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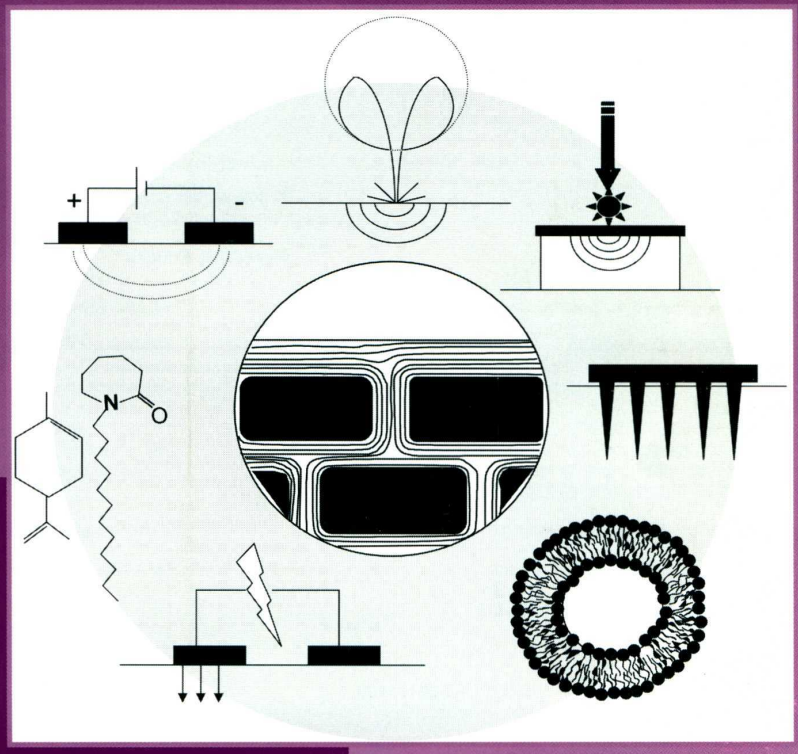

BREAKING THE SKIN BARRIER
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Penetration enhancers

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Abstract

One long-standing approach for improving transdermal drug delivery uses penetration enhancers (also called sorption promoters or accelerants) which penetrate into skin to reversibly decrease the barrier resistance. Numerous compounds have been evaluated for penetration enhancing activity, including sulphoxides (such as dimethylsulphoxide, DMSO), Azones (e.g. laurocapram), pyrrolidones (for example 2-pyrrolidone, 2P), alcohols and alkanols (ethanol, or decanol), glycols (for example propylene glycol, PG, a common excipient in topically applied dosage forms), surfactants (also common in dosage forms) and terpenes. Many potential sites and modes of action have been identified for skin penetration enhancers; the intercellular lipid matrix in which the accelerants may disrupt the packing motif, the intracellular keratin domains or through increasing drug partitioning into the tissue by acting as a solvent for the permeant within the membrane. Further potential mechanisms of action, for example with the enhancers acting on desmosomal connections between corneocytes or altering metabolic activity within the skin, or exerting an influence on the thermodynamic activity/solubility of the drug in its vehicle are also feasible, and are also considered in this review.

Keywords: Penetration enhancers; Accelerants; Skin; Lipids; Azone; Dimethylsulphoxide; Terpenes

Contents

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1. Introduction

Human skin is a remarkably efficient barrier, designed to keep "our insides in and the outsides out". This barrier property causes difficulties for transdermal delivery of therapeutic agents. One long-standing approach to increase the range of drugs that can be effectively delivered via this route has been to use penetration enhancers, chemicals that interact with skin constituents to promote drug flux. To-date, a vast array of chemicals has been evaluated as penetration enhancers (or absorption promoters), yet their inclusion into topical or transdermal formulations is limited since the underlying mechanisms of action of these agents are seldom clearly defined. In this article we review some uses of the more widely investigated chemical penetration enhancers and discuss possible mechanisms of action.

2. General principles

Although many chemicals have been evaluated as penetration enhancers in human or animal skins, to-date none has proven to be ideal. Some of the more desirable properties for penetration enhancers acting within skin have been given as [1];

- They should be non-toxic, non-irritating and non-allergenic.
- They would ideally work rapidly, and the activity and duration of effect should be both predictable and reproducible.
- They should have no pharmacological activity within the body—i.e. should not bind to receptor sites.
- The penetration enhancers should work unidirectionally, i.e. should allow therapeutic agents into the body whilst preventing the loss of endogenous material from the body.
- When removed from the skin, barrier properties should return both rapidly and fully.
- The penetration enhancers should be appropriate for formulation into diverse topical preparations, thus should be compatible with both excipients and drugs.
- They should be cosmetically acceptable with an appropriate skin 'feel'.

Not surprisingly, no such material has yet been discovered that possesses the above ideal properties although some chemicals demonstrate several of the above attributes.

Penetration enhancers may be incorporated into formulations in order to improve drug flux through diverse membranes including gastric epithelia or nasal membranes. Diffusion through skin, controlled by the outer most layer, the stratum corneum, can be regarded as diffusion through a passive membrane. The steady state flux (J) of a drug through skin can be approximated by Fick's second law of diffusion;

$$\frac{\delta C}{\delta t} = D\,\frac{\delta^2 C}{\delta x^2} \tag{1}$$

where C is the concentration of the diffusing substance, x the space co-ordinate measured normal to the section, D the diffusion coefficient, and t is time.

With skin permeation studies, investigators often use an in vitro protocol with a membrane clamped between two compartments, one of which contains a drug formulation (the donor) and the other compartment holding a receptor solution which provides sink conditions (essentially zero concentration). With sufficient time, steady state diffusion across the membrane prevails. Under these conditions Eq. (1) may be simplified to;

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{DC_0}{h} \tag{2}$$

where m is the cumulative mass of permeant that passes per unit area through the membrane in time t, C_0 is the concentration of diffusant in the first layer of the membrane at the skin surface contacting the source of the penetrant, and h is the membrane thickness.

