Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 291–299
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Review article

Skin deep

Jonathan Hadgraft*

Medway Sciences, University of Greenwich, Kent, UK

Received 12 January 2004; accepted in revised form 9 February 2004

Available online 15 April 2004

Abstract

Over the past 30 or so years there has been a considerable advance in our knowledge of the mechanisms of skin permeation. This has largely been brought about by the development of sophisticated biophysical techniques and increased computing powers. The advanced technology has clearly provided indications, at a molecular level, about routes of permeation and how the barrier function can be modulated by excipients with which actives are formulated. This publication reviews some of the advances that have been made and mathematical models that have been constructed to predict percutaneous penetration and transdermal delivery. The models also indicate the various enhancement strategies that can be used in dermal penetration. In the past, it has been difficult to identify precise mechanisms of action of the different classes of enhancer but a combination of appropriate biophysical techniques, mathematical modelling and chemometric analysis can help identify the contributing processes. The models can also be used to indicate rate control in transdermal delivery, whether it is in the applied delivery device or in the skin.

Keywords: Skin penetration; Enhancers; Transdermal; Percutaneous absorption

1. Introduction

The skin is our largest organ and forms a fascinating and unique interface between the outside world and us. It is accessible and it is relatively easy to interrogate it in vivo. Over the past decades research groups with whom I have been associated have been examining dermal tissue using a range of sophisticated and sensitive biophysical techniques. As a result of these investigations its properties are being revealed in increasing detail. As we learn more about the skin's structure its complexity becomes more apparent and its unique properties revealed [1]. In the context of drug delivery the outer layer, the stratum corneum, is the most important as this serves as the barrier to both the ingress of xenobiotics and the egress of water. When the barrier function is severely compromised, as in the case of generalised psoriasis, up to 6 l of water may be lost per day through the skin alone [2]. The epidermis also serves as a means of reducing the damaging impact of solar UV radiation. The significance of the stratum corneum cannot be overstated and any breach in its integrity can have profound effects. These also include invasion by possible pathogens with additional defence from 'defensins' and 'toll receptors' in the epidermis [3,4].

Increased modern hygiene results in frequent washing of infants and it is interesting to note that there has been a significance increase in the prevalence of eczema over the past decades with up to 25% incidence in the current infant population [5]. In fact dermatological disorders are more commonplace than may be anticipated with 60% of the population suffering. Of these ~25% should receive medical intervention and this results in ~15% of a general practitioner's workload. The effects that dermatological disorders have on quality of life is often underestimated and not fully understood [6–8]. For these reasons alone it is important that the barrier properties are investigated. With a mechanistic and physicochemical knowledge of these it is possible to design more effective topical medicines, taking into consideration both active agent and the formulation.

The last quarter of a century has also seen an increase in the numbers of transdermal systems. The promise of the early years has not been fulfilled but it is still a substantial market. The reason for this lack of fulfilment can be explained partly as a result of the excellent barrier properties

* Present address: The School of Pharamacy, University of London, 29-39 Brunswick Square, London, WCIN 1AX, UK. Tel.: +44 207 753 5800; fax: +44 8701 659275.

E-mail address: jonathan.hadgraft@btinternet.com (J. Hadgraft).

0939-6411/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2004.03.002

of the skin. It is also because of the way in which the inner regions of the skin 'sees' the active and produces irritant or allergic responses. This is related to the ability of the active to permeate and also the sensitivity with which the defence mechanisms elicit the unwanted response. Permeation will be a function of the physicochemical properties of the active and their role needs to be understood in light of the mechanisms of penetration across the stratum corneum.

2. Route of penetration

There has been much debate over the past decades on the route of penetration but experimental evidence suggests that, under normal circumstances, the predominant route is through the intercellular spaces [9–13]. The diffusional pathlength is therefore much longer than the simple thickness of the stratum corneum (~20 μm) and has been estimated as long as 500 μm. Importantly, the intercellular spaces contain structured lipids and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before it reaches the junction between the stratum corneum and the viable epidermis. The nature of the barrier is thus very heterogeneous and it is perhaps surprising that diffusion through it can be described by simple solutions to Fick's laws of diffusion [14].

