamellar lipid bilayers and constitute the outer 10–20 μm of the epidermis, called the stratum corneum [3].

3. The skin barrier

The barrier function of mammalian skin is principally attributed to the stratum corneum [4]. Barrier properties are based on the specific content and composition of the stratum corneum lipids [5] and, in particular, the exceptional structural arrangement of the intercellular lipid matrix and the lipid envelope surrounding the cells [6]. The lipids form bilayers surrounding the corneocytes, producing a 'brick-and-mortar' model with the corneocytes as the bricks and the intercellular lipids providing the mortar [7]. The intercellular lipids are primarily ceramides, cholesterol,

* Corresponding author. Centre Interuniversitaire de Recherche et d'Enseignement, Campus Universitaire, Parc d'Affaires International, F-74166 Archamps, France. Tel.: +33-450-31-50-21; fax: +33-450-95-28-32.
E-mail address: richard.guy@pharm.unige.ch (R.H. Guy).

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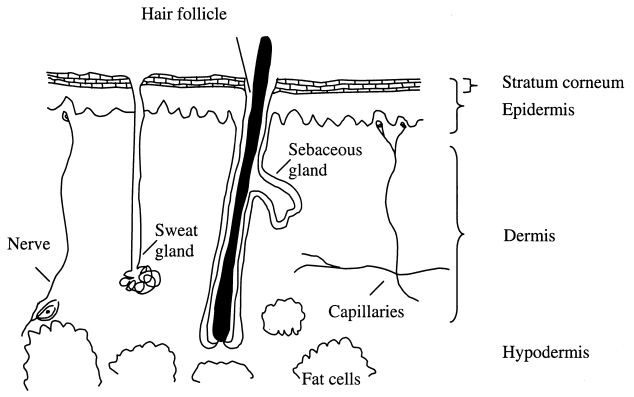

Fig. 1. Structure of the skin (adapted from Ref. [2]).

cholesteryl esters, fatty acids, and a small fraction of cholesterol sulfate [8].

4. Drug permeation routes

The permeation of drugs through the skin includes the diffusion through the intact epidermis and through the skin appendages, i.e. hair follicles and sweat glands, which form shunt pathways through the intact epidermis. However, these skin appendages occupy only 0.1% of the total human skin surface and the contribution of this pathway is usually considered to be small (with only a few exceptions having been noted [9]). As stated above, drug permeation through the skin is usually limited by the stratum corneum. Two pathways through the intact barrier may be identified (Fig. 2): the intercellular lipid route between the corneocytes and the transcellular route crossing through the corneocytes and the intervening lipids; that is, in both cases the permeant must diffuse at some point through the intercellular lipid matrix, which is now recognized as the major determinant of percutaneous transport rate [11].

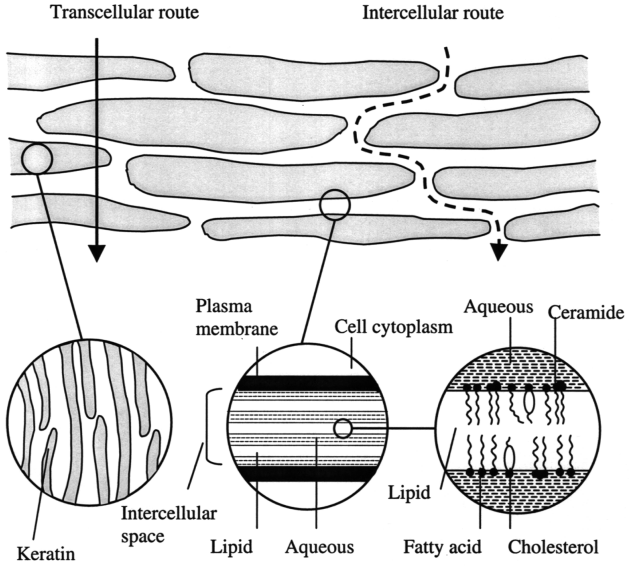

Fig. 2. Permeation routes through the stratum corneum: (i) via the lipid matrix between the corneocytes (intercellular route) and (ii) across the corneocytes and the intercellular lipid matrix (transcellular route) (adapted from Ref. [10]).