In most experimental protocols it is difficult to measure C_0 but C_0', the concentration of diffusant in the donor phase, which bathes the membrane, is usually known. C_0 and C_0' are related by;

$$C_0 = PC_0' \tag{3}$$

where P is the partition coefficient of the diffusant between the membrane and bathing solution. Substituting Eq. (3) into Eq. (2) gives:

$$\frac{dm}{dt} = \frac{DC_0' P}{h} \tag{4}$$

From Eq. (4), the classic equation used to analyse skin permeation data, it can be seen that the flux (dm/dt) is governed by the diffusion coefficient of the drug in the stratum corneum, the dissolved effective concentration of the drug in the vehicle, the partition coefficient between the formulation and the stratum corneum and the membrane thickness. This equation thus illustrates some of the properties that may be manipulated on application of a penetration enhancer to the skin. Thus, an effective penetration enhancer may increase the diffusion coefficient of the drug in the stratum corneum (i.e. disrupt the barrier nature of the stratum corneum), may act to increase the effective concentration of the drug in the vehicle (for example acting as an anti-solvent), could improve partitioning between the formulation and the stratum corneum (perhaps by altering the solvent nature of the skin membrane to improve partitioning into the tissue) or, less likely, by decreasing the skin thickness (perhaps by providing a permeation 'shortcut' as opposed to a tortuous pathway for a permeant).

The modes of action of penetration enhancers in general are complex. As will be seen below, at clinically acceptable concentrations, most enhancers interact with the intercellular lipid domain of the stratum corneum. Fig. 1 is a modification of a scheme

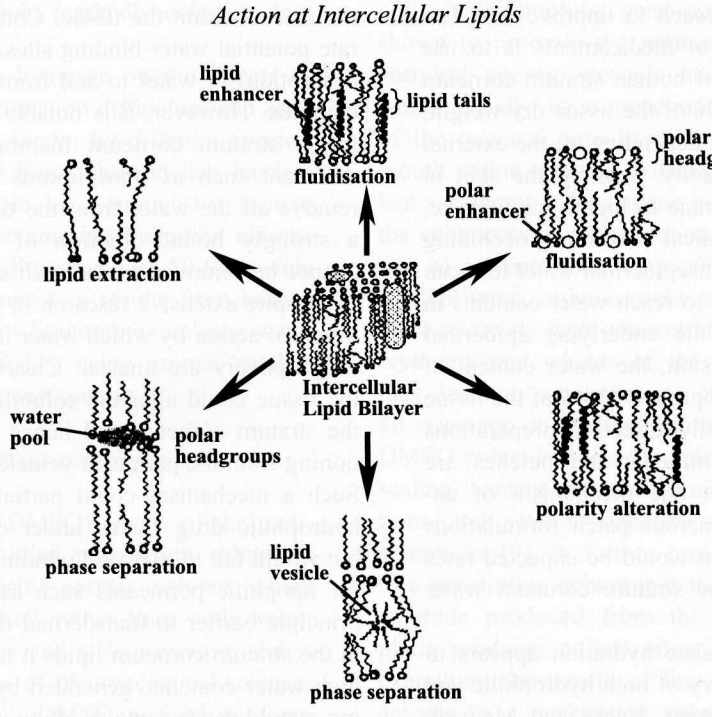

Fig. 1. Actions of penetration enhancers within the intercellular lipid domain (modified from [2]).

proposed by Menon and Lee [2] for the action of solvents on this tissue. However, the diagram is useful for accelerants in general as a visual aid to much of the discussion following below. Further consideration of penetration enhancer mechanisms of action is given in Section 4.

3. Penetration enhancers

The literature contains reports describing various elegant formulations that may contain materials which have penetration enhancing activity. For example, vesicles are often prepared from phospholipids; phospholipids themselves have some penetration enhancing activity (see below). Likewise, penetration enhancers have been formulated into eutectic systems or into slow or sustained release delivery systems. The focus of this chapter is not the formulations per se, rather the penetration enhancing activity of these materials.

3.1. Water

One long-standing approach to improve transdermal and topical delivery of medicaments is to use water. The water content of human stratum corneum is typically around 15–20% of the tissue dry weight, although clearly this varies depending on the external environment such as humidity. Soaking the skin in water, exposing the membrane to high humidities or, as is more usual under clinical conditions, occluding the tissue so preventing transepidermal water loss can allow the stratum corneum to reach water contents in equilibrium with that of the underlying epidermal skin cells. Thus, on occlusion, the water content of this outer membrane can approach 400% of the tissue dry weight. Many clinically effective preparations and products such as ointments and patches are occlusive, which provides one mechanism of enhanced drug delivery; numerous patch formulations deliver drugs at higher than would be expected rates due to modification of the stratum corneum water content.

In general, increased tissue hydration appears to increase transdermal delivery of both hydrophilic and lipophilic permeants. However, Bucks and Maibach cautioned against such a generalisation, stating that occlusion does not necessarily increase percutaneous absorption, and that transdermal delivery of hydrophilic compounds may not be enhanced by occlusion [3]. Further, they warn that occlusion could cause some local skin irritation with clear implications for the design and manufacture of transdermal and topical preparations.

Considering the heterogeneous nature of human stratum corneum it is not surprising that water within this membrane is found in several 'states'. Typically, from thermal analysis and spectroscopic methodologies, some 25–35% of the water present in stratum corneum can be assessed as 'bound', i.e. is associated with some structural elements within the tissue [4]. The remaining water within the tissue is 'free' and is available to act as a solvent within the membrane for polar permeants. Human skin also contains a hygroscopic humectant mixture of amino acids, amino acid derivatives and salts termed the natural moisturising factor (NMF). This material retains water within the stratum corneum and helps to maintain tissue pliability. Further, the keratin-filled corneocytes containing functional groups such as $-OH$ and $C-OOH$ are also expected to bind water molecules within the tissue. Considering such disparate potential water binding sites, the absorption (and desorption) of water to and from stratum corneum is complex. However, it is notable that even maintaining a stratum corneum membrane over a strong dessicant such as phosphorous pentoxide, will not remove all the water from the tissue—there remains a strongly bound fraction of 5–10% water that cannot be removed under such conditions.