3. Physicochemical parameters important in dermal absorption

The most basic diffusion equation is Fick's 1st law which describes steady state flux per unit area (J) in terms of the partition of the permeant between the skin and the applied formulation (K), its diffusion coefficient (D) in the intercellular channels of diffusional pathlength (h), the applied concentration of the permeant in the vehicle (c_{app}) and the concentration of the permeant in the receptor phase (c_{rec})

$$J = KD(c_{app} - c_{rec})/h \quad (1)$$

In most circumstances $c_{rec} \ll c_{app}$ and Eq. (1) is often simplified to

$$J = k_p c_{app} \quad (2)$$

where $k_p (= KD/h)$ is the permeability coefficient. This parameter (from an aqueous donor phase) may be estimated by an empirical relationship described by Potts and Guy [15]

$$\log[k_p/(\mathrm{cm\,h^{-1}})] = -2.7 + 0.71 \log K_{oct} - 0.0061\mathrm{MW} \quad (3)$$

where K_{oct} is the octanol water partition coefficient and MW the molecular weight.

The maximum flux for a compound is when c_{app} is equal to the solubility. Simple inspection of the equations shows that the important physicochemical properties are partition coefficient, diffusion coefficient, and solubility. Large molecules will tend to diffuse slowly, hence the MW term in Eq. (3), molecules with good solubility in both oils and water will permeate well. These tend to be compounds with low melting point. Eq. (1) or (3) would tend to indicate that a high partition coefficient will favour a high flux, however, large values of K tend to produce molecules that have poor solubility and in general molecules with a $\log K_{oct} \sim 1-3$ have the optimum partition behaviour. This also fits with the notion, stated nearly half a century ago, that a balanced solubility in both oils and water is desirable [16].

Many permeants are weak acids or weak bases. Permeation will depend on the degree of ionisation and how ionisation influences the solubility in the applied phase and its partition into the skin. There have been few studies investigating this but those that have been conducted indicate that it may be beneficial to apply the drug in its ionised form, in which state it will be much more soluble but with a lower permeability coefficient. Using information from two permeants, ibuprofen and lidocaine, there is an indication that equations such as Eq. (3) exist for ionisable species where $\log P$ is replaced by $\log D$ [17]. There are insufficient data to estimate accurately all the coefficients in Eq. (3) for ionisable materials. One of the problems involved in interpreting permeation data of ionised compounds is that the species that permeate will be a composite of the free acid (or base) of the ionised material and ion pairs that can exist with counter ions present either in the formulation or in the skin. Ion-pair mechanisms have been proposed for permeation enhancement [18–21]. It is also possible to modify the properties of the skin with agents such as phloretin or ketocholestanol. These influence the polarity properties of the lipid bilayers and can hence aid the permeation of charged molecules [22,23].

4. Model membranes

Human skin is often difficult to obtain and many studies have been conducted using surrogates. Liquid membranes have been considered, but their structural simplicity cannot provide an adequate model of the heterogeneous nature of the intercellular channels [24]. They can be made more realistic by the incorporation of lipids, which will form structured domains but even so there is little evidence to show that they can be used with confidence as models for human skin. Polymeric systems have also been considered with the most simple being silicone membranes. Under limited conditions, correlations can be obtained between transfer rates through silicone and skin but these are when the chemical potential is the dominant effect controlling transport through the skin [25]. More complex membranes such as Carbosil have also generated interesting correlations [26]. Animal skin has been used extensively and the most reliable tissue appears to be from pig ear. Lastly advances

have been made using cultured skin but this tends to have more limited barrier function than the genuine tissue. This generally means that the 'gold star' studies are in vivo human followed by in vitro human. Important issues concerning these studies are experimental design and data interpretation.