5. Permeation through the stratum corneum and its enhancement

The flux J of a drug through the stratum corneum can be described most simply by Fick's first law [12]:

$$J = \frac{D_m c_{s,m}}{L} \cdot \frac{c_v}{c_{s,v}} \quad (1)$$

In Eq. (1), D_m represents the diffusion coefficient of the drug in the membrane, $c_{s,m}$ its solubility in the membrane, L the diffusion path length across the membrane, c_v the concentration of the drug dissolved in the vehicle and $c_{s,v}$ the solubility of the drug in the vehicle. Three permeation enhancement strategies may be postulated based on Fick's first law (Fig. 3): (i) increase D_m, (ii) increase $c_{s,m}$, i.e. increase drug partitioning into the membrane, and (iii) increase the ratio $c_v/c_{s,v}$, i.e. the degree of saturation of the drug in the vehicle (the supersaturation approach). The latter strategy is based on interaction between the drug and the vehicle, the first two approaches imply an effect of the vehicle on the barrier function of the stratum corneum (for example, via penetration of chemical penetration enhancer into the stratum corneum and subsequent disordering of the intercellular stratum corneum lipids, or the extraction of such lipids by a solvating component of the formulation). A recent review has considered the interactions of different chemical penetration enhancers with the membrane [13]. In the following sections, the influence of penetration enhancers on the diffusion coefficient and solubility of the drug in the stratum corneum is evaluated and the use of supersaturation to increase the diffusive driving force for drug delivery is reviewed (Fig. 4).

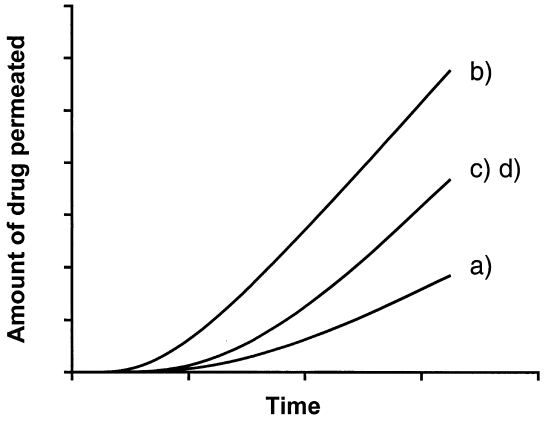

Fig. 3. Skin permeation of a drug substance from a standard vehicle (a) and the influence of a two-fold increase of the diffusion coefficient (b), of the solubility of the drug in the skin (c), or of the degree of saturation of the drug in the vehicle (d). The curves were modelled using Eq. (4).

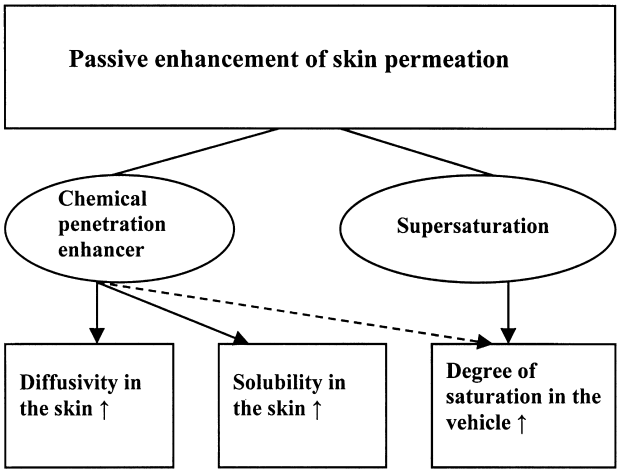

Fig. 4. Strategies for enhancement of skin permeation according to Fick's first law of diffusion and the modes of action of chemical permeation enhancers and supersaturation.

5.1. Increasing the diffusion coefficient

The diffusion coefficient can be increased by disordering the stratum corneum lipids. Fatty acids are a class of compounds frequently used to increase skin permeation and they are generally believed to increase diffusivity across the stratum corneum. Oleic acid is a good example [14] and has been shown to induce phase separation in the stratum corneum lipid domains, thereby reducing barrier function [15,16]. This mechanism of enhancement has been established for the increased permeation of cyanophenol [17] and of 6-mercaptopurine [18] in the presence of oleic acid. However, a further study with 6-mercaptopurine [19] implied that oleic acid also had an effect on the drug's partitioning into the stratum corneum.

Azone, like oleic acid composed of a polar head group and a long alkyl chain (Fig. 5), is another penetration enhancer, which increases lipid disorder in the stratum corneum [20] and hence increases drug diffusivity. A saturated solution of cyanophenol containing azone resulted in enhanced drug diffusivity across the stratum corneum compared to the enhancer-free control [21]. Azone also enhanced the percutaneous flux of diazepam; at concentrations up to 12%, drugs stratum corneum/vehicle partition coefficient remained constant, while the diffusion coefficient increased significantly and accounted exclusively for the observed permeation enhancement (Fig. 6) [22].

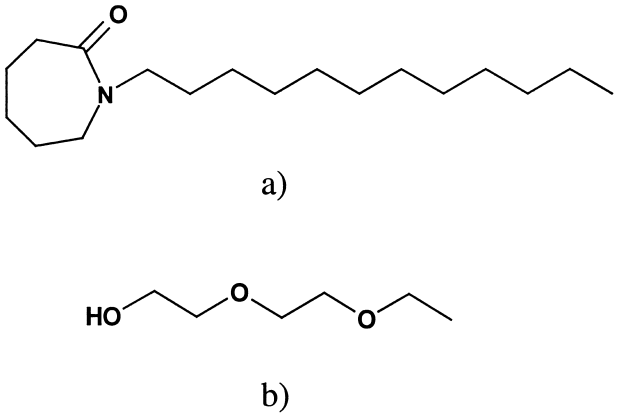

Fig. 5. Chemical structures of: (a) azone and (b) transcutol.