Despite extensive research in the area, the mechanisms of action by which water increases transdermal drug delivery are unclear. Clearly free water within the tissue could alter the solubility of a permeant in the stratum corneum and hence could modify partitioning from the permeant vehicle into the membrane. Such a mechanism could partially explain elevated hydrophilic drug fluxes under occlusive conditions but would fail to explain hydration-enhanced delivery for lipophilic permeants such as steroids. Since the principle barrier to transdermal drug delivery resides in the stratum corneum lipids it may be expected that high water contents, generated by occlusion or soaking, would cause some swelling and hence disruption to these domains possibly by swelling the polar head

group regions of the bilayers. However, investigations by Bouwstra and co-workers using diffractometry methods have shown that water does not cause modification to the lipid bilayer packing [5]. Such findings raise the question "Where does the water go?". Clearly the corneocytes take up water and swell. One may expect that such swelling of cells would impact upon the lipid structure between the corneocytes causing some disruption to the bilayer packing. Again the experimental evidence contradicts this view. Data from freeze fracture electron microscopy of fully hydrated stratum corneum shows that the intercellular lipid bilayers contain water pools with vesicle-like structures occasionally found but no gross distortion to the lipid domains [6].

Elias et al. [7] consider the presence of an aqueous pore pathway in the stratum corneum, consisting of lacunar domains (sites of corneodesmosome degradation) embedded within the lipid bilayers. Although scattered and discontinuous under normal physiological conditions, they suggest that under high stress conditions (such as extensive hydration, iontophoresis or ultrasound) the lacunae expand, interconnect and form a continuous "pore pathway". The formation of such a route would markedly enhance drug penetration.

When examining the literature on the effects of water on transdermal permeation difficulties can arise from variable responses shown by different species. For example, Bond and Barry showed that hairless mouse skin is unsuitable as a model for human stratum corneum when examining hydration effects; the rodent skin permeability rose over 50-fold when hydrated for 24 h in contrast to results from human skin membranes [8]. Thus literature examining water effects on skin permeability using animal models should be viewed with some caution.

3.2. Sulphoxides and similar chemicals

Dimethylsulphoxide (DMSO) is one of the earliest and most widely studied penetration enhancers (Fig. 2). It is a powerful aprotic solvent which hydrogen bonds with itself rather than with water; it is colourless, odourless and is hygroscopic and is often used in many areas of pharmaceutical sciences as a "universal solvent". DMSO is used as a co-solvent in a vehicle for a commercial preparation of

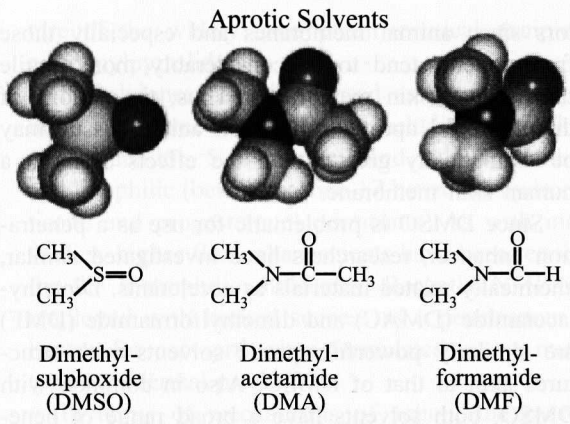

Fig. 2. Aprotic solvents which act as potent penetration enhancers.

idoxuridine, used to treat severe herpetic infections of the skin, particularly those caused by herpes simplex. DMSO alone has also been applied topically to treat systemic inflammation, although currently it is used only to treat animals.

A vast array of literature describes the penetration enhancing activities of DMSO, and studies have shown it to be effective in promoting both hydrophilic and lipophilic permeants. Thus, it has been shown to promote the permeation of, for example, antiviral agents, steroids and antibiotics. DMSO works rapidly as a penetration enhancer—spillage of the material onto the skin can be tasted in the mouth within seconds. Although DMSO is an excellent accelerant it does create problems. The effects of the enhancer are concentration dependent and generally co-solvents containing >60% DMSO are needed for optimum enhancement efficacy. However, at these relatively high concentrations DMSO can cause erythema and wheals of the stratum corneum and may denature some proteins. Studies performed over 40 years ago on healthy volunteers painted with 90% DMSO twice daily for 3 weeks resulted in erythema, scaling, contact uticaria, stinging and burning sensations and several volunteers developed systemic symptoms [9]. A further problem with DMSO use as a penetration enhancer is the metabolite dimethylsulphide produced from the solvent; dimethylsulphide produces a foul odour on the breath. When examining the wealth of literature reporting DMSO activity as a penetration enhancer it is essential to consider the membrane employed by the investiga-

tors since animal membranes and especially those from rodents tend to be considerably more fragile than human skin membranes. Thus, the actions of this powerful aprotic solvent on animal tissue may be dramatically greater than the effects seen on a human skin membrane.

Since DMSO is problematic for use as a penetration enhancer, researchers have investigated similar, chemically related materials as accelerants. Dimethylacetamide (DMAC) and dimethylformamide (DMF) are similarly powerful aprotic solvents with structures akin to that of DMSO. Also in common with DMSO, both solvents have a broad range of penetration enhancing activities, for example, promoting the flux of hydrocortisone, lidocaine and naloxone through skin membranes. However, Southwell and Barry, showing a 12-fold increase in the flux of caffeine permeating across the DMF treated human skin, concluded that the enhancer caused irreversible membrane damage [10]. Despite the evidence that DMF irreversibly damaged human skin membranes, this penetration enhancer has been used in vivo and promoted the bioavailability of betamethasone-17-benzoate as judged by the vasoconstrictor assay [11,12]. Further structural analogues have been prepared including alkylmethylsulphoxides such as decylmethylsulphoxide (DCMS). This analogue has been shown to act reversibly on human skin and, like its parent DMSO, also possesses a concentration dependent effect. The majority of available literature shows that DCMS is a potent enhancer for hydrophilic permeants but is less effective at promoting transdermal delivery of lipophilic agents.

The mechanisms of the sulphoxide penetration enhancers, and DMSO in particular, are complex. DMSO is widely used to denature proteins and on application to human skin has been shown to change the intercellular keratin confirmation, from α helical to a β sheet [13,14]. As well as an effect on the proteins, DMSO has also been shown to interact with the intercellular lipid domains of human stratum corneum. Considering the small highly polar nature of this molecule it is feasible that DMSO interacts with the head groups of some bilayer lipids to distort to the packing geometry. Further, DMSO within skin membranes may facilitate drug partitioning from a formulation into this "universal solvent" within the tissue.