5. Mathematical models and data interpretation

In one of the seminal papers in transdermal delivery, Michaels and co-workers [27] described the skin as a 'bricks and mortar' structure. This structure was subsequently used in data analysis of the in vivo permeation of esters of nicotinic acid. The results indicated that the major route of permeation was through the intercellular channels. This observation was confirmed by visualisation of a number of permeants such as butanol [10], mercury chloride [11] and estradiol [28] in the intercellular channels. It is also suggested in work by Rougier and co-workers [29] who demonstrated that skin sites where the corneocyte size is large tend to be the least permeable. Also the thermodynamics of water transfer suggest a very tortuous pathway for permeation [13]. However, the route is still under debate.

The solutions to the diffusion equations in the publications by Albery and Hadgraft [30] include both non-steady and steady state regions. Often in vitro diffusion experiments are conducted in which the non-steady state region is ignored, steady state is assumed and permeability coefficients calculated using Eq. (2) from the steady state flux J. Extrapolation of the linear portion of the diffusion profile to the abscissa is unreliable because of the inherent biovariability in skin permeability. An alternative analysis is to use a curve fitting procedure [31]. This provides values of $\alpha(= D/h^2)$ and $\beta(= KD/h)$. If these values can be obtained with confidence it is possible to use them to indicate what effects a formulation has on the barrier function of the skin. For example, if penetration enhancers are included they can influence either D or K or both. This will be manifest in changes to the values of α and β. An interpretation of this type has been used to show how different commercial formulations of ibuprofen may influence the barrier properties of the stratum corneum and hence delivery [32]. Accurate values of β rely on data in the non-steady state region. Often data at early times are sparse because of the sampling techniques used in conventional Franz type diffusion cells. This can be improved using continuous spectroscopic sampling as in a novel diffusion cell that is directly mounted in a diode array UV detector [33].

There is a substantial collection of permeability coefficient data in the literature. This formed the basis for formulating Eq. (3). Since the data have been collected from a variety of sources it is possible that some of the results are anomalous and it is sometimes difficult to have confidence in some of the QSAR interpretations that have been derived from incorrect data [34].

Analysis of subsets of the data reveals that 'ab initio' calculations are possible in which molecular fragments are assigned specific values that contribute to the permeability coefficient [35]. Since the permeability coefficient is a function of both K and D it is possible to separate the two values and show that the diffusional parameter is related to hydrogen bonding effects [36–38]. Because hydrogen bonding can be related to a partial charge interaction, molecular graphics software has also been used to predict the partial charges around a molecule. These are also related to diffusion in the skin lipids [39]. These two factors would tend to indicate that diffusion is sensitive to the interaction between the permeant and the polar head groups of the lipids in the intercellular channels. The role of hydrogen bonding in membrane permeation has been considered [40,41].

It is also possible to use subsets of the permeability data as training values in artificial neural networks (ANNs). There are sufficient data to test the ANN and this approach may prove valuable in the future, especially if it can be used in conjunction with formulation design [42].

Molecular graphics have also been useful in considering the structure of penetration enhancers such as Azone analogues. Partial charge calculations have provided indications about the interaction of the polar region of the ring structures in the Azone derivatives and ceramide head groups [43]. The lower partial charge on the sulphur analogue is probably responsible for its inactivity as an enhancer. The charges on opposite sides of the ring in N-0915 have the potential to 'attract' adjacent head groups together. This makes the alkyl chains in proximity to the head groups more rigid and permeation is retarded (see Fig. 1).

Molecular simulations are complex but as computing power increases and packages become more sophisticated it is possible to get reasonable representations of the bilayer lipid arrays [44]. It is also important to remember that formulation design plays an important role in overall ability to promote drug transfer across the skin. Molecular simulations can also be used to optimise polymer choice in transdermal systems. Modern transdermal systems are as manufactured so that they are as small and as thin as possible with a concomitant high drug load. The way in which the drug interacts with the polymer matrix can be modelled and the polymer chosen so that it can accommodate high loading of the active [45]. This is exemplified in the delivery of nitroglycerin (GTN) where, for example, the original Deponit system was 300 μm thick whereas Deponit NT is only 90 μm.