Dimethyl sulfoxide (DMSO), at concentrations above 50%, increased the diffusivity of bepridil in the skin [23]. At even higher levels, DMSO decreased the time necessary for tetrachlorsalicylanilid and demethylchlortetraycline hydrochloride to penetrate to the lower stratum corneum [24], indicating an increase in the apparent diffusion coefficient. More recently, with DMSO $>$ 40%, interaction of the enhancer with stratum corneum lipid alkyl chains was observed, implying a decreased diffusion resistance of the barrier. Increased drug partitioning into the skin has also been suggested [25].

The effect of different terpenes on the skin permeation of 5-fluorouracil (5-FU) was investigated by Williams and Barry [26]. They observed a linear relationship between the enhancement in skin permeation and diffusivity, whereas the partitioning was not enhanced. In a separate study [27], the sesquiterpenes farnesol and neridol were found to significantly increase the diffusion coefficient of 5-FU and this was suggested to be the main mechanism responsible for permeation enhancement. The effect on the partition coefficient was minor. The permeation of tamoxifen has also been increased by different terpenes: eugenol, D-limonene and menthone had only a minor influence on drug partitioning, but caused a 10–30-fold permeability enhancement presumably via an effect on lipid extraction [28]. In addition, improvement in the partitioning of the drug from the vehicle into the skin has been proposed for several terpenes [29].

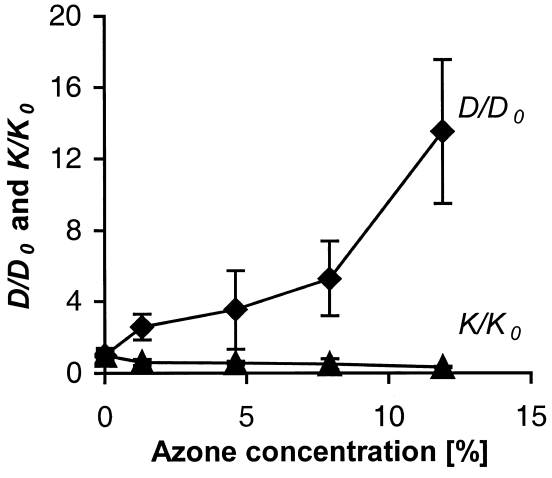

Fig. 6. Skin permeation of diazepam: influence of azone on the diffusivity (D/D_0) and the partitioning (K/K_0). The ratio of the parameters observed with enhancer-containing solutions (D and K, respectively) against the enhancer-free solution (D_0 and K_0, respectively) is shown as a function of the enhancer concentration. ($n = 4$, mean ± SD, adapted from Ref. [22].)

5.2. Increasing drug solubility in the skin

Agents, which are typically thought to act in this way are propylene glycol, ethanol, transcutol, and *N*-methyl pyrrolidone [30].

The permeation of ibuprofen through skin from saturated propylene glycol–water mixtures increased with increasing volume fraction of propylene glycol. This observation could be explained by an increase in the solubility of ibuprofen in the skin caused by the stratum corneum uptake of propylene glycol (Table 1) [31]. In another study, propylene glycol increased skin permeation of metronidazole [32], a result explained on the basis of solubility parameters [33]; that is, the solubility parameter of stratum corneum (9.8) being shifted in the direction of that of the drug (13.8), by propylene glycol (solubility parameter 14). In addition, a non-specific mechanism, i.e. the solvent drag effect is thought to participate in skin permeation enhancement of propylene glycol [34,35].

Different mechanisms have been proposed to explain the action of ethanol. According to Pershing et al. [36], ethanol enhanced the flux of estradiol through human skin without influencing the diffusion coefficient and the increased transport was correlated with an increase in the apparent partition coefficient; it was concluded that ethanol enhanced the solubility of the drug in the membrane. Similarly, increased permeation of nifedipine through skin by the addition of ethanol was attributed to the same mechanism [37]. In the case of levonorgestrel, ethanol again increased the drug's solubility in the skin, but also increased the diffusion coefficient [38]. On the other hand, the enhanced diffusion via the lipid pathway was proposed as the main mechanism to explain the effect of ethanol (at concentrations of up to 60%) for various drugs [39]. Finally, ethanol has been reported to penetrate into the skin and reduce barrier function by extraction of stratum corneum lipids [40].

