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Invited review

Skin, the final frontier☆

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1. Introduction

The skin covers an area of approximately 2 m^2 and provides the contact between our bodies and the external environment. It prevents the loss of water and the ingress of foreign materials. Without it, water loss would be so great that we could not survive. It is a complex organ having a greater variety of cell types than the brain. Even though it is relatively accessible for research, it still holds a large number of secrets. One of the main puzzles has been to determine the precise route by which compounds penetrate through it and how this can be affected by formulations. The object of this publication is to summarise the physicochemical determinants that control drug delivery through the skin and the advances that have been made over the last decade or so to an understanding of skin permeation and its modulation. Particular emphasis has been placed on subjects with which my research group has been involved.

The skin is a very heterogeneous membrane but the layer that controls absorption is the outermost layer, the stratum corneum. The stratum corneum is only 15–20 μm thick but provides a very effective barrier to penetration. The exact nature of the barrier function has been investigated over many years and recent advances in biophysical techniques have provided interesting insights into the mechanisms of absorption at a molecular level.

2. Mechanisms of skin penetration

There are a number of routes by which a molecule can cross the stratum corneum, these are, intercellular, transcellular and appendageal (through either the eccrine (sweat) glands or hair follicles). Under normal conditions the appendageal route is not thought to be very significant, in part this is due to the low surface area occupied by the appendages. It is more difficult to determine differences between the transcellular and intercellular route. Research work, in vivo, on the skin absorption of methyl nicotinate was analysed using solutions to Fick's laws of diffusion and the best fit to the data was found for a diffusional pathlength of 350 μm (Albery and Hadgraft 1979a). Since the thickness of the skin is

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approximately 1/20 of this, it was postulated that the intercellular route was important. This value was similar to that calculated by a 'bricks and mortar' model of the stratum corneum proposed by Michaels et al. (1975). However, at the time the nature of the intercellular channels was unclear. Later experiments visualised diffusing molecules in the intercellular channels (Nemanic and Elias, 1980; Bodde et al., 1989) and work on the diffusion of water suggested that even a small polar molecule transferred along a tortuous pathway. The pathlength for diffusion of water was quoted as 500 μm (Potts and Francoeur, 1991).

Developments in analytical techniques have shown that the intercellular spaces contain a mixture of ceramides, free fatty acids (and their esters), cholesterol (and its sulfate). Interestingly, advances in microscopy, and the application of X-ray diffraction have shown that these lipids are arranged into structured bilayers. The reasons, therefore, for the impermeability of the skin are:

1. the tortuous route; and
2. the problem of repeated partition and diffusion across structured bilayers.

The impermeability is a considerable problem in the delivery of medicines both to and through the skin. It has been estimated that only a few percent of the active material reaches its target site when it is delivered topically. As an example only 1.7% of hydrocortisone alcohol is absorbed (Feldmann and Maibach, 1967). If this could be improved dermatological formulations could be made very much more efficient and hence effective. For this reason there have been many attempts to identify safe compounds that can be applied to the skin and promote the absorption of the active drug. The mechanisms by which these penetration enhancers act have their basis in the underlying physical chemistry that controls percutaneous absorption. A schematic of the skin is given in Fig. 1.

3. The physical chemistry of percutaneous absorption

There has been little evidence to suggest that there are any active processes involved in skin permeation, therefore the underlying transport process is controlled by simple passive diffusion. Fick's laws of diffusion can be used to analyse permeation data and can be used predictively. Fick's first law is used to describe steady state diffusion and can be simplified to:

$$J = \frac{DK\Delta c}{h} \tag{1}$$

where J is the flux per unit area, D is the diffusion coefficient in the skin, K is the skin–vehicle partition coefficient, Δc is the concentration difference across the skin and h is the diffusional pathlength. Under normal circumstances the applied concentration (c_{app}) is very much larger than the concentration under the skin and Eq. (1) is often simplified to

$$J = k_p \cdot c_{app} \tag{2}$$

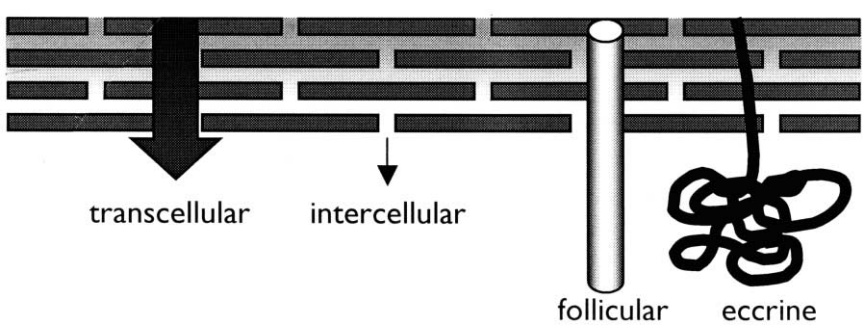


Fig. 1. A schematic representation of the skin showing the different possible routes of penetration. The follicular area accounts for approximately 0.1% and the eccrine 10^{-3}% of the total surface area.

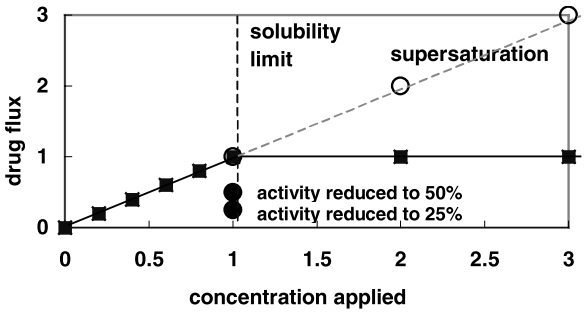


Fig. 2. The relationship between flux and concentration for a hypothetical drug. The saturated solubility of the drug is unity, hence the flux is invariant with concentration above the solubility limit. Also shown is the effect of maintaining the drug concentration but lowering the driving force for diffusion [the thermodynamic activity (solid circles)]. If the drug can be supersaturated, fluxes greater than those for the saturated solution can be achieved (open circles).

where k_p is a permeability coefficient ($=KD/h$) and is a heterogeneous rate constant having the units, for example, cm h^{-1}. As will become apparent, it is often difficult to separate K and D and their calculated magnitude will depend on h. h cannot be accurately estimated as it is the tortuosity of the intercellular channels, which is imprecise.

The driving force for diffusion is often simplified as the concentration gradient ($\partial c/\partial x$). Strictly it should be the chemical potential gradient ($\partial \mu/\partial x$). This leads to confusion when concentrated solutions are used and often mention is made of the thermodynamic activity. Fick's laws of diffusion show that the flux (J) should increase linearly with concentration until c_{app} reaches the solubility limit. This means that a 1, 5, and 10% suspension of the same drug in the same vehicle will give the same flux. This is shown schematically in Fig. 2. It has been demonstrated in vivo for corticosteroids (Lippold and Schneemann, 1984). In some circumstances there may be excipients in the formulation, e.g. surfactants, cyclodextrins, that will reduce the thermodynamic activity of the drug. Although the concentration of the drug present in the formulation will be the same, its flux through the skin will be reduced as its activity has been reduced.

There are also specific conditions when the drug can be supersaturated. This state is inherently unstable but if the supersaturation can be maintained for the lifetime of the application, fluxes greater than those from a saturated solution can be achieved.

It is possible to apply saturated solutions of a drug in different vehicles to a membrane. If the components of the vehicle do not alter the properties of the membrane, the flux will be the same, although the applied concentrations can vary by orders of magnitude. This was elegantly shown using silicone membranes (Fig. 3) (Twist and Zatz, 1986) and is a direct consequence of the fact that saturated solutions of a drug all possess the same chemical potential whereas the concentrations can vary considerably. This has been demonstrated in vivo for simple solutions of methyl nicotinate (Hadgraft et al., 1973).

The important physicochemical determinants that control diffusion of xenobiotics through the skin are therefore, partition, diffusion and solubility. If formulations need to be optimised, either to promote or retard absorption, it is these variables that can be modified.