The ultimate ultrathin transdermal system is when the drug is sprayed in an appropriate solvent onto the skin. This has been achieved in the metered dose transdermal spray (MDTS) for a variety of drugs [46–48]. The loading dose would suggest an initial applied thickness of 20 μm which is

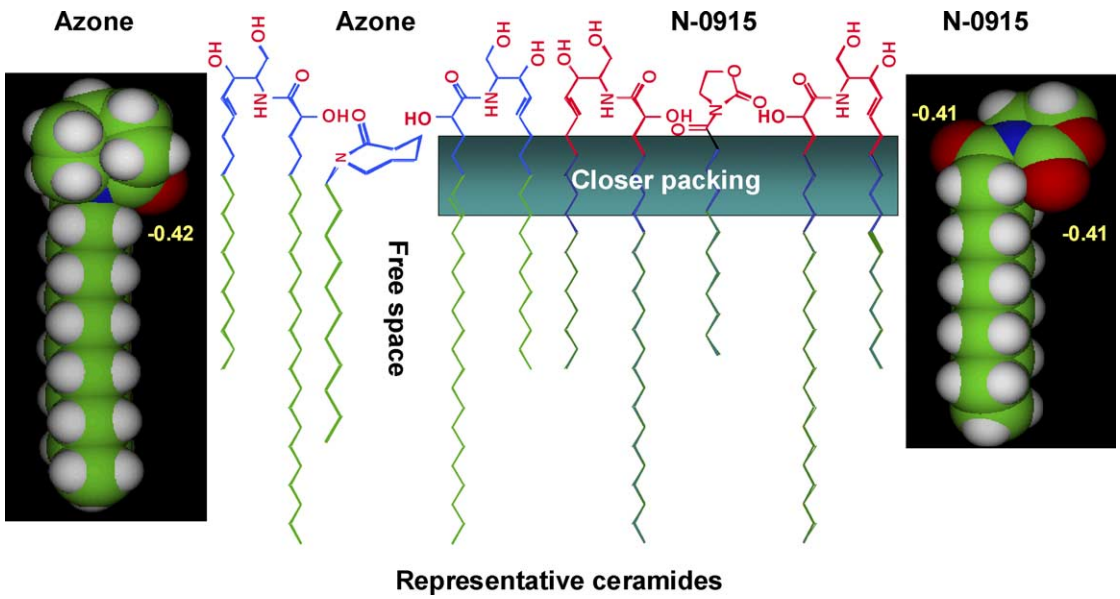

Fig. 1. A schematic representation of Azone and N-0915 interacting with the ceramides in the intercellular spaces.

comparable to a topically applied cream. This means that the amount of drug is limited and significant depletion occurs during the application time. Depletion can be modelled and the results indicate that there is significant attenuation in the partition of the drug into the outer layers of the skin. For example, if the partition coefficient increases by a factor of 100, there will be less than a 2-fold increase in the concentration of the drug in the stratum corneum. This has implications in the selection of drugs both for spray technology and also in conventional topical delivery systems, such as gels, creams, ointments and lotions.

Modelling diffusion and partition from thin films also provides an insight into the concentration profiles within the stratum corneum. These can be measured by careful experimentation using skin stripping. Traditionally the total amount obtained in the skin strips has been equated to the bioavailability of a topical product. However, mathematical simulations question this premise [49]. With careful experimental design it is possible to deconvolve the data to give values of K and D. If these are known they can be used in diffusion equations to give an accurate description of a drug's bioavailability [50].

The influence that permeation enhancers have on K and D can be monitored using attenuated total reflectance-Fourier transform infra red (ATR-FTIR) spectroscopy. It is often very difficult to separate these factors but the IR analysis provides unequivocal information and has shown that enhancers such as Transcutol can improve the solubility properties of the skin to the permeant (i.e. it increases K) whereas Azone has little influence on K but improves D by disrupting the lipids in the intercellular channels of the stratum corneum [51]. In the initial studies, the data were interpreted by examination of a single IR band associated with the permeant. However, the spectrometer captures the entire spectrum and a chemometric deconvolution can be used to separate signals from the permeant, the formulation components and also the membrane. This type of analysis is under development but should provide information, at a molecular level, on the mechanism of skin permeation and the modulation of its barrier properties [52].