Transcutol, a monoethyl ether of diethylene glycol (Fig. 5), is another penetration enhancer, which can increase the solubility of drugs in the skin. This mechanism was demonstrated for the model compound, cyanophenol [21]. As mentioned above, terpenes can increase drug partitioning into the stratum corneum. The permeation enhancement of 5-FU by several terpenes was well correlated with the increase in the drug's solubility in isooctane, provoked by addition of the enhancers, suggesting their potential to increase permeation by improving partitioning into the skin [29].

Table 1
Influence of propylene glycol on the permeation of ibuprofen from saturated solutions through rat skin (adapted from Ref. [31])

Propylene glycol (%)	Flux[a] ($\times 10^3$ μmol cm^{-2} h^{-1})	$C_{s,v}K_m$[b] (mg ml^{-1})
0	25 ± 6	3
10	33 ± 7	7
20	79 ± 8	10
30	51 ± 6	14
40	59 ± 5	16
50	70 ± 4	13
60	100 ± 33	23

[a] $n = 4$–16, mean ± SD.
[b] Product of vehicle solubility and partition coefficient [vehicle–skin], i.e. ibuprofen solubility in the skin.

5.3. Combined increase in membrane solubility and diffusion coefficient

As we have seen in the preceding section, many penetration enhancers, depending on their concentration and on the drug substance, can act upon both the drug's solubility and diffusion coefficient. Therefore, it is not surprising that synergistic effects can be observed by combining penetration enhancers [41]. For example, combination of azone, which typically increases D_m, and propylene glycol, which enhances drug solubility in the stratum corneum, increased significantly the skin permeation of metronidazole, 5-FU, and estradiol [32,42].

5.4. Increasing the degree of saturation

Optimization of the degree of drug saturation is important for simple formulations as well as those containing chemical penetration enhancers. The degree of saturation can be increased by increasing the drug concentration in the vehicle or by decreasing the solubility of the drug in the vehicle. Both approaches lead to an enhanced thermodynamic activity of the drug in the formulation and therefore to an increased skin permeation.

The pharmacological effect of betamethasone-17-benzoate from ointments was investigated as a function of the composition of the formulation. With a given vehicle composition, increasing the concentration of betamethasone-17-benzoate, until the solution was saturated, increased its effect in accordance with Fick's law of diffusion. When more drug was added creating suspensions which contained progressively larger total amounts of the drug, the effect remained constant. Identical effects were observed for solutions and suspensions of the steroid in other vehicle compositions though the amount of drug needed for the maximal effect differed according to the solubility of the drug in the vehicle (Fig. 7) [43]. The results demonstrate clearly, therefore, that it is the drug's thermodynamic activity in the vehicle, which is important, not its absolute concentration.

A saturated solution implies a thermodynamic activity of unity. A transient increase of the degree of saturation to greater than one can be achieved via supersaturation, which is, on the surface, a very simple method to increase skin permeation; however, such formulations are thermodynamically unstable and drug crystallization occurs over time. The stability of supersaturated formulations can sometimes be prolonged by the addition of certain polymers [44–53] but, even then, these systems are not stable enough for

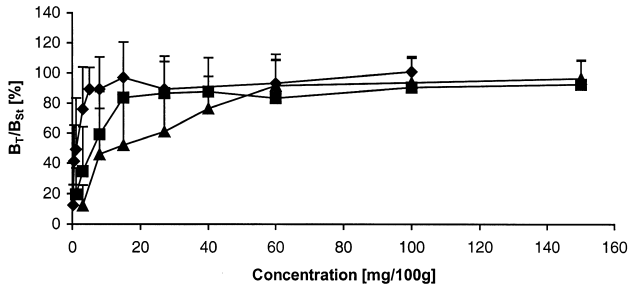

Fig. 7. Dose–response relationship of betamethasone-17-benzoate when delivered from different vehicles: (◆) neutral oil–mineral oil 10:90, solubility 2.8 ± 0.7 mg/100 g; (■) neutral oil–mineral oil 40:60, solubility 29 ± 3 mg/100 g; (▲) neutral oil–mineral oil 60:40, solubility 59 ± 1 mg/100 g. The blanching response, normalized to that induced by a standard formulation (B_t/B_{St}), increased until the corresponding solubility was reached and remained then constant. (n = 8–18, mean + SD, adapted from Ref. [41].)

long-term storage. To circumvent this problem, supersaturation can be achieved by different methods directly before or during application of the formulation: (a) by water uptake from the skin [54]; (b) through evaporation of a volatile formulation component during application [44,45,55–57], or (c) using the method of mixed cosolvent systems wherein vehicle changes are produced immediately prior to administration of the formulation [46–53,58–60]. The last method (Fig. 8), in particular, has enabled the investigation of the quantitative relationship between degree of saturation and permeation. The delivery of drugs such as hydrocortisone acetate, piroxicam, oestradiol, and a lipophilic lavendustin derivative through artificial membranes and/or skin has been shown to increase linearly with increasing degree of saturation in the vehicle [46–50,58–60]. For the lavendustin derivative, the diffusion coefficient and the partition coefficient between vehicle and skin were individually extracted and no influence of supersaturation on these parameters was found, implying that supersaturation acted purely via a thermodynamic effect [58,60].