4. Supersaturation

There have been a number of attempts to show the role of supersaturation in optimising dermal drug delivery. Supersaturated states can be produced by loss of volatile solvent (Coldman et al.,

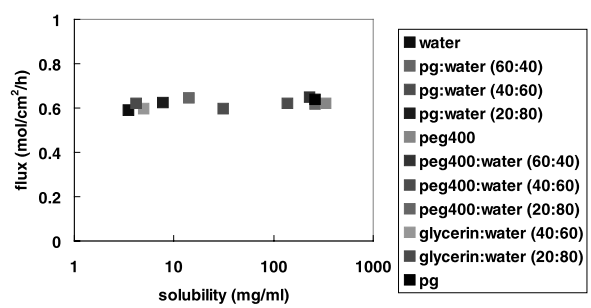


Fig. 3. The flux of paraben through a silicone membrane from saturated solutions. Data adapted from Twist and Zatz (1986). The flux is constant even though the applied concentration varies by over 100-fold.

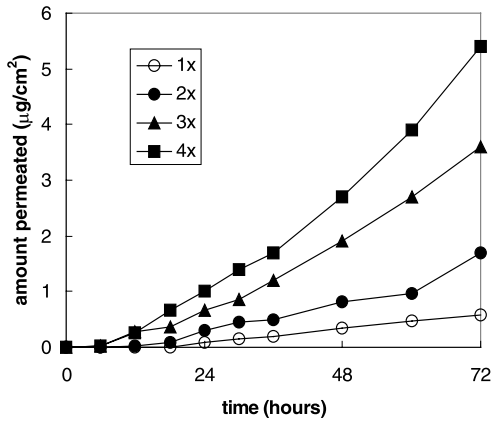


Fig. 4. The permeation of piroxicam through human epidermis in vitro, showing the effect of the degree of supersaturation. Data adapted from Pellett et al. (1994).

1969), use of cosolvent mixtures (Davis and Hadgraft, 1991), temperature changes (Henmi et al., 1994) and uptake of water into the formulation from the skin (Kemken et al., 1992). The thermodynamic states are unstable but the addition of anti-nucleant polymers such as hydroxypropyl methyl cellulose (HPMC) can impart stability for many hours (Iervolino et al., 2000; Raghavan et al., 2000). This is dependent on the nature of the drug but the precise reasons for this are unclear. It is possible that there are molecular interactions between the polymer and the drug molecules that prevent subsequent drug molecules interacting with each other. For example hydrocortisone acetate can be prevented from nucleating if small amounts of HPMC are present. There is infra red evidence to suggest that there is hydrogen bonding between the –OH groups on the drug and the polymer and the presence of the polymer prevents crystal growth from occurring (Raghavan et al., 2001).

Figs. 4 and 5 show that supersaturation can produce enhanced permeation through, and uptake into, human skin in vitro. The utility of the approach has also been demonstrated in vivo where a supersaturated hydrocortisone preparation is bioactive at 1/20th the concentration of a conventional formulation (Marks et al., 1992).

5. Penetration enhancement: diffusion effects

Diffusion through the stratum corneum will be controlled by the slowest step as the molecule passes through the intercellular structured lipids. Electron spin resonance studies have shown that the methylene groups adjacent to the ceramide polar head groups are the most rigid (Gay et al., 1989) a fact confirmed by fluorescence spectroscopy (Azimi et al., 1992). This is shown diagrammatically in Fig. 6. It is thought that hydrogen bonding between the polar head groups 'pulls' the alkyl chains together. One of the ways in which permeation modulators can work is therefore by their intercalation into the bilayers with subsequent disruption to the structure. Two of the most researched compounds that disrupt the structure of the bilayers are Azone and oleic acid (Fig. 7). These appear to act by two distinct mechanisms. In some very elegant Fourier transform infra red (FTIR) studies oleic acid was found to form pools within the bilayers (Ongpipattanakul et al., 1991) and a diffusing molecule would therefore 'find' an energetically favourable route by diffusing down the interfacial defects between the pools of oleic acid and the ceramides or by diffusing through the pools, which at body temperature, would be liquid. On the other hand Azone appears to distribute homogeneously (Harrison et al., 1996a). The difference appears to be the *cis* double bond in the oleic acid. It would be

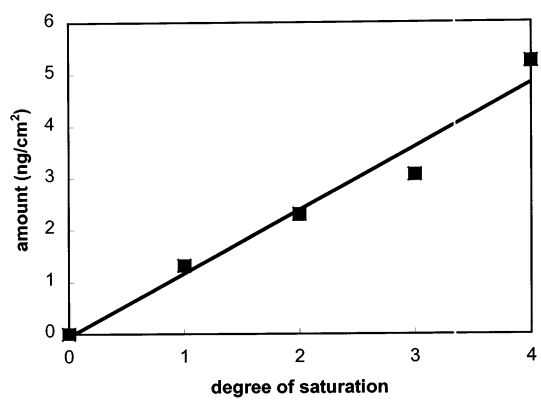


Fig. 5. The effect of degree of supersaturation on the uptake of piroxicam into the stratum cornuem. Data adapted from Pellett et al. (1997a).

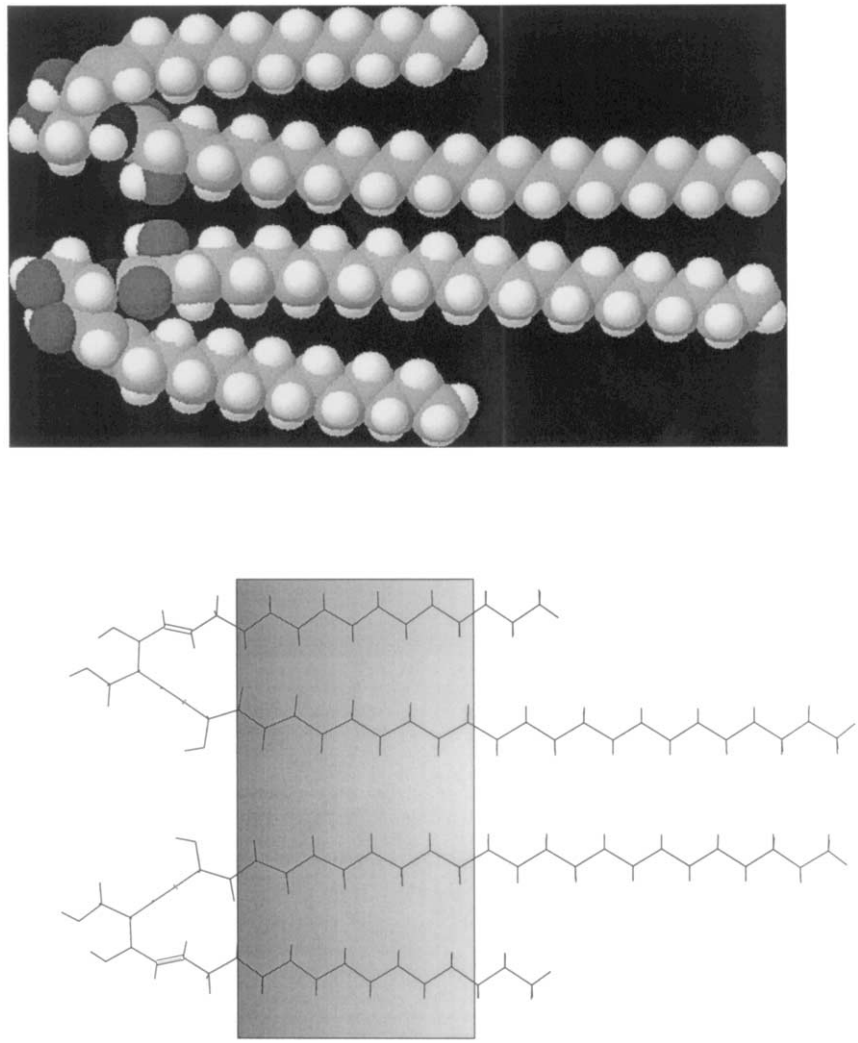
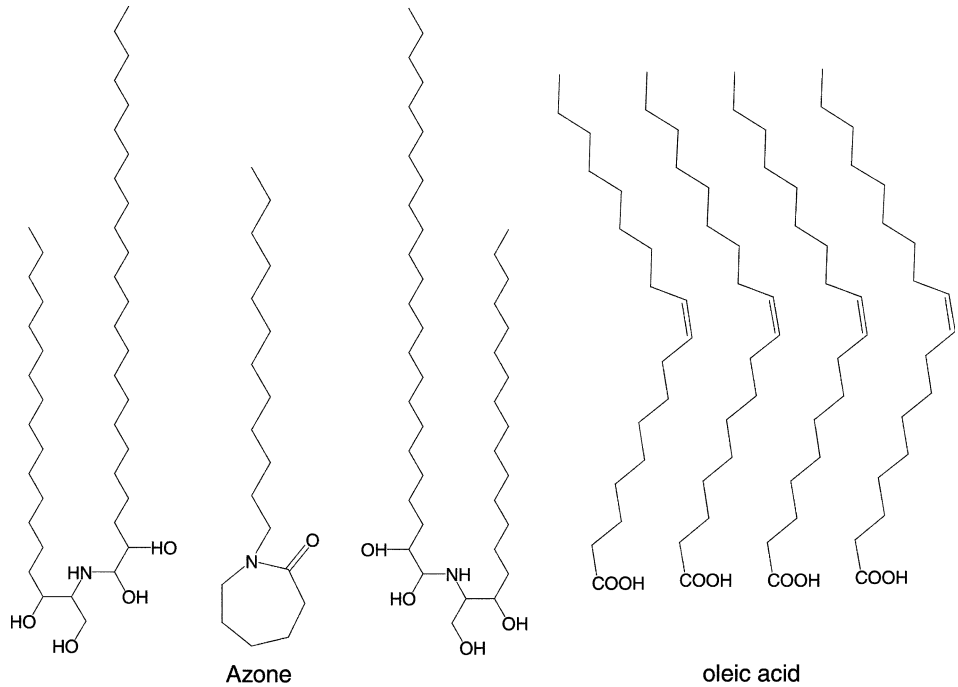