6. Enhancement strategies

As mentioned above there are various ways of enhancing permeation through the skin. Since the stratum corneum is such a good barrier this is necessary to improve bioavailability, but even though enhancement strategies have been developed over the years, topical delivery remains relatively inefficient. Examination of Eq. (1) shows that the variables that can be affected are c_{app}, K and D. In simplest terms, the applied concentration can be increased up to the solubility of the permeant. Thereafter increases in concentration should have no effect on permeation rate. Therefore, a 1% suspension should be equally effective as a 5% suspension [53]. The real driving force for diffusion is the chemical potential which will be identical for any given drug in suspension. This has been demonstrated in model experiments by Twist and Zatz [54] in which the authors applied saturated solutions of methyl paraben in various solvents to a silicone membrane. The applied concentration varied by over 100-fold but the flux remained the same. The invariant flux with applied concentration also demonstrated that the solvents selected had no effect on the barrier properties of the silicone.

It is possible, by the process of supersaturation, to produce solutions that have a high chemical potential but these are thermodynamically unstable [55,56]. Various techniques can be used to produce supersaturated states for example, solvent evaporation, mixing of two solvents

where the solubility in one is very much less than the other, e.g. piroxicam in water and propylene glycol, mixing of solutions of different pH where there is a large difference in solubility at the two pH's. Instantaneous crystallisation of the active can be prevented by the addition of anti-nucleant polymers. Stability can be conferred for hours, days or weeks depending on various factors such as degree of supersaturation, the polymer selected and its concentration. In general, only small amounts of polymer are required ($<1\%$), the mechanism of action is thought to be adsorption of the polymer onto the nuclei as they form and subsequent inhibition of their growth [57,58]. This technique can also be used in the controlled production of submicron particles [59]. The morphology of the crystals produced is often altered by the presence of the polymer; this may influence dissolution kinetics. The nucleation process is difficult to follow but it may be possible to gain more insight by following the small thermal changes that occur by using microcalorimetry. This technique has proved useful in assessing instability of topical products caused by either chemical degradation or crystallisation [60,61].

Delivery into and through the skin is directly related to the degree of supersaturation [62]. The main problem with this strategy is the inherent instability of the product and ideally the supersaturated states should be produced in situ. This is possible for solvent evaporation but for a binary solvent mixture a twin pack design is required. It is possible that the MDTS system, as mentioned above, produces a film on the skin surface in which the drug is supersaturated.

The other major ways to influence permeation are to include penetration enhancers that co-diffuse into the skin and alter the properties of the stratum corneum. They achieve this by either altering the lipid packing of the bilayers and hence influence D or change the solubility properties and hence influence K. Oleic acid (OA) intercalates into the lipids and appears to alter D [63,64]. FTIR studies using perdeuterated OA indicate that enhancer forms pools in the skin and the permeant diffuses faster either through these or through the defects between the pools of OA and the structured lipids of the skin [65]. The size of the pools has not been determined although thermal studies have suggested that they may be microscopic in size rather than comprising of a few molecules [66]. This contrasts with the penetration enhancer Azone, which appears to distribute itself homogeneously [67]. The difference between Azone and OA is possibly a result of the *cis* double bond in OA, which may allow more favourable packing with itself rather than the chains on the skin lipids, which do not have the *cis* double bond. In general, structures which disrupt the skin lipids tend to be 'surfactant' type in nature with polar headgroups and long alkyl chains. It must be remembered, however, that this class of molecule also tends to be associated with skin irritancy.