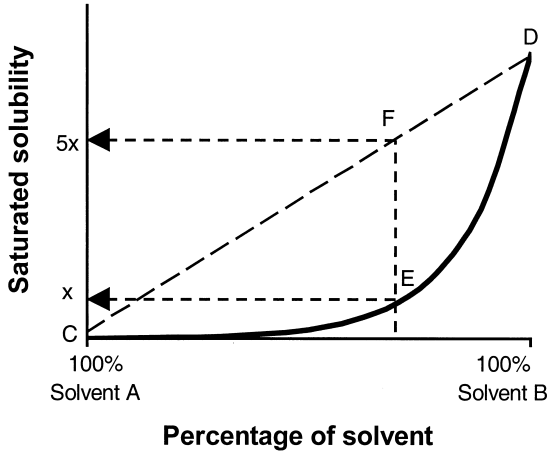

Fig. 8. Method of mixed co-solvents for preparation of supersaturated formulations: the exponential curve represents the solubility of the drug in the mixtures. When a saturated solution of drug in solvent B (D) is diluted with solvent A, which shows a very low solubility for the drug (C), concentrations on the dashed line results. For a solvent mixture with 70% B, the resulting concentration (F) is five times higher than the saturated solubility in the same mixture (E) (adapted from Ref. [45]).

When a penetration enhancer is added to a vehicle, the solubility of the drug may be altered, thereby increasing or decreasing the degree of saturation. It should also be noted that the degree of saturation of the enhancer itself can be optimized. Clearly, when the degree of saturation of the chemical penetration enhancer is high, it will better penetrate into the skin and increase drug permeation to a greater extent. However, from a practical standpoint, the 'delivery' of many enhancers is limited by the fact that they frequently elicit skin irritation in vivo. Thus, some in vitro results of apparent promise may not be realizable in the real world because of local and undesirable effects.

6. Quantification of skin penetration/permeation in vitro

In this section, three different techniques to measure skin penetration and permeation in vitro are discussed, with emphasis on how the effects of enhancers on the diffusion and partition parameters can be separated. This deconvolution provides insight into the mechanism of enhancer action by distinguishing between the kinetic and thermodynamic parameters that govern membrane transport.

The diffusion cell method is the standard for measuring drug permeation across the skin. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy is a more recently developed method for assessing skin permeation. The various techniques for determining drug concentrations in the skin were discussed in a recent review by Touitou et al. [61]; here, we focus on the tape-stripping approach, which is easy to perform and which allows facile determination of drug concentration in the stratum corneum.

6.1. Permeation through skin: diffusion cell technique

The classic diffusion cell consists of donor and receptor compartments separated by the skin sample. The permeation rate of the drug from the donor chamber through the skin and into the receptor is determined by measuring the amount of drug permeated over time with e.g. high-performance liquid chromatography (HPLC) as analytical method (Fig. 9). Clearly, with appropriate experimental design, this experiment can provide useful information on the effects of penetration enhancers on topical drug delivery. The source of skin (animal vs. human skin), the skin layers used (i.e., stratum corneum alone, epidermis, dermatomed skin, or full thickness skin) and the properties of the receptor solution can have a significant effect on the permeation. Therefore, these factors are discussed in the following section:

6.1.1. Skin source

The best and most relevant, of course, is human skin [62].

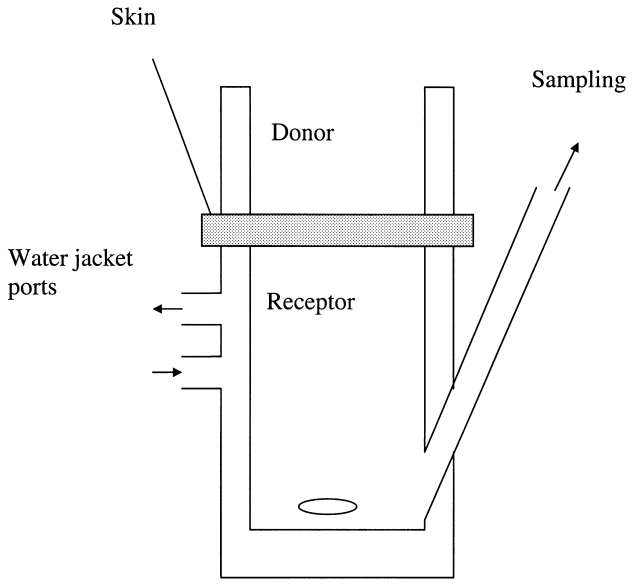

Fig. 9. Schematic representation of a static diffusion cell to measure the skin permeation of a drug. The formulation is placed in the donor chamber and the diffusion of the drug through the skin into the receptor is measured by taking samples at different time points.