Fig. 6. A space filling molecular model of a representative ceramide and a diagram showing the region of maximum rigidity in the methylene groups adjacent to the polar head groups of the ceramide, implying condensation.

interesting to determine the energies involved in the insertion of the modulator. It is likely that the *cis* double bond requires some considerable energy and therefore pool formation and phase separation is favoured. The manner in which Azone inserts itself is interesting. A number of experiments have been conducted to show that the ring structure probably adopts a higher energy state and forms roughly a right angle to the alkyl chain. This exposes the lactam functionality to the more polar environment of the polar head groups, where it will gain energy from hydration. The area per molecule can be seen clearly in simple Π–A experiments where Azone monolayers are formed at an air water interface (Lewis and Hadgraft, 1990; Lewis et al., 1991). The area per molecule correlates well with that determined by molecular graphics for the bent conformation. A similar area per molecule was determined, by neutron scattering, when Azone is present in phospholipid vesicles (Watkinson and Hadgraft, 1992; Watkinson et al., 1997).



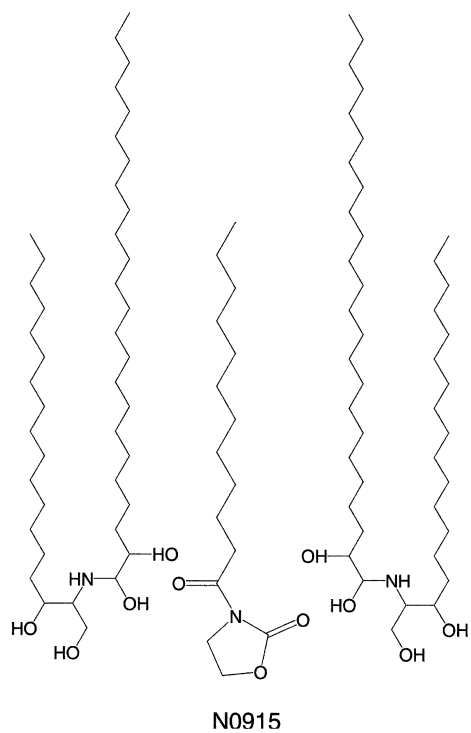


Fig. 7. Structures of some permeation modulators and a schematic showing their interaction with a representative ceramide.

Structure–activity relationships with Azone analogues have shown the importance of the functional groups in the polar head group region and also the ring size and chain length. A simple substitution of the oxygen in Azone with sulfur produces an inactive modulator. Molecular calculations on the two structures show that the presence of sulfur has little effect on the size of the molecule but there is a significant change in the negative partial charge. It is much less on the sulfur and this probably does not provide enough interaction with the –OH groups of the ceramides to locate it in the polar head group regions where it is required to act. Similarly the length of the alkyl chain is important and Azone analogues with an alkyl chain length of 12 or 14 appear optimum (Bodde et al., 1990). This indicates also that the ring structure needs to be 'anchored' in position. An alkyl chain that is too short or too long will not locate the ring structure in the polar head group regions.

A five membered ring structure (N0915), also with a 12 methylene alkyl chain has been examined (Hadgraft et al., 1996). The structure in Fig. 7 shows carbonyl groups on opposite sides of the ring. These can form a hydrogen bond bridge and condense the structured lipids. This is reflected in the Π–A curves where the mixed monolayers comprising N0915 and phosphatidyl choline show a negative deviation from ideality, and there is a condensation of the lipids.

Additionally N0915 increases the phase transition temperature of phospholipid vesicles (Hadgraft et al., 1996) and acts generally as a penetration retarder. This is useful when systemic absorption is undesirable, e.g. for UV filters and insect repellents.

Fig. 8 shows that the presence of N0915 in human skin significantly reduces the absorption of the insect repellent diethyl *m*-toluamide (DEET). It is possible that analogues can be designed that will have specific actions in reducing the absorption of many xenobiotics. It is interesting that DEET has also been used as a penetration enhancer (Kondo et al., 1988) but its mechanism of action is likely to be a result of its solvent action.

6. Penetration enhancement: partition effects

Another way of improving the flux of the drug is to improve the ability of the permeant to partition into the outer layers of the skin. If an excipient in the formulation permeates into the skin it should alter the solubility characteristics of the skin. For example, propylene glycol is often used as a formulation component. It is a small molecule that is known to transfer through the skin. It is also often a good solvent for the permeant. Its presence in the skin should therefore improve the absorption rate. There has been little systematic work conducted on the effect of vehicle excipients on solubility of permeants in skin. This is largely due to the problems of identifying appropriate techniques to separate solvent and lipid disordering behaviour. One way of considering solubility effects is to examine the solubility parameters of the skin, the permeant and the solvent and to make some judgement about the appropriate choice. The skin is thought to have a solubility parameter (δ) of ~ 10 $(\text{cal/cm}^3)^{1/2}$ (Liron and Cohen, 1984). If propylene glycol is applied ($\delta = 14$) to the skin surface it will permeate into the skin and δ would be anticipated to be shifted from 10 towards 14. Any drugs having a δ between 10 and 14 would be expected to have an enhanced flux. In practice the results are less clear. Fig. 9 shows some results for the permeation of ibuprofen and paracetamol. Skin was treated with saturated solutions of either ibupro-

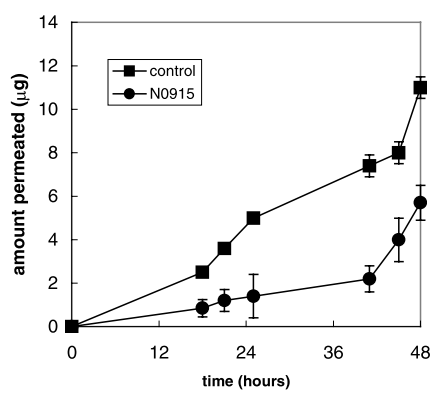
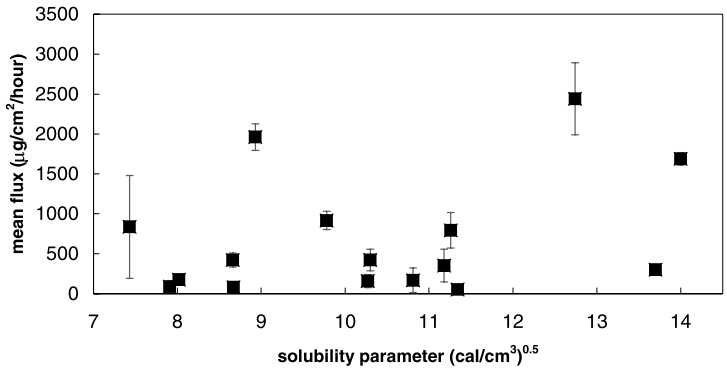


Fig. 8. The influence of N0915 on the skin permeation of DEET. Data adapted from Hadgraft et al. (1996).