The other mode of action is provided by the 'solvent' type, typically these will permeate into the skin and improve the solubility of the permeant. Examples include propylene glycol, Transcutol and ethanol. However, care should be taken in interpreting enhancement data since solvents such as ethanol can also abstract skin lipids making the stratum corneum more permeable [68]. Separating the different enhancement effects is difficult but in some circumstances it can be resolved using appropriate biophysical techniques.

7. Biophysical techniques

Over the past decade there have been advances made in understanding diffusion mechanisms through the skin as a result of applying modern biophysical techniques. These have become more sensitive and sophisticated and have, as a result, provided a lot of information. One spectroscopic technique that has proved particularly useful is FTIR spectroscopy. As described above it has been used to investigate enhancer mechanisms. It can be used both in vitro, as above, and in vivo using surface reflectance (ATR) technology. As an illustration, representative perdeuterated solvents have been applied to the skin in vivo. The IR signal from the skin surface can be monitored and if measurements are repeated following tape stripping, the signals provide information deeper into the stratum corneum. Since deuterated solvents were used, the discrete signal from the C–D stretch shows how much solvent has entered the SC as a function of depth. The decrease in the C–H stretch shows any lipid extraction from the skin and changes in the C–H frequency demonstrate how the solvent changes the intercellular lipid fluidity (and hence diffusion). Model solvents such as hexanol and decanol have different effects, with the longer chain C_{10} solvent disrupting the skin lipids (shifting the C–H frequency) in direct proportion to the amount taken up (increase in C–D). Hexanol extracts the skin lipids whereas decanol does not [69]. Clearly there are substantially different mechanisms involved and it would be useful to examine these for substances of more pharmaceutical relevance [1]. The problem is that deuterated excipients are required and these are often not readily available and are expensive.

Dermatological disease states are often associated with changes in SC lipids and it may be possible to use ATR-FTIR to monitor disease state and treatment. There are limited data that show that the IR signals from psoriatic skin change after UV radiation [70]. A similar IR technique has been used for the examination of cancerous tissue although in this case it was not related directly to skin [71]. Since IR microscopy is available, it is also possible to image the skin surface and recent developments give resolution of a few microns [72]. This should reveal spatial differences on the skin surface and if these can be linked to disease state it may give indications of the extent of cancerous tissue with significant resolution.

Imaging is also possible using laser confocal microscopy. In theory, this can provide a depth profile of the permeant of

interest, however, attenuation of the signal means that it is difficult to relate the signal to an absolute concentration. The inability to obtain exact concentrations is also a limitation of the ATR-FTIR experiments detailed above. The membrane dipole nature of the skin can be influenced by ketocholestanol, this will affect the interaction of hydrophobic ions and peptides such as bacitracin. Confocal microscopy has shown that fluorescently labelled bacitracin has an enhanced permeation into the skin as a result of treating the skin with liposomes loaded with 6-ketocholestanol [73]. This confirms the fact that enhanced permeation of charged molecules can be influenced by changing the dipolar nature of the SC lipids. Imaging into the skin is also possible using nuclear magnetic resonance (NMR). In theory, a number of nuclei can be examined, e.g. fluorine, however, the dermal concentrations following topical application are very low and monitoring a permeant that contains a fluorine atom remains elusive with current sensitivity. Protons are much easier to detect and stray field NMR gives hydration profiles in the skin. This procedure can be applied both in vitro and in vivo and should be useful in assessing products that are claimed to hydrate the outer layers of the skin [74]. Attenuation of the signal is also a problem and has to be taken into account when deeper layers are being probed.

X-ray scattering, both small and wide angle shows structural features of the SC lipids such as the spacing between the lamellae [75]. Microscopy resolution is such that sophisticated techniques can now be used to visualise the lipid layers and confirm some of the distances found by scattering techniques [76]. Neutron scattering can also be used to probe skin samples and the interaction of enhancers with model lipid membranes representative of the stratum corneum lipids [77]. The results confirm the fact that oleic tends to distribute in pools. Using neutron reflectometry it is also possible to show that water transfers deeper into the alkyl chains of a model lipid when a small amount of OA is present. The implications are that the OA packs in such a way to disrupt the packing of the polar headgroups providing more disorder in the methylene groups immediately adjacent to the headgroups. The rigidity along the alkyl chains of the lipids can be examined using electron spin resonance and appropriately substituted spin probes such as doxyl stearic acid [78]. Disruption of the headgroup regions after the incorporation of enhancers such as OA and decylmethyl sulphoxide can be demonstrated.