3.3. Azone

Azone (1-dodecylazacycloheptan-2-one or laurocapram) was the first molecule specifically designed as a skin penetration enhancer (Fig. 3). Chemically it may be considered to be a hybrid of a cyclic amide, as with pyrrolidone structures (see Section 3.4 below) with an alkylsulphoxide but is missing the aprotic sulphoxide group that provides some of the disadvantages listed above for DMSO. Azone is a colourless, odourless liquid with a melting point of −7 °C and it possesses a smooth, oily but yet non-greasy feel. As would be expected from its chemical structure, Azone is a highly lipophilic material with a $\log_{Poctanol/water}$ around 6.2 and it is soluble in and is compatible with most organic solvents including alcohols and propylene glycol (PG). The chemical has low irritancy, very low toxicity (oral LD_{50} in rat of 9 g/kg) and little pharmacological activity although some evidence exists for an antiviral effect. Thus, judging from the above, Azone appears to possess many of the desirable qualities listed for a penetration enhancer in Section 2.

Azone enhances the skin transport of a wide variety of drugs including steroids, antibiotics and antiviral agents. The literature contains reports describing activity in promoting flux of both hydrophilic and lipophilic permeants. As with many penetration enhancers, the efficacy of Azone appears strongly concentration dependent and is also influenced by the choice of vehicle from which it is applied. Surprisingly, Azone is most effective at low concentrations, being employed typically between

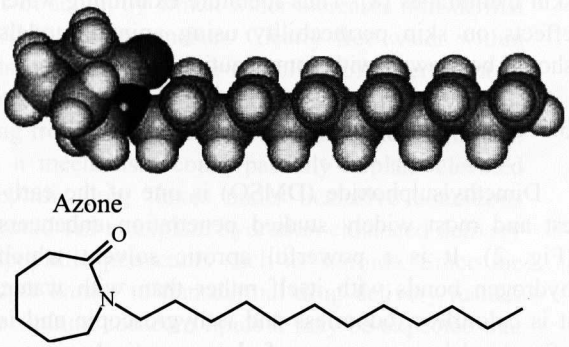

Fig. 3. Azone, the first molecule to be synthesised so as to act as a skin penetration enhancer.

0.1% and 5%, often between 1% and 3%. Although Azone has been in use for some 25 years, researchers continue to investigate its mechanism of action. Azone probably exerts its penetration enhancing effects through interactions with the lipid domains of the stratum corneum. Considering the chemical structure of the molecule (possessing a large polar head group and lipid alkyl chain) it would be expected that the enhancer partitions into the bilayer lipids to disrupt their packing arrangement; integration into the lipids is unlikely to be homogeneous considering the variety of compositional and packing domains within stratum corneum lipid bilayers. Thus, Azone molecules may exist dispersed within the barrier lipids or in separate domains within the bilayers. A 'soup spoon' model for Azone's confirmation within stratum corneum lipids supports the above mechanisms of action [15] and electron diffraction studies using lipids isolated from human stratum corneum provides good evidence that Azone exists (or partially exists) as a distinct phase within the stratum corneum lipids [16]. Extensive discussion concerning the metabolism and fate of Azone and on its use as a penetration enhancer has been reviewed and the molecule is still being investigated presently [17–19].

3.4. Pyrrolidones

A range of pyrrolidones and structurally related compounds have been investigated as potential penetration enhancers in human skin. As with Azone and many other penetration enhancers, they apparently have greater effects on hydrophilic permeants than for lipophilic materials, although this may be attributable to the greater enhancement potential for the poorer hydrophilic permeants. *N*-methyl-2-pyrrolidone (NMP) and 2-pyrrolidone (2P) are the most widely studied enhancers of this group. NMP is a polar aprotic solvent and is used to extract aromatic moieties from oils, olefins and animal feeds. It is a clear liquid at room temperature and is miscible with most common solvents including water and alcohols. Likewise 2P is miscible with most solvents, again including water and alcohols, and is a liquid above 25 °C. 2P also finds commercial use as a solvent in oil production and is useful as a solvent for sugars, iodine and polymers. 2P is an intermediate in the manufacture of the widely used pharmaceutical excipients polyvinylpyrrolidone.

Pyrrolidones have been used as permeation promoters for numerous molecules including hydrophilic (e.g. mannitol, 5-fluorouracil and sulphaguanidine) and lipophilic (betamethasone-17-benzoate, hydrocortisone and progesterone) permeants. As with many studies, higher flux enhancements have been reported for the hydrophilic molecules. Recently NMP was employed with limited success as a penetration enhancer for captopril when formulated into a matrix type transdermal patch [20].

In terms of mechanisms of action, the pyrrolidones partition well into human corneum stratum. Within the tissue they may act by altering the solvent nature of the membrane and pyrrolidones have been used to generate 'reservoirs' within skin membranes. Such a reservoir effect offers potential for sustained release of a permeant from the stratum corneum over extended time periods. However, as with several other penetration enhancers, clinical use of pyrrolidones is precluded due to adverse reactions. An in vivo vasoconstrictor bioavailability study demonstrated that pyrrolidones caused erythema in some volunteers, although this effect was relatively short lived. Also, a toxic hygroscopic contact reaction to *N*-methyl-2-pyrrolidone has recently been reported [21].

3.5. Fatty acids

Percutaneous drug absorption has been increased by a wide variety of long chain fatty acids, the most popular of which is oleic acid. It is of interest to note that many penetration enhancers such as Azone contain saturated or unsaturated hydrocarbon chains and some structure activity relationships have been drawn from the extensive studies of Aungst who employed a range of fatty acids and alcohols, sulphoxides, surfactants and amides as enhancers for naloxone [22,23]. From these extensive experiments, it appears that saturated alkyl chain lengths of around C_{10}–C_{12} attached to a polar head group yields a potent enhancer. In contrast, for penetration enhancers containing unsaturated alkyl chains, then C_{18} appears near optimum. For such unsaturated compounds, the bent *cis* configuration is expected to disturb intercellular lipid packing more so than the

trans arrangement, which differs little from the saturated analogue (Fig. 4).