a) ibuprofen



b) paracetamol

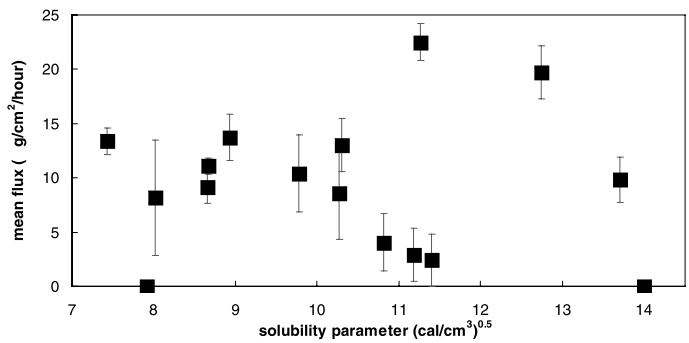


Fig. 9. The steady state flux of (a) ibuprofen ($\delta = 10.2$) and (b) paracetamol ($\delta = 15$) across human epidermis. The donor solutions are saturated with respect to the permeant and represent a range of different solvents spanning solubility parameters from 7 to 14. Data adapted from Rosado (2000).

fen ($\delta = 10.2$) or paracetamol ($\delta = 15$) in a variety of solvents (δ ranging from 7.5 to 14). There is no simple relationship between the δ of the solvent its ability to enhance flux. Equally there is no significant difference for the two permeants even though they possess different δ values. If the solvent was having no effect on the barrier properties of the skin, the flux should be independent of δ, as in Fig. 3. Similar findings have been found for other permeants and for model membranes such as polydimethylsiloxane. Hydrocortisone permeation across silicone membranes showed that the enhancement effects are related to the sorption of the solvent into the membrane and this is not a simple function of the solubility (Cross et al., in press).

More work needs to be conducted so that the optimum choice of solvent type enhancers can be rationalised. Preliminary work indicates that mixed solvents may be better but it is possible that they may be acting by extracting some of the lipid components of the intercellular channels.

7. Separation of diffusion and partition effects

It is possible to devise experiments that can discriminate enhancers that act on diffusion and

partition. The approach involves the use of attenuated total reflectance-Fourier transform infra red (ATR-FTIR). A membrane (e.g. a piece of skin) is placed on a ZnSe crystal (Harrison et al., 1996b). Above the piece of skin is a donor chamber that contains a saturated solution of a permeant. The permeant is chosen such that it has an IR signature that is discrete from the skin spectrum. The arrival of the permeant at the interface between the crystal and the skin is recorded by monitoring the IR signal from the permeant. The signal increases sigmoidally with time. This is shown schematically in Fig. 10.

The reason for the sigmoid profile is that there is a lag phase, a pseudo steady state phase and then an equilibration as the skin becomes saturated with the permeant. If the skin is pre-treated with an enhancer that modifies the diffusional properties of the skin, the plateau reached will be the same but it will occur earlier. If an enhancer alters the solubility in the skin the plateau will be affected whereas the time to reach the plateau will not. This is shown in Fig. 11.

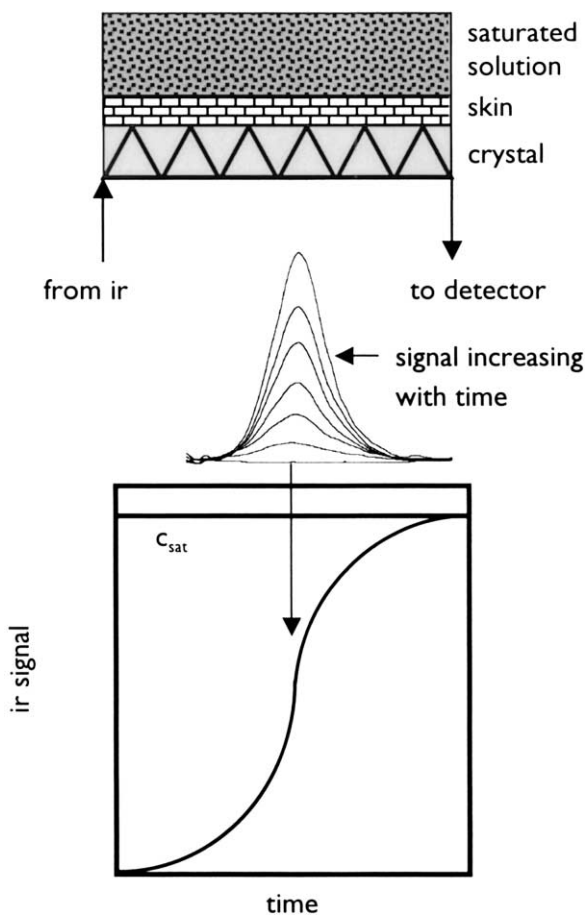


Fig. 10. A schematic of the ATR-FTIR experiment showing the build up in IR signal with time.

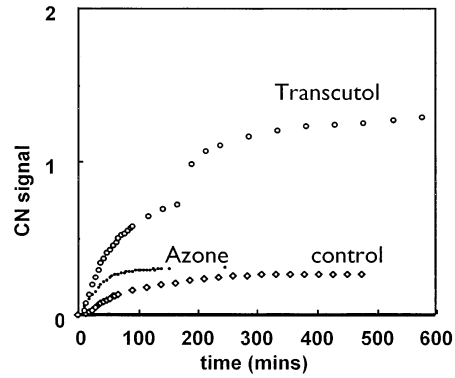


Fig. 11. The effect of Azone and Transcutol on the permeation of cyanophenol through human skin.

Analysis of the data in Fig. 11 using an appropriate solution to Fick's second law of diffusion shows that Azone is acting solely to increase the diffusion (by approx. a factor of 3) of the cyanophenol in the skin and Transcutol is increasing the solubility of the permeant in the skin (also by a factor of 3). The two distinct enhancement mechanisms are therefore separated (Harrison et al., 1996b).

8. Penetration enhancement: synergy

A simple consideration of Eq. (1) shows that it is possible to obtain synergy if more than one enhancement strategy is used. For example if D can be improved by a factor of 3 and if supersaturation can be used to increase the effective concentration by a factor of 3, the overall enhancement will be ninefold. In the above example, if Azone and Transcutol are combined, each effect is threefold and therefore the combination should also provide a ninefold enhancement. As a second, different, example, Fig. 12 shows the effect of oleic acid (increases D) and supersatura-

tion on the in vitro permeation of flurbiprofen (Pellett et al., 1997b).

Substantial enhancement can be achieved using the combination and this type of approach could be used to improve the very poor bioavailability seen with normal topical products.

9. Penetration enhancement: lipid extraction

It has been known for a long time that the skin can be treated with various solvents and these can extract the lipids in the stratum corneum. The most effective extractant is a mixture of methanol and chloroform, which will remove all but the lipids that are covalently bonded to the corneocyte envelope. The barrier function of the skin is considerably reduced but it can be restored by reintroduction of the lipids. Ethanol will extract some of the lipids but it is a very much milder solvent. The skin recognises that its barrier function has been impaired and it rapidly restores itself by synthesis of lipids to replace those that have been extracted (Bommannan et al., 1991). Little systematic studies have been conducted on the degree to which solvents interact with the skin lipids. More quantitative data can be obtained using ATR-FTIR and perdeuterated solvents (so that their signal can be distinguished from the CH stretch of the skin lipids) coupled to an in vivo tape stripping technique (Dias, 2001). It is possible to examine the effect of the solvent on both the skin lipid mobility and extraction. A series of alkanols was selected as representative solvents. *n*-Hexanol and octanol were found to decrease the amount of intercellular lipids whereas *n*-decanol does not. The degree of extraction was found to be time dependent. In contrast to the other two alkanols, the uptake of *n*-decanol was higher. The disordering of the skin lipids was in proportion to the solvent uptake. An example of this is shown in Fig. 13.

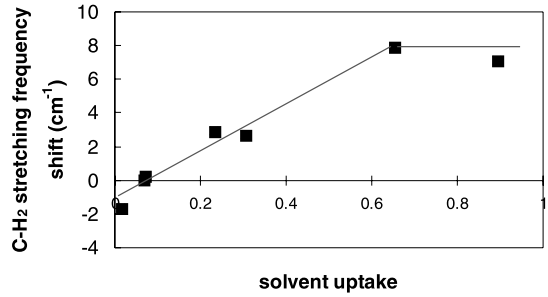


Fig. 13. Relationship between the solvent uptake (normalised C–D peak area) and the C–H frequency shift after 30 min exposure to D-hexanol. Data adapted from Dias (2001).