Headgroup interactions can also be inferred using monolayers spread in a Langmuir trough. In this type of experiment, penetration enhancers such as Azone can be seen to ‘push’ the polar head groups of model skin lipids apart whereas penetration retarders such as N-0915 ‘pull’ the headgroups together [79,80]. This means that the methylene groups adjacent to the headgroups are less rigidly packed in the presence of Azone (*D* of the penetrant is increased) but more rigidly packed in the presence of N-0915 (*D* of the penetrant is reduced). These interactions are shown schematically in Fig. 1.

Changes in the ‘fluidity’ of the lipids have also been studied using differential scanning calorimetry. The characteristic transitions associated with lipid melting occur at a lowered temperature when the skin is treated with a penetration enhancer such as OA [81]. Novel silicone enhancers have been identified and screened using this technique [82].

8. In vivo evaluations

It is often difficult to measure the in vivo absorption of a topical product intended for local action. The amount absorbed are very low and non-invasive monitoring has yet to provide the sensitivity required. Relatively non-invasive techniques such as tape stripping are advancing [50] but there are still problems in data interpretation [49]. Despite the problem of low amount absorbed, a wealth of information has been gained using permeants such as the corticosteroids and esters of nicotinic acid, which provide noticeable physiological responses when they are absorbed locally through the skin. The responses are vasoconstriction and vasodilatation, respectively. The response from the nicotinates has been used to show the influence of chemical potential and the results confirm those given in the silicone experiments described previously [83]. The diffusion of the nicotinates has also inferred the tortuous pathway [9]. The radial spread of the response cannot be attributed solely to a slow diffusion mechanism, there must be a more rapid distribution from transport in the peripheral blood supply [84]. Enhancement mechanisms can also be suggested by analysing the time of onset of erythema as a function of applied concentration. It is possible to determine if the enhancer is altering *D* or *K* if appropriate solutions to non-steady state diffusion equations are used. In the case of urea as an enhancer it is the diffusion that appears to be altered [85]. The mechanisms of physical enhancement, such as ultrasound, can also be probed by investigating the erythema induced by the nicotinates. The more lipophilic the ester the less ultrasound improved absorption [86].

It is far easier to investigate the in vivo performance for transdermal delivery where significant blood levels can be measured. It is also possible to compare in vitro and in vivo data and demonstrate that in vitro experiments do provide a very good indication of in vivo performance. This can be seen in the case of GTN where in vitro results can be coupled with clearance data to give good estimates of blood levels [87]. In vitro data are also shown to be directly related to the residual amount of GTN in the patches that are applied in vivo. Comparisons of in vitro release rates into an inert sink and through excised skin can be used to show the relative control of the device and the skin to the delivery of the active [88]. For example NitroDur II has little influence on the overall rate of GTN permeation through skin

whereas for Deponit there is considerable control as a result of slow diffusion through the device. The skin has even less effect as a barrier in preterm infants but in vitro models can be used to predict in vivo performance provided the reduction in barrier function is taken into account. The modelling has been useful in predicting the blood levels of theophylline after transdermal delivery to preterm infants [89,90]. Other actives where the in vivo blood levels have been effectively modelled include clonidine [91], rolipram [92] and bupranolol [93]. It should also be remembered that, in the toxicological area, dermal risk analysis can be conducted successfully using this type of approach [94].