Again from extensive literature reports fatty acids have been used to improve transdermal delivery of, amongst others, estradiol, progesterone, acyclovir, 5-fluorouracil and salicylic acid, indicating that these enhancers can be used to promote delivery of both lipophilic and hydrophilic permeants. Lauric acid in PG enhanced the delivery of highly lipophilic anti-estrogens [24]. Fatty acid effects on drug delivery to and through human skin can vary. For example, Santoyo and Ygartua, employed the mono-unsaturated oleic acid, polyunsaturated, linoleic and linolenic acids and the saturated lauric acid enhancers for promoting piroxicam flux [25]. Pre-treating the tissue with the fatty acids increased the amount of piroxicam retained within the skin and also decreased the lag time to pseudo steady state flux. Oleic acid has been shown to be effective for many drugs, for example increasing the flux of salicylic acid 28-fold and 5-flourouracil flux 56-fold through human skin membrane in vitro [26]. As with Azone, oleic acid is effected at relatively low concentrations (typically less than 10%) and can work synergistically when delivered from vehicles such as PG or ternary systems with dimethyl isosorbide [27]. Various analogues of fatty acids have been researched as penetration enhancers, for example diesters increased the permeation of non-steroidal anti-inflammatory drugs through rat skin [28].

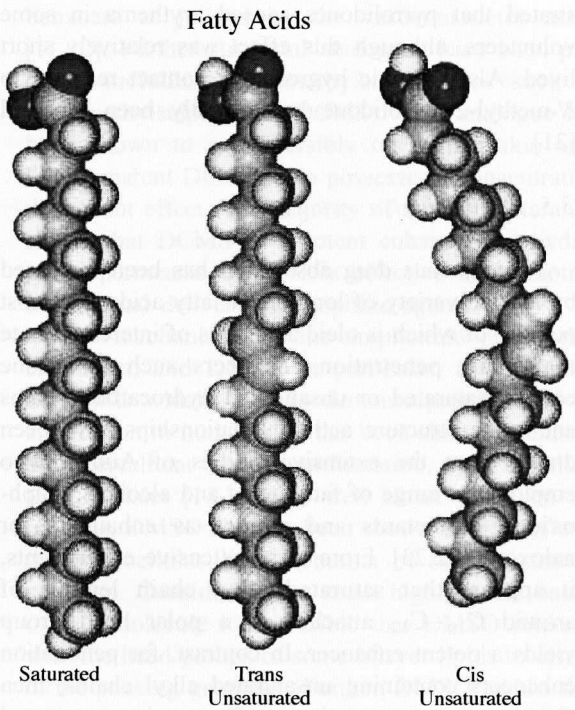

Fig. 4. Fatty acids, illustrating the different space-filling properties of saturated and *trans/cis* unsaturated hydrocarbon tails.

Considerable efforts have been directed at investigating the mechanisms of action of oleic acid as a penetration enhancer in human skin. It is clear from numerous literature reports that the enhancer interacts with and modifies the lipid domains of the stratum corneum, as would be expected for a long chain fatty acid with a *cis* configuration (Fig. 4). Spectroscopic investigations using deuterated oleic acid in human stratum corneum indicates that oleic acid at higher concentration can also exist as a separate phase (or as 'pools') within the bilayer lipids [29]. More recently, electron microscopic studies have shown that a discreet lipid domain is induced within stratum corneum bilayer lipids on exposure to oleic acid [30]. The formation of such pools would provide permeability defects within the bilayer lipids thus facilitating permeation of hydrophilic permeants through the membrane.

3.6. Alcohols, fatty alcohols and glycols

Ethanol is commonly used in many transdermal formulations and is often the solvent of choice for use in patches. It is also commonly employed as a co-solvent with water for ensuring sink conditions during in vitro permeation experiments. As with water, ethanol permeates rapidly through human skin with a steady state flux of approximately 1 mg cm^2/h [31].

Ethanol has been used to enhance the flux of levonorgestrel, estradiol, hydro-cortisone and 5-fluorouracil through rat skin [32] and of estradiol through human skin in vivo [33]. However, when using an ethanol water co-solvent vehicle, the enhancement effect of ethanol appears to be concentration dependent. Salicylate ion diffusion across human epidermal membranes was promoted up to an ethanol:water composition of 0.63 whereas higher levels of the alcohol decreased permeation [34]. Similar results have been reported for nitroglycerine [31] and estradiol [35] and zidovudine [36]. It is probable that at

higher ethanol levels dehydration of the biological membrane reduced permeation across the tissue.

Ethanol can exert its permeation enhancing activity through various mechanisms. Firstly, as a solvent, it can increase the solubility of the drug in the vehicle—although at steady state the flux of a permeant from any saturated, non-enhancing, vehicle should be equivalent. However, for poorly soluble permeants that are prone to depletion within the donor during a steady state permeation study, then ethanol can increase permeant solubility in the donor phase [33]. Further, permeation of ethanol into the stratum corneum can alter the solubility properties of the tissue with a consequent improvement for drug partitioning into the membrane [35]. Additionally, it is also feasible that the rapid permeation of ethanol, or evaporative loss of this volatile solvent, from the donor phase modifies the thermodynamic activity of the drug within the formulation. Such an effect is most apparent when applying a finite dose of a formulation onto the skin surface before evaporation of the alcohol; as ethanol is lost, drug concentration can increase beyond saturated solubility providing a supersaturated state with a greater driving force for permeation. Such a mechanism may operate for transdermal delivery from patches where ethanol, typically included to solubilise the drug or to apply the adhesive, may traverse the stratum corneum rapidly leaving behind a metastable supersaturated permeant which is inhibited from crystallising by polymers that are typically incorporated into patches. A further potential mechanism of action arising as a consequence of rapid ethanol permeation across the skin has been reported; solvent 'drag' may carry permeant into the tissue as ethanol traverses, although such a mechanism has been discounted for morphine hydrochloride permeation from ethanol and methanol containing formulations [37]. In addition, ethanol as a volatile solvent may extract some of the lipid fraction from within the stratum corneum when used at high concentration for prolonged times; though not a 'enhancing' effect, such a mechanism would clearly improve drug flux through skin.