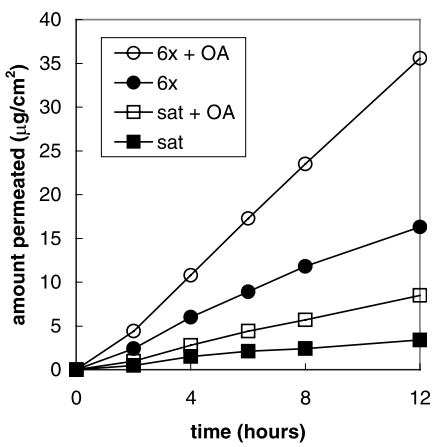


Fig. 12. The in vitro permeation of flurbiprofen and how it is affected by oleic acid or sixfold saturation. Synergy is seen when both enhancement strategies are used. Data adapted from Pellett et al. (1997b).

10. Modelling percutaneous absorption

Since the overall absorption process is a sequence of partition and diffusion events it should be possible to build physicochemical models for skin penetration. A number of different approaches have been adopted for this. Complex solutions to Fick's laws of diffusion have been used to infer mechanisms of absorption (Albery and Hadgraft, 1979a,b; Albery et al., 1983) and to show that urea exerts its enhancing effect by altering the diffusional resistance of the skin (Beastall et al., 1986). They have also been used to interpret concentration profiles found across the stratum corneum (Watkinson et al., 1992). However, since the data are subject to considerable biological variability the data analysis has to be performed with caution.

A more general approach to modelling skin absorption has been to build a 'pharmacokinetic' model in which various compartments represent the delivery device, the stratum corneum, the viable tissue and the blood. Normal elimination kinetics are assumed from the blood compartment. The rate constants connecting the compartments can be ascribed physical significance, e.g. the slowest step, transfer across the stratum corneum k_1 is equal to h^2/D_{sc} where h is the diffusional pathlength and D_{sc} the diffusion coefficient of the drug in the stratum corneum. A ratio of first order rate constants k_3/k_2 is related to the partition coefficient between the lipophilic stratum corneum and the aqueous viable epidermis. The model has been used successfully to predict the transdermal delivery of a number of drugs (Guy et al., 1982; Guy and Hadgraft, 1983, 1985a,b, 1986).

However, the data bank available for transdermal delivery is relatively small and it is helpful to be able to model a wide spectrum of xenobiotics. Permeability data exist, in the literature, for an extensive range of chemical entities and a simple equation has been shown to exist for these data (Potts and Guy, 1992)

$$\log k_p(\text{cm/h}) = -2.7 + 0.71 \log K_{oct} - 0.0061\ \text{MW} \quad (3)$$

where K_{oct} is simply the octanol water partition coefficient and MW, the molecular weight. It should be noted that this equation is used for predicting the permeability coefficient from an aqueous solution of the diffusant. The physical significance of this empirical equation is clear. As the molecule becomes more lipophilic its permeability increases due to better partitioning into the skin. As it becomes larger its diffusion in the skin is reduced. Although a very successful equation there are difficulties as the two constants, K_{oct} and MW, are not totally independent. For this reason further analysis of the data has been conducted to try and separate the diffusion and partition elements (Pugh et al., 1996; Roberts et al., 1996, 1995). This has revealed some interesting factors, the most significant of which is that diffusion within the skin is affected significantly by the number of functional groups on the permeant that are capable of hydrogen bonding. Fig. 14 shows that, as the number of hydrogen bonding groups increase the diffusion coefficient decreases until three are present, thereafter there is little significant effect. It is probable that the effect is related to the ability of the diffusant to hydrogen bond with the polar head groups of the ceramides. Fig. 14 also shows the relative degrees to which some simple functional groups retard penetration with a carboxyl group having the most effect and ether, the least.

For this reason the effect of partial charge on diffusion was examined (Pugh et al., 2000). The partial charges around a molecule can easily be calculated using computer algorithms and diffusional effects therefore calculated 'ab initio'. The following relationship was found with the charge and molecular weight being equally significant factors:

$$\log(D/h) = -2.66 - 0.00269\ \text{MW}*\text{charge} \quad (4)$$

where charge is defined as the simple sum of the moduli of the partial charges on the constituent atoms of the molecule.

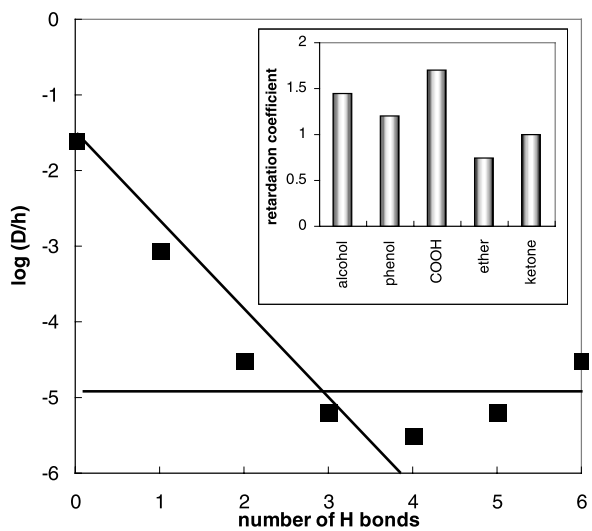


Fig. 14. The effect of increasing the number of hydrogen bonding functional groups on diffusion through the skin. The insert shows that the addition of a carboxyl functional group has the largest effect on reducing permeation whereas ether has the least. Data adapted from Pugh et al. (1996) and Roberts et al. (1996).

The mass of a molecule absorbed across the skin is important in determining therapeutic effect and also in risk assessment. It is related to the permeability coefficient and the applied concentration. The maximum flux will therefore be the product of the permeability coefficient, as predicted by Eq. (3), and the aqueous solubility. There are equations for predicting aqueous solubility (S_w) at temperature T, an example (Valvani et al., 1981) is:

$$\log S_w = -\log K_{oct} \frac{1.11 \Delta S_f (mp - T)}{4.577(273 + T)} - 0.54 \qquad (5)$$

where ΔS_f is the entropy of fusion and mp is the melting point of the compound. A comparison of Eqs. (3) and (5) shows the problem in selecting ideal candidates for dermal delivery. As K_{oct} increases, permeability rises, however, solubility decreases. In general compromises have to be made and an ideal permeant probably has a log K_{oct} value of approximately 2. Eq. (5) also indicates that it should have a low melting point. It is therefore not surprising that nitroglycerin (mp = 13.5 °C, log $K_{oct} = 2.2$) and nicotine (oil, log $K_{oct} = 1.17$) permeate the skin extremely well. There is often a parabolic dependency between the amount of drug absorbed and log K_{oct}, this has been observed for the non-steroidal anti-inflammatory agents (Yano et al., 1986) and also analysed theoretically for the same compounds (Degim et al., 1995). However, the overall effectiveness of a topical NSAID will depend both on permeation and its pharmacological potency. These can be considered in combination to provide guidelines for the appropriate NSAID selection (Hadgraft et al., 2000).

Since the skin behaves as a lipophilic membrane the ionisation state of the permeant is also important. The surface of the skin has a pH of approximately 4–5 and appears to have a good buffer capacity. Formulations placed on the skin should possess a pH between 4 and 7 and therefore the pK_a of the permeant can also be important. It is perhaps surprising that not much systematic work has been conducted on the pH partition behaviour of skin. Ibuprofen and lignocaine have been examined in some detail and simple relationships between the observed permeability and log D (allowing for ionisation) found (Hadgraft and Valenta, 2000). Again there appears to be optimum conditions. At low pH the ionisation of ibuprofen (p$K_a = 4.4$) is suppressed and log D is high but the solubility is low. In contrast at high pH, log D is low whereas the solubility is high. It appears that the effect of solubility is greater than partition and that more efficient delivery of ibuprofen can be achieved by using the higher concentrations of the salt that can be achieved at the higher pH values. Recent work (Smith and Irwin, 2000) however, has not found the same type of effect and more research is required to determine the exact nature of pH partition effects in skin.

Evidence exists that suggest that ionised compounds do permeate the skin and it is probably the dipolar nature of the skin that is important in controlling the absorption steps. In classic bilayer membrane studies, compounds such as phloretin can be used to change the dipolar nature of the lipids and improve permeation of lipophilic ions. Similar effects have been seen in skin where the presence of phloretin improved the absorption of ionised lignocaine (Valenta et al., 2001). This type of enhancement strategy has not been explored in any detail but warrants further investigation.