However, its availability is sometimes limited and animal skin is therefore frequently used. The most relevant animal model for human skin is the pig. It is readily available from abattoirs and its histological and biochemical properties have been shown to be similar to human skin [63–65]. In addition, the permeability of drugs through pig skin has been repeatedly shown to be similar to that through human skin [66–69]. Porcine ear skin is particularly well-suited for permeation studies and gives comparable results to human skin [70,71]. Skin from mouse, rat, and guinea pig, which are commonly used, generally show higher permeation rates [66–69].

6.1.2. Skin section

Considerable data exists in the literature now to show, beyond question, that the permeation of lipophilic drugs (crudely those with log[octanol–water partition coefficients] greater than 3 or 4) is significantly, and artificially impeded in vitro when the skin membrane includes a significant part of the dermis [72–75]. This essentially aqueous tissue mass, which is not, of course, richly perfused with blood as it is in vivo, provides an important barrier to compounds of low water solubility. For such lipophilic drugs, simply use of the separated epidermis is recommended. On the other hand, for polar drugs of high aqueous solubility, the stratum corneum barrier is formidable and completely rate limiting, such that the presence or absence of dermal tissue has negligible effect on the permeation rate [68].

6.1.3. Receptor solution

The receptor compartment solution should provide sink conditions. For hydrophilic compounds, a buffer solution is suitable, whereas for lipophilic compounds the use of a solubilizing additive is sometimes necessary [76]. While ethanol (40%) and methanol (50%) have been so used, their back-diffusion into the skin reduces the barrier function of the stratum corneum; on the other hand, polyethylene glycol 20 oleyl ether (6%) and γ-cyclodextrin (0.1%), which are also useful, do not undermine the integrity of the skin [77,78]. A more physiological solubilizer, which again has no effect on the barrier function of the skin, is bovine serum albumin (BSA). For example, at a concentration of 4.5% the in vitro skin permeation of xylene was comparable to that when whole blood was used as receiver medium; lower BSA concentrations in the receptor resulted in lower xylene permeabilities [79].

6.1.4. Data analysis

The permeation rate of the drug from the donor through the skin into the receptor compartment is determined by measuring the amount permeated as a function of time. In the case of an infinite donor solution (drug concentration in the vehicle, c_v = constant), the cumulative drug permeated is plotted against time and the steady-state permeation rate (J) is calculated from the slope of the linear portion of the curve according to Fick's first law of diffusion [12]:

$$J = \frac{D_m K}{L} c_v \tag{2}$$

where D_m is the diffusion coefficient of the drug in the membrane, K the drug's partition coefficient between the stratum corneum and the vehicle, and L the diffusion path length in the membrane.

However, while this approach yields a permeability coefficient (K_p which is a composite parameter, KD_m/L), further separation of diffusion and partitioning requires determination of the so-called lag-time (t_{lag}), which is obtained by back-extrapolation of the steady-state slope to its intersection with the time-axis [80]:

$$t_{lag} = \frac{L^2}{6D_m} \tag{3}$$

The problem is that this method of dissecting diffusion and partition parameters depends strongly on the attainment of steady-state conditions (which may require too long to be reliably measured in a diffusion cell experiment). A better approach, therefore, is to fit the entire cumulative permeation vs. time curve to the appropriate solution of Fick's second law of diffusion [81]:

$$Q(t) = P_1 c_v \left[P_2 t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp(-P_2 n^2 \pi^2 t) \right] \tag{4}$$

where $Q(t)$ represents the amount of drug permeated at time t, $P_1 = KL$ and $P_2 = D_m/L^2$. While this method has been used by Harrison et al. [21] in an attempt to determine mechanism of action of different enhancers on the flux of

cyanophenol through skin, high standard errors of the fitted values have been observed thus rendering conclusions of only limited value.

6.2. Permeation through skin: ATR-FTIR spectroscopy

As the stratum corneum is the rate-limiting barrier for percutaneous absorption and it lies directly between the formulation and the viable skin layers, one can reasonably expect that the concentration of a topically applied drug in the stratum corneum will be related to its concentration in deeper skin layers [82].