Fatty alcohols (or alkanols) may also have penetration enhancing activity. These molecules are typically applied to the skin in a co-solvent—often PG—at concentrations between 1% and 10%. As with fatty acids described above (Section 3.5), some structure/activity relationships for fatty alcohol penetration enhancement have been drawn with lower activities reported for branched alkanols whereas 1-butanol was shown to be the most effective enhancer for levonorgesterol traversing rat skin [32]. Other workers have shown 1-octanol and 1-propranolol to be effective enhancers for salicylic acid and nicotinamide in hairless mouse skin. More recent structure activity relationships have been drawn for fatty alcohols using melatonin permeating through porcine and human skin in vitro [38]; comparing activities for saturated fatty alcohols from octanol to myristyl alcohol, a parabolic relationship was found with a maximum enhancement effect given by decanol. Enhancement activity also showed a general increase when adding up to two unsaturated bonds into the alcohols, but activity fell when three double bonds were introduced.

PG is widely used as a vehicle for penetration enhancers and shows synergistic action when used with, for example, oleic acid (see Section 3.5). However, PG has also been used as a penetration enhancer in its own right. Literature reports concerning the efficacy of PG as a permeation enhancer are mixed; evidence suggests at best only a very mild enhancement effect for molecules such as estradiol and 5-fluorouracil. As with ethanol, PG permeates well through human stratum corneum and its mechanisms of action are probably similar to those suggested above for ethanol. Permeation of the solvent through the tissue could alter thermodynamic activity of the drug in the vehicle which would in turn modify the driving force for diffusion, solvent may partition into the tissue facilitating uptake of the drug into skin and there may be some minor disturbance to intercellular lipid packing within the stratum corneum bilayers.

3.7. Surfactants

As with some of the materials described previously (for example ethanol and PG) surfactants are found in many existing therapeutic, cosmetic and agro-chemical preparations. Usually, surfactants are added to formulations in order to solubilise lipophilic active ingredients, and so they have potential to solubilise lipids within the stratum corneum. Typically composed of a lipophilic alkyl or aryl fatty chain, together with a hydrophilic head group, surfactants are often

described in terms of the nature of the hydrophilic moiety. Anionic surfactants include sodium lauryl sulphate (SLS), cationic surfactants include cetyltrimethyl ammonium bromide, the nonoxynol surfactants are non-ionic surfactants and zwitterionic surfactants include dodecyl betaine. Anionic and cationic surfactants have potential to damage human skin; SLS is a powerful irritant and increased the trans epidemeral water loss in human volunteers in vivo [39] and both anionic and cationic surfactants swell the stratum corneum and interact with intercellular keratin. Non-ionic surfactants tend to be widely regarded as safe. Surfactants generally have low chronic toxicity and most have been shown to enhance the flux of materials permeating through biological membranes.

Most studies evaluating enhancement activity have focussed on the use of anionic and non-ionic surfactants. Anionic materials themselves tend to permeate relatively poorly through human stratum corneum upon short time period exposure (for example when mimicking occupation exposure) but permeation increases with application time. Relatively few studies exist assessing non-ionic surfactant permeation through human skin but Watkinson et al. showed that around 0.5% of the applied dose of nonoxynol surfactant materials traversed human skin after 48 h exposure in vitro [40]. Surfactant facilitated permeation of many materials through skin membranes has been researched, with reports of significant enhancement of materials such as chloramphenicol through hairless mouse skin by SLS, and acceleration of hydrocortisone and lidocaine permeating across hairless mouse skin by the non-ionic surfactant Tween 80 [41,42].

However, as with several of the enhancers described above, the choice of model membrane can dramatically affect the scale of permeation enhancement. Tween 80 did not enhance nicardipine or ketorolac permeation in monkeys in vivo [43]. Likewise, 5-flourouracil permeation through human and snake skin in-vitro was not improved by 0.1% Tween 20 in normal saline [26,44], whereas the same enhancer formulation improved 5-flourouracil permeation across hairless mouse skin 6-fold [44]. From the literature it is apparent that, in general terms, non-ionic surfactants have only a minor enhancement effect in human skin whereas anionic surfactants can have a more pronounced effect.

3.8. Urea

Urea is a hydrating agent (a hydrotrope) used in the treatment of scaling conditions such as psoriasis, ichthyosis and other hyper-keratotic skin conditions. Applied in a water in oil vehicle, urea alone or in combination with ammonium lactate produced significant stratum cornum hydration and improved barrier function when compared to the vehicle alone in human volunteers in vivo [45]. Urea also has keratolytic properties, usually when used in combination with salicylic acid for keratolysis. The somewhat modest penetration enhancing activity of urea probably results from a combination of increasing stratum cornum water content (water is a valuable penetration enhancer, see Section 3.1) and through the keratolytic activity.

As urea itself possesses only marginal penetration enhancing activity, attempts have been made to synthesis analogues containing more potent enhancing moieties. Thus Wong and co-workers synthesised cyclic urea analogues and found them to be as potent as Azone for promoting indomethacin across shed snake skin and hairless mouse skin [46]. A series of alkyl and aryl urea analogies were moderately effective as enhancers for 5-flourouracil when applied in PG to human skin in vitro, though urea itself was ineffective [47].

3.9. Essential oils, terpenes and terpenoids

Terpenes are found in essential oils, and are compounds comprising only carbon, hydrogen and oxygen atoms, yet which are not aromatic. Numerous terpenes have long been used as medicines, flavourings and fragrance agents. For examples, menthol is traditionally used in inhalation pharmaceuticals and has a mild antipruritic effect when incorporated into emollient preparations. It is also used as a fragrance and to flavour toothpastes, peppermint sweets and menthylated cigarettes.

The essential oils of eucalyptus, chenopodium and ylang ylang were effective penetration enhancers for 5-flouorouracil traversing in human skin in vivo [48]. The most potent of these essential oils, eucalyptus, increased the permeability coefficient of the drug 34-fold. The principal terpene element within eucalyptus oil is 1,8-cineole and this molecule was one of a series