11. In vitro experiments

Modeling the permeation process is a very useful tool but confidence in the various mathematical models used is lacking. This is due to lack of reliable data and the inability to extrapolate from simple aqueous solutions to real formulations. A better indicator of what is likely to happen in vivo in humans is to use human skin in vitro. Considerable care needs to be taken in the experimental design of these studies since it is easy to generate erroneous results, particularly with an incorrect choice of receptor medium. A number of guidelines have been established (Howes et al., 1996; Skelly et al., 1987) which help in the experimental design but it is still difficult to find appropriate conditions when the permeant is very lipophilic in nature. Where correct conditions are used it is possible to obtain very good correlation between

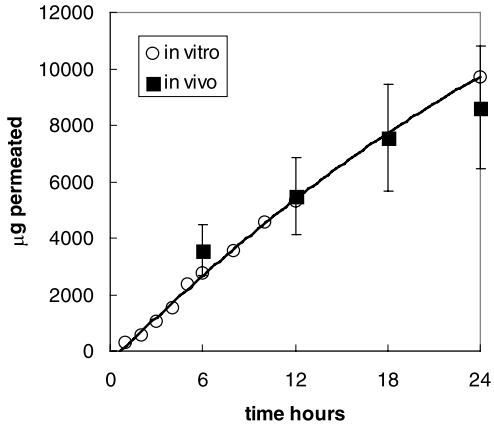


Fig. 15. A comparison of the permeation of nitroglycerin from a transdermal patch as measured both in vivo and in vitro. Data adapted from Hadgraft et al. (1993).

in vitro and in vivo data. An example of this is shown in Fig. 15 where the uptake of nitroglycerin from a patch was studied both in vitro and in vivo (Hadgraft et al., 1993).

Difficulties can arise in vitro when the permeant is subject to considerable skin metabolism. The skin is a metabolic organ with most of its activity in the viable tissue. However, even in the stratum corneum, there are enzymes present that are capable of breaking down proteins and substances such as cholesterol sulfate. It is possible to obtain fresh skin, mount it in a diffusion chamber and maintain viability over a short period of time but the methodology is complex (Collier et al., 1989).

It is also possible to make use of the metabolic activity and design prodrugs. These can be designed so that they have optimum physicochemical properties for skin absorption and are broken down at the site of action by endogenous enzymes (Martin et al., 1987).

12. Delivery of biotech drugs

There has been considerable interest in the delivery of peptides and proteins into and through the skin. Since these chemical entities are large and charged, there are considerable problems in getting them into the skin. The process that has attracted most attention is that of iontophoresis, where a small electric current is used to 'force' the ions through the skin. The skin is permselective and it is easier to deliver cationic drugs using iontophoresis. There has been research in passive delivery strategies and small amounts of oligonucleotides can be delivered if appropriate solvents are used (Nolen et al., 1994). This has been confirmed in our laboratories using a fluorescently labelled 12-mer oligonucleotides (Campbell et al., 1999). The 12-mer could be detected in lower layers of the skin using fluorescence microscopy but the amounts in a conventional Franz diffusion cell experiment were too low to be detected using a HPLC assay.

DNA has been delivered using a complex with DOTAP (Alexander and Akhurst, 1995; Birchall et al., 2000; Ramsay et al., 2000). The resulting complexes are quite large and the mechanisms of delivery through the skin are unclear. There are reports in the literature of delivery using flexible liposomes (Transfersomes). These can be used to deliver quantities of insulin large enough to invoke lowering of blood glucose levels (Cevc et al., 1995). Their mechanism of entry through the stratum corneum is also unclear but it is possible that they enter through defects in the intercellular lipids under the influence of the water concentration gradient that exists across the skin. Since the surface of the particle is highly charged, the energy of hydration is sufficient to 'pull' the deformable liposomes through the skin. The insulin is associated with the Transfersomes and is therefore delivered. A similar process may occur with the DNA complexes although direct entry via the hair follicles cannot be ruled out.

Other physical techniques of permeation enhancement include electroporation (Vanbever and Preat, 1995), ultra sound (McElnay et al., 1993), and physically blasting particles through the skin (Sarphie et al., 1993).

13. Conclusions and future

The process of skin permeation is complex but it is possible to identify the major physicochemical determinants that control the absorption process. Now that these are beginning to be

understood at a molecular level it is possible to make rational decisions about the design and formulation of topical and transdermal drugs. Formulation design, particularly for topical rather than transdermal patches, is complicated by the fact that the composition changes as the formulation is rubbed into the skin. Volatile components can evaporate and small solvent molecules will themselves be absorbed at an appreciable rate. It is important to make rational design strategies based on the formulation itself and the residual phase that will be left on the skin surface.

The understanding of the barrier properties of the skin has benefited from advances in the various biophysical techniques that have been used recently. Spectroscopic techniques have allowed an investigation of the molecular interactions that occur within the skin. Stray field NMR is being used to monitor water profiles in the various strata of the skin in vitro and in vivo. An example of water transferring out of a sample of skin is shown in Fig. 16. The NMR generated image reveals both the amount of water and its spatial distribution. As water is lost the signal decreases and also the membrane can be seen to be decreasing in thickness.

The research into iontophoresis and also the associated electro-osmosis has led to developments in non-invasive drug monitoring. Considerable effort has been concentrated on the development of a non-invasive method for following glucose levels in the plasma, which would have obvious benefits for diabetic patients (Garg et al., 1999). Once the technique is accepted there are many developments that could be made to measure other levels in the plasma. It may be possible to develop feedback drug delivery systems that monitor biochemical markers and deliver a drug in response to the perceived need. It may also be possible to develop these approaches to examine drug levels in the underlying skin tissue after drugs have been applied topically. One of the big problems at the moment is to relate changes in topical formulations to the levels of drug in the underlying skin tissue. Developments have been made using invasive techniques such as microdialysis (Anderson et al., 1992) but it may be possible to use non-invasive procedures as well. Examples with potential are optothermal techniques (Bindra et al., 1994; Xiao and Imhof, 1998), photoacoustic spectroscopy (Hanh et al., 2000) and possibly developments of stray field NMR monitoring nuclei other than the protons exemplified in Fig. 16.

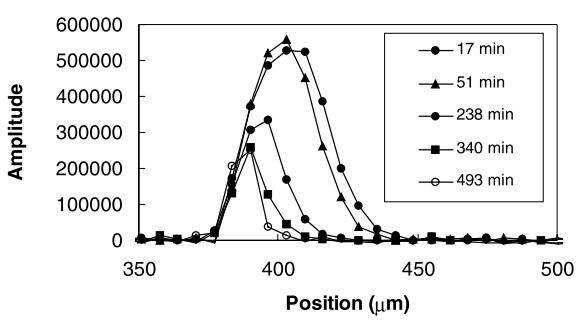


Fig. 16. The loss of water from hydrated skin as a function of time and position, as shown by stray field NMR. The edge of the stratum corneum is located at approximately 380 μm on the x-axis. Profiles can be obtained at closer time intervals than shown and therefore a more accurate kinetic analysis can be conducted on the data. Data adapted from Dias (2001).

In the late 1970s and early 1980s there was optimism about delivering many drugs using the transdermal route. Skin is the final frontier and it imposes many constraints on the numbers of drugs that can be delivered. However, advances in research have given us ways of monitoring glucose levels in the blood and also spraying drugs onto the skin so that they can be successfully delivered. Examples quoted in the literature include the sex hormones (Finnin and Morgan, 1999; Morgan et al., 1998). Fig. 17 shows an example of a 'state of the art' glucose-monitoring device.

Substantial problems do exist in transdermal delivery; the barrier property of the skin is one of these. Another very important issue is that of skin toxicity. Many drugs have been examined and shown to pass through the skin in sufficient quantity to produce a therapeutic effect, however, in vivo they fail due to adverse effects in the skin. There are probably ways of circumventing the irritant and allergic responses and their development will probably come early in the new millennium as a result of biotechnology advances.

It is interesting that nearly 50 years ago my father wrote, 'Maximal percutaneous absorption

Fig. 17. The GlucoWatch Biographer from Cygnus (www.cygn.com).

occurs when the medicament combines lipoid solubility with a moderate solubility in water' (Hadgraft and Somers, 1956). The advances in techniques since then have provided us with an insight into the underlying mechanisms of skin permeation. However, far more effective topical delivery systems could be produced if more attention was paid to the underlying physical chemistry involved. Rather than deliver 1–2% of the active drug it would be relatively easy to increase this by an order of magnitude and reduce the amount of drug applied accordingly. This would have obvious patient and environmental benefits.