As an alternative to the conventional diffusion cell measurement, drug permeation through stratum corneum can be directly measured with ATR-FTIR. The stratum corneum is sandwiched between a reflectance and a drug donor solution and the diffusion of the permeant into the deeper layers of the barrier is monitored by the appearance and increase of a drug-specific IR absorbance as a function of time (Fig. 10). By appropriate calibration, this absorbance is converted to a concentration ($c(t)$), which, again from the appropriate solution to Fick's second law of diffusion, can be shown to evolve with time as follows [83]:

$$c(t) = c_0\left[1 - \frac{4}{\pi}\sum_{n=0}^{1}\frac{(-1)^n}{2n+1}\exp\left(\frac{-D_m(2n+1)^2\pi^2 t}{4L^2}\right)\right] \quad (5)$$

where c_0 is the maximum drug concentration achieved in the stratum corneum (i.e. its solubility, corresponding to the plateau in the IR-absorbance vs. time profile; such a plateau must be reached, of course, because the skin–crystal interface is impermeable and drug therefore cumulates in the stratum corneum until a pseudo-equilibrium with the donor is achieved). It follows that fitting the experimental data to Eq. (5) provides c_0 (and hence the partition coefficient (K) of the drug between stratum corneum and the vehicle; i.e. $K = c_0/c_v$) and the characteristic diffusion parameter (D/L^2).

Although the technique is limited to IR-active permeants, it offers the potential to monitor and to quantify multiple species (e.g. co-permeants, co-solvents) and their interactions simultaneously, in real-time, without elaborate sample preparation, in a single experiment integrating the diffusion and detection protocols. This method has been successfully used to measure the permeation of drugs and solvents and the influence of permeation enhancers (Fig. 11) [21,84,85].

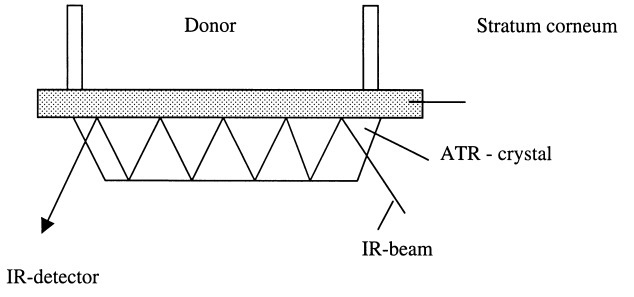

Fig. 10. Schematic representation of the method of ATR-FTIR for measuring drug permeation through the stratum corneum. The formulation is applied in the donor compartment and the diffusion of the drug into the lowest part of the stratum corneum is measured by the IR-beam.

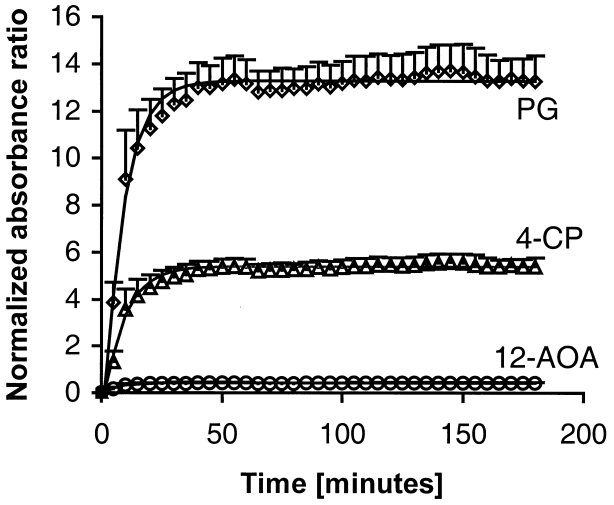

Fig. 11. Stratum corneum penetration of the solute 4-cyanophenol (4-CP), the enhancer 12-azido oleic acid (12-AOA), and the solvent propylene glycol (PG), monitored simultaneously using ATR-FTIR spectroscopy. 4-CP was saturated in 5% 12-AOA/propylene glycol. ($n = 3$, mean + SE, adapted from Ref. [83].)

6.3. Tape stripping and determination of drug concentration profiles

The tape stripping technique is being increasingly used to measure drug concentration and its concentration profile across the stratum corneum [50,82,86–88]. The stratum corneum is progressively removed by serial adhesive tape-stripping (Fig. 12), and the determination of (a) the drug amount in each tape and (b) the thickness of stratum corneum removed by each tape-strip, enable the diffusivity and solubility of the permeant within the stratum corneum to be calculated. This time, the appropriate solution to Fick's second law of diffusion predicts concentration of the drug (c_x) as a function of position (x) within stratum corneum according to the following equation [89]:

$$c_x = c_{x=0}\left\{\left(1 - \frac{x}{L}\right) - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\sin\left(\frac{n\pi x}{L}\right)\exp\left(\frac{-Dn^2\pi^2 t}{L^2}\right)\right\} \quad (6)$$

The concentration of drug in the outermost layer of the stratum corneum ($c_{x=0}$) divided by its concentration in the applied vehicle (c_v) gives the partition coefficient (K). Fitting experimental concentration profiles to Eq. (6) therefore provides, as before, both K and D/L^2.