of 17 monoterpenes and terpenoids evaluated as enhancers for the model hydrophilic drug 5-flourouracil in human skin in vitro [49]—see Fig. 5. Some structure activity relationships were apparent from the data in that hydrocarbon terpenes were less potent enhancers for this hydrophilic drug than were alcohol or ketone containing terpenes, and the greatest enhancement activity was shown by the oxide terpenes and terpenoids. Within this oxide subclass, some potency variation was also apparent with ring-bridged oxides (cyclic ethers) being more potent than 1,2-oxygen linked (epoxide) molecules; pre-treatment of human epidermal membranes with 1,8-cineole provided a near 100-fold increase in the permeability coefficient of the model drug. However, such tentative structure activity relationships appear to be drug specific. The same terpenes were employed in an identical protocol for the lipophilic drug estradiol [50]. The results show that, unlike for 5-flourouracil where alcohol and ketone terpenes have moderate enhancement activities (10–40 fold increases in permeability coefficient), these same agents had no accelerent activity towards the lipophilic model drug and indeed appeared to retard its permeation. The cyclic ethers, so potent for 5-fluorouracil, provided only moderate enhancements for estradiol permeation and, in contrast to the hydrophilic drug, hydrocarbon terpenes (such as D-limonene) were generally the most effective terpene enhancers for the steroid. Similar results were reported for the permeation of another lipophilic molecule, indomethacin, traversing rat skin; hydrocarbon terpenes, especially limonene, were as effective as Azone in promoting drug flux and oxygen containing terpenes (carvone, 1,8-cineole) were ineffective [51,52]. Other hydrophilic drugs such as propanolol and diazepam are also enhanced by terpenes that contained no polar groups. As with many enhancers described above, a synergistic effect for terpene efficacy has also been shown when PG is used as the vehicle [53]; with this co-solvent, enhancer efficacies for carveol, carvone, pulegone and 1,-8 cineole rose approximately 4-fold, explained by improved partitioning of the enhancer into the stratum corneum. Cyclic monoterpenes generally showed stronger enhancement of curcumin than other terpenes, flavanoids and cholestanol [54].

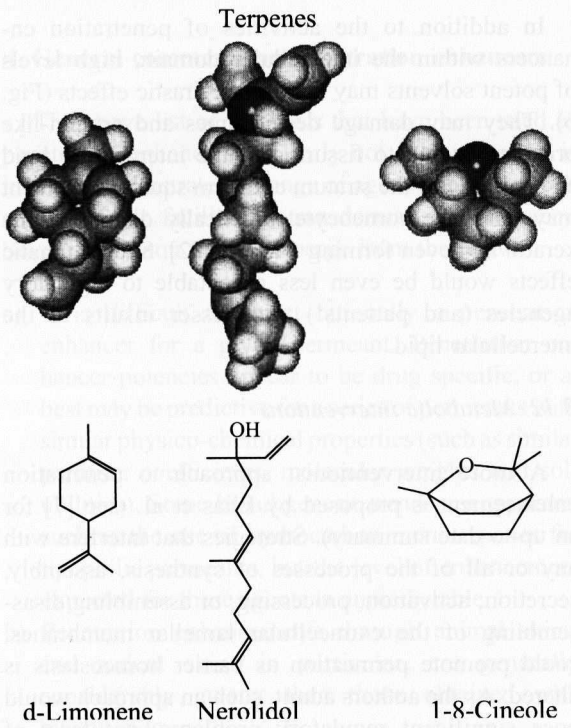

Fig. 5. Example terpenes that promote skin penetration of a variety of drugs.

Beyond the relatively small monoterpenes described above, larger terpene molecules (sesquiterpenes) have also been evaluated as enhancers for molecules permeating human skin membranes. Thus, materials such as nerolidol (Fig. 5) were shown to enhance 5-flourouracil permeability over 20-fold through human skin in vitro [55]. As larger more lipophilic enhancers, these agents exerted their effects over prolonged periods—for up to 5 days—in contrast to the monoterpenes that tended to wash out relatively easily from the stratum corneum. Moderate enhancement activity has also been reported for the cosmetically valuable terpene α-bisabolol [56].

Terpenes continue to be a popular choice of enhancer for delivering materials across skin membranes. For example, L-menthol has been used to facilitate in vitro permeation of morphine hydrochloride through hairless rat skin [37] imipramine hydrochloride across rat skin [57] and hydrocortisone through hairless mouse skin [58]. Recently, niaouli oil was the effective of six essential oils in promoting estradiol penetration through hairless mouse skin [59]. It is interesting that there is currently little control on the topical use of most terpenes, and

many 'aromatherapy' oils and formulations contain appreciable quantities of these enhancers. Their excessive use offers potential for permeation of hazardous compounds from the same formulations into the skin; some terpenes also have pharmacological activity.

From the above it is apparent that the smaller terpenes tend to be more active permeation enhancers than the larger sequiterpenes. Further, it also appears that hydrocarbon or the 'non-polar group containing' terpenes, such as limonene provide better enhancement for lipophilic permeants than do the 'polar' terpenes. Conversely, the polar group containing terpenes (such as menthol, 1,8-cineole) provide better enhancement for hydrophilic permeants. Such as relationship tends to imply that one mechanism by which this agents operate is to modify the solvent nature of the stratum corneum, improving drug partitioning into the tissue. Many terpenes permeate human skin well [55], and large amounts of terpenes (up to 1.5 $\mu g/cm^2$) were found in the epidermis after application from a matrix type patch [60]. With loss of terpenes, which are generally good solvents, from a formulation there could be an alteration to the thermodynamic activity of the permeant in the formulation as was described for ethanol. Terpenes may also modify drug diffusivity through the membrane. During steady state permeation experiments using terpenes as penetration enhancers, the lag time for permeation is usually reduced, indicating some increase in diffusivity of the drug through the membrane following terpene treatment. Small angle X-ray diffraction studies have also indicated that D-limonene and 1,8-cineole disrupt stratum corneum bilayer lipids, whereas nerolidol, a long chain sesquiterpene, reinforces the bilayers, possibly by orientating alongside the stratum corneum lipids [61]. Spectroscopic evidence has also suggested that, as with Azone and oleic acid, terpenes could exist within separate domains in stratum corneum lipids.

3.10. Phospholipids

Many studies have employed phospholipids as vesicles (liposomes) to carry drugs into and through human skin. However, a few studies have used phospholipids in a non-vesicular form as penetration enhancers. For example, theophylline was enhanced through hairless mouse skin by 1% phosphatidylcholine in PG, a concentration at which liposomes would not form [62]. Similarly, indomethacin flux was enhanced through rat skin by the same phospholipid and hydrogenated soy bean phospholipids have been reported to enhance diclofenac permeation through rat skin in vivo.