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References

Albery, W.J., Guy, R.H., Hadgraft, J., 1983. Percutaneous absorption: transport in the dermis. Int. J. Pharm. 15, 125–148.

Albery, W.J., Hadgraft, J., 1979a. Percutaneous absorption: in vivo experiments. J. Pharm. Pharmacol. 31, 140–147.

Albery, W.J., Hadgraft, J., 1979b. Percutaneous absorption: theoretical description. J. Pharm. Pharmacol. 31, 129–139.

Alexander, M.Y., Akhurst, R.J., 1995. Liposome-mediated gene-transfer and expression via the skin. Human Molecular Genetics 4, 2279–2285.

Anderson, C., Andersson, T., Andersson, R.G., 1992. In vivo microdialysis estimation of histamine in human skin. Skin Pharmacol. 5, 177–183.

Azimi, N.A., Spencer, I.S., Potts, R.O., Lyttle, F.E., Chen, D.A., 1992. Fluorescence spectroscopic evaluation of the fluidity gradient in stratum-corneum lipids. J. Invest. Dermatol. 98, 641.

Beastall, J., Guy, R.H., Hadgraft, J., Wilding, I., 1986. The influence of urea on percutaneous-absorption. Pharmaceut. Res. 3, 294–297.

Bindra, R.M.S., Imhof, R.E., Eccleston, G.M., 1994. In-vivo optothermal measurement of epidermal thickness. J. Phys. IV 4, 445–448.

Birchall, J.C., Marichal, C., Campbell, L., Alwan, A., Hadgraft, J., Gumbleton, M., 2000. Gene expression in an intact ex-vivo skin tissue model following percutaneous delivery of cationic liposome–plasmid DNA complexes. Int. J. Pharm. 197, 233–238.

Bodde, H.E., Kruithof, M.A., Brussee, J., Koerten, H.K., 1989. Visualisation of normal and enhanced $HgCl_2$ transport through human skin in vitro. Int. J. Pharm. 53, 13–24.

Bodde, H.E., Hoogstraate, A.J., Verhoef, J.C., 1990. Structure-dependent enhancement by azones of peptide (Dgavp) transport through human stratum-corneum. J. Invest. Dermatol. 95, 463–463.

Bommannan, D., Potts, R.O., Guy, R.H., 1991. Examination of the effect of ethanol on human stratum-corneum in vivo using infrared-spectroscopy. J. Control. Release 16, 299–304.

Campbell, L., Hadgraft, J., Gumbleton, M., 1999. Unpublished results.

Cevc, G., Schatzlein, A., Blume, G., 1995. Transdermal drug carriers — basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. J. Control. Release 36, 3–16.

Coldman, M.F., Poulsen, B.J., Higuchi, T., 1969. Enhancement of percutaneous absorption by the use of volatile: nonvolatile systems as vehicles. J. Pharm. Sci. 58, 1098–1102.

Collier, S.W., Sheikh, N.M., Sakr, A., Lichtin, J.L., Stewart, R.F., Bronaugh, R.L., 1989. Maintenance of skin viability during in vitro percutaneous absorption–metabolism studies. Toxicol. Appl. Pharmacol. 99, 522–533.

Cross, S.E., Pugh, W.J., Hadgraft, J., Roberts, M.S., in press. Probing the effect of vehicles on topical delivery: understanding the basic relationship between solvent and solute penetration kinetics using silicone membranes. Pharm. Res.

Davis, A.F., Hadgraft, J., 1991. Effect of supersaturation on membrane transport. 1. Hydrocortisone acetate. Int. J. Pharm. 76, 1–8.

Degim, T., Hadgraft, J., Pugh, W.J., 1995. Modelling the transdermal delivery of non-steroidal anti-inflammatory agents. Proc. Int. Symp. Control. Release Bioact. Mater. 22, 652–653.

Dias, M.M., 2001. Facilitated Percutaneous Penetration. Cardiff University.

Feldmann, R.J., Maibach, H.I., 1967. Regional variation in percutaneous penetration of ^{14}C cortisol in man. J. Invest. Dermatol. 48, 181–183.

Finnin, B.C., Morgan, T.M., 1999. Transdermal penetration enhancers: Applications, limitations, and potential. J. Pharm. Sci. 88, 955–958.

Garg, S.K., Potts, R.O., Ackerman, N.R., Fermi, S.J., Tamada, J.A., Chase, H.P., 1999. Correlation at fingerstick blood glucose measurements with GlucoWatch biographer glucose results in young subjects with type 1 diabetes. Diabetes Care 22, 1708–1714.

Gay, C.L., Murphy, T.M., Hadgraft, J., Kellaway, I.W., Evans, J.C., Rowlands, C.C., 1989. An electron spin resonance study of skin penetration enhancers. Int. J. Pharm. 49, 39–45.

Guy, R.H., Hadgraft, J., 1983. Physicochemical interpretation of the pharmacokinetics of percutaneous-absorption. J. Pharmacokinet. BioPharm. 11, 189–203.

Guy, R.H., Hadgraft, J. 1985a. Kinetic-analysis of transdermal nitroglycerin delivery. Pharm. Res. 5, 206–211.

Guy, R.H., Hadgraft, J., 1985b. Pharmacokinetic interpretation of the plasma-levels of clonidine following transdermal delivery. J. Pharm. Sci. 74, 1016–1018.

Guy, R.H., Hadgraft, J., 1986. Interpretation and prediction of the kinetics of transdermal drug delivery-estradiol, hyoscine and timolol. Int. J. Pharm. 32, 159–163.

Guy, R.H., Hadgraft, J., Maibach, H.I., 1982. A pharmacokinetic model for percutaneous-absorption. Int. J. Pharm. 11, 119–129.

Hadgraft, J.W., Somers, G.F., 1956. Review article percutaneous absorption. J. Pharm. Pharmacol. 8, 625–634.

Hadgraft, J., Valenta, C., 2000. PH, pK(a) and dermal delivery. Int. J. Pharm. 200, 243–247.

Hadgraft, J., Hadgraft, J.W., Sarkany, I., 1973. The effect of thermodynamic activity on the percutaneous absorption of methyl nicotinate from water glycerol mixtures. J. Pharm. Pharmacol. 25, 122P–123P.

Hadgraft, J., Beutner, D., Wolff, H.M., 1993. In vivo–in vitro comparisons in the transdermal delivery of nitroglycerin. Int. J. Pharm. 89, R1–R4.

Hadgraft, J., Peck, J., Williams, D.G., Pugh, W.J., Allan, G., 1996. Mechanisms of action of skin penetration enhancers retarders — Azone and Analogs. Int. J. Pharm. 141, 17–25.

Hadgraft, J., Du Plessis, J., Goosen, C., 2000. The selection of non-steroidal anti-inflammatory agents for dermal delivery. Int. J. Pharm. 207, 31–37.

Hanh, B.D., Neubert, R.H.H., Wartewig, S., Christ, A., Hentzsch, C., 2000. Drug penetration as studied by noninvasive methods: Fourier transform infrared-attenuated total reflection, Fourier transform infrared, and ultraviolet photoacoustic spectroscopy. J. Pharm. Sci. 89, 1106–1113.

Harrison, J.E., Groundwater, P.W., Brain, K.R., Hadgraft, J., 1996a. Azone(R) induced fluidity in human stratum corneum. A Fourier transform infrared spectroscopy investigation using the analogue. J. Control. Release 41, 283–290.

Harrison, J.E., Watkinson, A.C., Green, D.M., Hadgraft, J., Brain, K., 1996b. The relative effect of azone(R) and transcutol(R) on permeant diffusivity and solubility in human stratum-corneum. Pharm. Res. 13, 542–546.

Henmi, T., Fujii, M., Kikuchi, K., Yamanobe, N., Matsumoto, M., 1994. Application of an oily gel formed by hydrogenated soybean phospholipids as a percutaneous absorption-type ointment base. Chem. Pharm. Bull. 42, 651–655.

Howes, D., Guy, R., Hadgraft, J., Heylings, J., Hoeck, U., Kemper, F., et al., 1996. Methods for assessing percutaneous absorption — the report and recommendations of ECVAM workshop 13. Atla-Alternatives Laboratory Animals 24, 81–106.

Iervolino, M., Raghavan, S.L., Hadgraft, J., 2000. Membrane penetration enhancement of ibuprofen using supersaturation. Int. J. Pharm. 198, 229–238.

Kemken, J., Ziegler, A., Muller, B.W., 1992. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. Pharm. Res. 9, 554–558.