Tape stripping, in combination with an appropriate analytical method, especially HPLC can be quite generally used both in vitro and in vivo. This method has been applied recently with some success to the skin penetration of cyano-

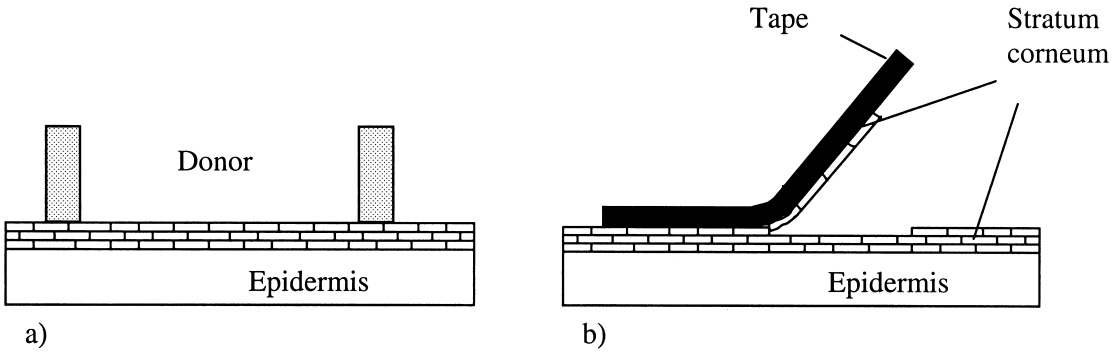

Fig. 12. Schematic representation of the method of tape stripping for determination of the amount of drug penetrated into the skin. After application of the formulation at the donor site (a) and removal of the formulation, the stratum corneum is progressively removed by tape stripping (b).

phenol, terbinafine, and a lavendustin derivative (Fig. 13) [58,60,87,90–92].

7. Conclusions

In gross physicochemical terms, penetration enhancement can be achieved by (a) increasing the degree of saturation of the drug in the vehicle, (b) increasing the drug's solubility in the stratum corneum, and (c) increasing the drug's diffusivity through the barrier. Some enhancers, of course, may operate via more than one of these mechanisms. The first approach can be realized by simply increasing the drug's concentration in the vehicle, or by changing the formulation to lower its solubility (i.e. increase its thermodynamic activity) in the applied delivery system. The method of supersaturation can also be used to create formulations in which the thermodynamic activity of the drug exceeds unity for a certain period of time. The physical chemistry involved in this first category centres on the drug–vehicle interaction; on the other hand, approaches to change the drug's solubility and/or diffusivity in the stratum corneum concern the three-way interaction drug–vehicle–skin. With respect to the use of specific penetration enhancers, the delivery of the enhancer must also be considered (i.e. its thermodynamic activity in the vehicle, its solubility in the skin and how this may interact synergistically with co-solvents in the formulation), together with a thought towards the ultimate in vivo use and the issue of skin tolerability.

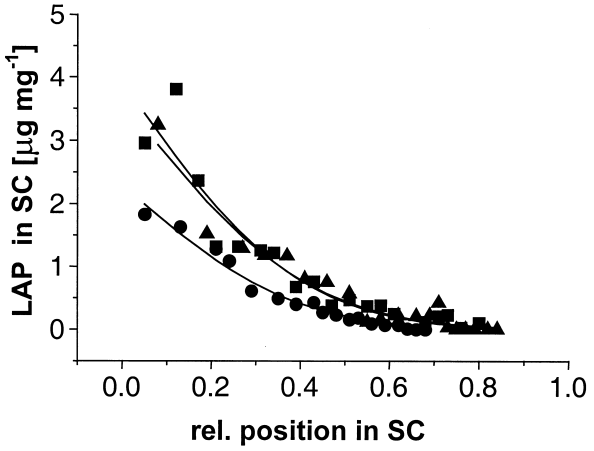

Fig. 13. Concentration profile of a lavendustin derivative (LAP, 0.6 mg/ml propylene glycol–water 7:3, 3-h application) in pig ear stratum corneum, determined by tape stripping. The concentration of LAP measured in three experiments (different symbols) is plotted against relative position within the stratum corneum. The lines drawn through the data represent the best fit of Eq. (6) for each skin sample (adapted from Ref. [58]).

Experimental methodology to evaluate skin penetration enhancement in vitro has evolved from the classic diffusion cell approach to more sensitive and sophisticated techniques that can be used to separate the physicochemical mechanisms of increased permeation discussed above. Steady-state diffusion may no longer be necessary in order to distinguish the kinetic and thermodynamic effects of different enhancers. In addition, the ATR-FTIR and skin stripping procedures appear well-suited not only to in vitro evaluations but also to in vivo measurements in human subjects. The stage is set, therefore, for the more rational and objective selection and optimization of chemical enhancement approaches to improve the skin penetration and permeation of therapeutically useful drugs.

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