There is no compelling evidence to suggest that phospholipids interact directly with stratum corneum packing, though this may be expected when considering their physico-chemical properties and structures. However, phospholipids can occlude the skin surface and thus can increase tissue hydration, which, as shown above, can increase drug permeation. When applied to the stratum corneum as vesicles, phospholipids can fuse with stratum corneum lipids. This collapse of structure liberates permeant into the vehicle in which the drug may be poorly soluble and hence thermodynamic activity could be raised so facilitating drug delivery.

3.11. Solvents at high concentrations

In addition to the activities of penetration enhancers within the intercellular domain, high levels of potent solvents may have more drastic effects (Fig. 6). They may damage desmosomes and protein-like bridges, leading to fissuring of the intercellular lipid and splitting of the stratum corneum squames. Solvent may enter the corneocyte, drastically disrupting the keratin and even forming vacuoles [2]. Such dramatic effects would be even less acceptable to regulatory agencies (and patients!) than lesser insults to the intercellular lipid.

3.12. Metabolic interventions

A more interventionist approach to penetration enhancement is proposed by Elias et al. (see [7] for an up-to-date summary). Strategies that interfere with any or all of the processes of synthesis, assembly, secretion, activation, processing, or assembling/disassembling of the extracellular lamellar membranes, could promote permeation as barrier homeostasis is altered. As the authors admit, such an approach would pose significant regulatory problems, not least of which would be issues related to increased xenobiotic or microbial access. The concept of interfering with

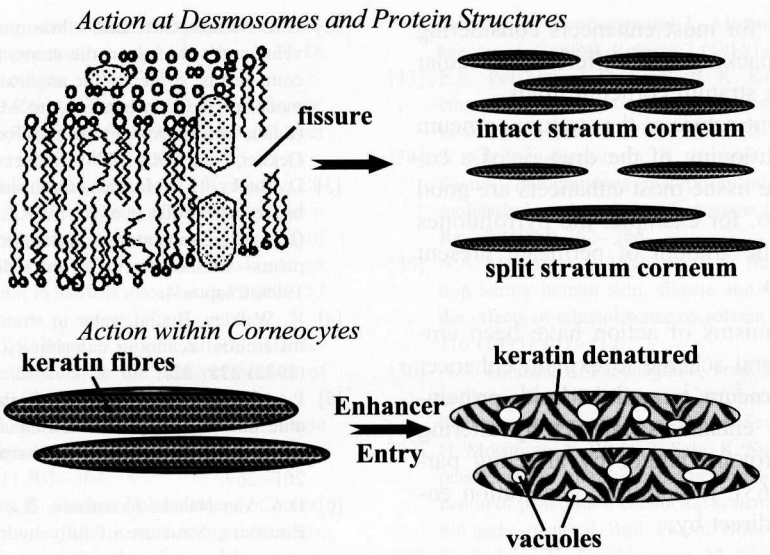

Fig. 6. Dramatic action of enhancers (particularly solvents) on the integrity of stratum corneum adhesion (modified from [2]).

barrier homeostasis on a relatively long time scale poses a myriad of clinical considerations.

4. General comments on penetration enhancers

The above list of materials that have been used as penetration enhancers is clearly not exhaustive but is intended to illustrate the range of agents that have been employed for facilitating transdermal drug delivery. Several common themes emerge from the above.

a) It is difficult to select rationally a penetration enhancer for a given permeant. Penetration enhancer potencies appear to be drug specific, or at best may be predictive for a series of permeants with similar physico-chemical properties (such as similar partition coefficients, molecular weights and solubilities). Some broad generic trends are apparent, such as the use of hydrocarbon monoterpenes for lipophilic permeants, but the level of enhancement expected for these agents is unpredictable.
b) Penetration enhancements through animal skins, and rodent skins in particular, are generally considerably greater than those obtained with human skin.
c) Penetration enhancers tend to work well with co-solvents such as PG or ethanol. Synergistic effects are found between enhancers such as Azone, oleic acid (and other fatty acids) and terpenes with PG.
d) Most penetration enhancers have a complex concentration dependent effect. This is shown clearly by Azone which is effective in promoting the transdermal flux for many drugs when used at 1% in PG but which is far less effective when applied at higher concentrations or neat (also relates to the use of co-solvents as above).
e) Potential mechanisms of action of enhancers are varied, and can range from direct effects on the skin to modification of the formulation. Thus, directly acting on the skin, enhancers can:
   i. Act on the stratum corneum intracellular keratin, denature it or modify its conformation causing swelling and increased hydration.
   ii. Affect the desmosomes that maintain cohesion between corneocytes.
   iii. Modify the intercellular lipid domains to reduce the barrier resistance of the bilayer lipids. Disruption to the lipid bilayers could be homogeneous where the enhancer distributes evenly within the complex bilayer lipids but more likely the accelerant will be heterogeneously concentrated within domains of the bilayer lipids. Such 'pooling' phenomenen has been shown for oleic acid and Azone, and is

likely to occur for most enhancers considering the range of packing and different molecular domains in the stratum corneum lipids.

iv. Alter the solvent nature of the stratum corneum to modify partitioning of the drug or of a co-solvent into the tissue most enhancers are good solvents and so, for example, the pyrrolidones can increase the amount of permeant present within the skin.

The above mechanisms of action have been embraced within a general scheme to explain enhancer effects on stratum corneum, termed the lipid–protein-partitioning theory; enhancers can act by altering skin lipids and/or proteins and/or by affecting partitioning behaviour [63]. In addition, penetration enhancement can be indirect by;

i. Modification of thermodynamic activity of the vehicle. Rapid permeation of a good solvent from the donor solution, such as ethanol, can leave the permeant in a more thermodynamically active state than when the solvent was present—even to the point of supersaturation.
ii. It has been suggested that solvent permeating through the membrane could 'drag' the permeant with it, though this concept is somewhat controversial and remains to be proven.
iii. Solubilising the permeant in the donor (e.g. with surfactants), especially where solubility is very low as with steroids in aqueous donor solutions, can reduce depletion effects and prolong drug permeation.

f) Many of the chemicals described above are used for alternative reasons within topical and transdermal preparations. For example, a topical prepartion could contain PG as a vehicle, a surfactant to solubilise the drug and a terpene as a fragrance material. The efficacy of some topical preparations is probably due to penetration enhancement by these types of agents, though no commercial preparations yet claim to incorporate an agent used specifically for this purpose.

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