Kondo, S., Mizuno, T., Sugimoto, I., 1988. Effects of penetration enhancers on percutaneous absorption of nifedipine. Comparison between Deet and Azone. J. Pharmacobio-Dynamics 11, 88–94.

Lewis, D., Hadgraft, J., 1990. Mixed monolayers of dipalmitoylphosphatidylcholine with Azone or oleic acid at the air–water interface. Int. J. Pharm. 65, 211–218.

Lewis, D., Hadgraft, J., Bodde, H.E., 1991. Mixed monolayer studies of DPPC with *N*-aklyl-aza-cycloheptanones at the air–water interface. Prediction Percutaenous Penetration 355, 364.

Lippold, B.C., Schneemann, H., 1984. The influence of vehicles on the local bioavailability of betamethasone-17-benzoate from solution-type and suspension-type ointments. Int. J. Pharm. 22, 31–43.

Liron, Z., Cohen, S., 1984. Percutaneous absorption of alkanoic acids II: Application of regular solution theory. J. Pharm. Sci. 73, 538–542.

Marks, R., Dykes, P.J., Gordon, J., Hanlan, G., Davis, A.F., 1992. Percutaneous Penetration from a Low Dose Supersaturated Hydrocortisone Acetate Formulation. British Society of Investigative Dermatology, Sheffield.

Martin, R.J., Denyer, S.P., Hadgraft, J., 1987. Skin metabolism of topically applied compounds. Int. J. Pharm. 39, 23–32.

McElnay, J.C., Benson, H.A.E., Harland, R., Hadgraft, J., 1993. Phonophoresis of methyl nicotinate — a preliminary-study to elucidate the mechanism of action. Pharm. Res. 10, 1726–1731.

Michaels, A.S., Chandrasekaran, S.K., Shaw, J.E., 1975. Drug permeation through human skin: Theory and in vitro experimental measurement. Am. Inst. Chem. Eng. J. 21, 985–996.

Morgan, T.M., Reed, B.L., Finnin, B.C., 1998. Enhanced skin permeation of sex hormones with novel topical spray vehicles. J. Pharm. Sci. 87, 1213–1218.

Nemanic, M.K., Elias, P.M., 1980. In situ precipitation: A novel cyotchemical technique for visualization of permeability pathways in mammalian stratum corneum. J. Histochem. Cytochem. 28, 573–578.

Nolen, H.W., Catz, P., Friend, D.R., 1994. Percutaneous penetration of methyl phosphonate antisense oligonucleotides. Int. J. Pharm. 107, 169–177.

Ongpipattanakul, B., Burnette, R.R., Potts, R.O., Francoeur, M.L., 1991. Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm. Res. 8, 350–354.

Pellett, M.A., Davis, A.F., Hadgraft, J., 1994. Effect of supersaturation on membrane transport: 2. Piroxicam. Int. J. Pharm. 111, 1–6.

Pellett, M.A., Roberts, M.S., Hadgraft, J., 1997a. Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique. Int. J. Pharm. 151, 91–98.

Pellett, M.A., Watkinson, A.C., Brain, K.R., Hadgraft, J., 1997b. Diffusion of flurbiprofen across human stratum corneum using synergistic methods of enhancement. In: Brain, K.R., James, V.J., Walters, K.A. (Eds.), Perspectives in Percutaneous Penetration. STS Publishing, Cardiff, p. 86.

Potts, R.O., Francoeur, M.L., 1991. The influence of stratum-corneum morphology on water permeability. J. Invest. Dermatol. 96, 495–499.

Potts, R.O., Guy, R.H., 1992. Predicting skin permeability. Pharm. Res. 9, 663–669.

Pugh, W.J., Degim, I.T., Hadgraft, J., 2000. Epidermal permeability-penetrant structure relationships: 4, QSAR of permeant diffusion across human stratum corneum in terms of molecular weight, H-bonding and electronic charge. Int. J. Pharm. 197, 203–211.

Pugh, W.J., Roberts, M.S., Hadgraft, J., 1996. Epidermal permeability-penetrant structure relationships. 3. The effect of hydrogen-bonding interactions and molecular-size on diffusion across the stratum-corneum. Int. J. Pharm. 138, 149–165.

Raghavan, S.L., Trividic, A., Davis, A.F., Hadgraft, J., 2000. Effect of cellulose polymers on supersaturation and in vitro membrane transport of hydrocortisone acetate. Int. J. Pharm. 193, 231–237.

Raghavan, S.L., Kieper, B., Davis, A.F., Kazarian, S.G., Hadgraft, J., 2001. Membrane transport of hydrocortisone acetate from supersaturated solutions; the role of polymers. Int. J. Pharm. 221, 95–105.

Ramsay, E., Hadgraft, J., Birchall, J., Gumbleton, M., 2000. Examination of the biophysical interaction between plasmid DNA and the polycations, polylysine and polyornithine, as a basis for their differential gene transfection in-vitro. Int. J. Pharm. 210, 97–107.

Roberts, M.S., Pugh, W.J., Hadgraft, J., 1996. Epidermal permeability: Penetrant structure relationships. 2. The effect of H-bonding groups in penetrants on their diffusion through the stratum corneum. Int. J. Pharm. 132, 23–32.

Roberts, M.S., Pugh, W.J., Hadgraft, J., Watkinson, A.C., 1995. Epidermal permeability penetrant structure relationships.1. An analysis of methods of predicting penetration of monofunctional solutes from aqueous solutions. Int. J. Pharm. 126, 219–233.

Rosado, C.B.F., 2000. Formulation Strategies in Transdermal Delivery. Cardiff University.

Sarphie, D.F., Bellhouse, B.J., Alexander, Y., Brain, K.R., Hadgraft, J., James, V., et al., 1993. Gas propulsion of microprojectiles for the transdermal delivery of powder form drugs. In: Brain, K.R., James, V.J., Walters, K.A. (Eds.), Prediction of Percutaneous Penetration. STS Publishing, Cardiff, pp. 360–368.

Skelly, J.P., Shah, V.P., Maibach, H.I., Guy, R.H., Wester, R.C., Flynn, G., et al., 1987. FDA and AAPS report of the workshop on principles and practices of in vitro percutaneous penetration studies: Relevance to bioavailability and bioequivalence. Pharm. Res. 4, 265–267.

Smith, J.C., Irwin, W.J., 2000. Ionisation and the effect of absorption enhancers on transport of salicylic acid through silastic rubber and human skin. Int. J. Pharm. 210, 69–82.

Twist, J.N., Zatz, J.L., 1986. Influence of solvents on paraben permeation through idealized skin model membranes. J. Soc. Cosmetic Chem. 37, 429–444.

Valenta, C., Cladera, J., O'Shea, P., Hadgraft, J., 2001. Effect of phloretin on the percutaneous absorption of lignocaine across human skin. J. Pharm. Sci. 90, 485–492.

Valvani, S.C., Yalkowsky, S.H., Roseman, T.J., 1981. Solubility and partitioning. 4. Aqueous solubility and octanol–water partition-coefficients of liquid non-electrolytes. J. Pharm. Sci. 70, 502–507.

Vanbever, R., Preat, V., 1995. Factors affecting transdermal delivery of metoprolol by electroporation. Bioelectrochem. Bioenergetics 38, 223–228.

Watkinson, A.C., Hadgraft, J., 1992. Small angle neutron scattering (SANS) studies on DPPC/Azone® multilamellar systems. In: Hadgraft, J., Kellaway, I.W., Parr, G.D. (Eds.), Proceedings of the 1st Annual UKaps Conference. STS Publishing, Cardiff 16 pp.

Watkinson, A.C., Bunge, A.L., Hadgraft, J., Naik, A., 1992. Computer simulation of penetrant concentration-depth profiles in the stratum corneum. Int. J. Pharm. 87, 175–182.

Watkinson, A.C., Hadgraft, J., Street, P.R., Richards, R.W., 1997. Neutrons, surfaces, and skin. In: Potts, R.O., Guy, R.H. (Eds.), Mechanisms of Transdermal Drug delivery. Marcel Dekker, New York, pp. 231–265.

Xiao, P., Imhof, R.E., 1998. Optothermal measurement of water distribution within the stratum corneum. Skin Bioeng. 26, 48–60.

Yano, T., Nakagawa, A., Tsuji, M., Noda, K., 1986. Skin permeability of various non-steroidal anti-inflammatory drugs in man. Life Sci. 39, 1043–1050.