9. Conclusions

The past decades have given us a wealth of information about how molecules cross the skin. The barrier function does provide a formidable barrier and the use of physical techniques such as iontophoresis, electroporation and ultrasound will undoubtedly have a future impact on delivery of drugs into and through the skin. Likewise, physical techniques which involve breaching the barrier such as microneedles and microprojections will also find their niche. Interesting developments in non-invasive drug monitoring following the approaches of the GlucoWatch® G2™ Biographer can also be anticipated. However, these will only be possible with a fundamental understanding of the properties of the skin. It will be necessary to understand how the physicochemical properties of the penetrant impact on the transport rate. This will be achieved through ever sophisticated biophysical techniques which will undoubtedly become more refined in the future decades.

References

[1] J. Hadgraft, Skin, the final frontier, Int. J. Pharm. 224 (2001) 1–18.
[2] R. Marks, The stratum corneum barrier—the final frontier, J. Nutr. (2004) in press.
[3] A.L. Hughes, Evolutionary diversification of the mammalian defensins, Cell. Mol. Life Sci. 56 (1999) 94–103.
[4] J.L. Imler, J.A. Hoffmann, Toll and Toll-like proteins: an ancient family of receptors signaling infection, Rev. Immunogenet. 2 (2000) 294–304.
[5] M.J. Cork, R. Murphy, J. Carr, D. Buttle, S. Ward, C. Bavik, R. Tazi-Ahnini, The rise of atopic eczema, Dermatol. Pract. 10 (2002) 22–26.
[6] A.Y. Finlay, Effect of acne on the quality of life, J. Invest. Dermatol. 108 (1997) 110–110.
[7] A.Y. Finlay, E.C. Coles, The effect of severe psoriasis on the quality-of-life of 369 patients, Br. J. Dermatol. 132 (1995) 236–244.
[8] A.Y. Finlay, Quality of life assessments in dermatology, Semin. Cutan. Med. Surg. 17 (1998) 291–296.
[9] W.J. Albery, J. Hadgraft, Percutaneous absorption: in vivo experiments, J. Pharm. Pharmacol. 31 (1979) 140–147.
[10] M.K. Nemanic, P.M. Elias, In situ precipitation: a novel cyotchemical technique for visualization of permeability pathways in mammalian stratum corneum, J. Histochem. Cytochem. 28 (1980) 573–578.
[11] H.E. Bodde, I. Van den Brink, H.K. Koerten, F.H.N. Dehaan, Visualization of in vitro percutaneous penetration of mercuric chloride transport through intercellular space versus cellular uptake through desmosomes, J. Control. Release 15 (1991) 227–236.
[12] M. Heisig, R. Lieckfeldt, G. Wittum, G. Mazurkevich, G. Lee, Non-steady-state descriptions of drug permeation through stratum corneum. 1. The biphasic brick and mortar model, Pharm. Res. 13 (1996) 421–426.
[13] R.O. Potts, M.L. Francoeur, The influence of stratum corneum morphology on water permeability, J. Invest. Dermatol. 96 (1991) 495–499.
[14] B.W. Barry, Reflections on transdermal drug delivery, Pharm. Sci. Technol. Today 2 (1999) 41–43.
[15] R.O. Potts, R.H. Guy, Predicting skin permeability, Pharm. Res. 9 (1992) 663–669.
[16] J.W. Hadgraft, G.F. Somers, Percutaneous absorption, J. Pharm. Pharmacol. 8 (1956) 625–634.
[17] J. Hadgraft, C. Valenta, pH, pKa and dermal delivery, Int. J. Pharm. 200 (2000) 243–247.
[18] C. Valenta, U. Siman, M. Kratzel, J. Hadgraft, The dermal delivery of lignocaine: influence of ion pairing, Int. J. Pharm. 197 (2000) 77–85.
[19] J. Hadgraft, P.G. Green, P.K. Wotton, Facilitated percutaneous absorption of charged drugs, in: R. Bronaugh, H.I. Maibach (Eds.), Percutaneous Absorption, Marcel Dekker, New York, 1989, pp. 555–565.
[20] P.G. Green, J. Hadgraft, G. Ridout, Enhanced in vitro skin permeation of cationic drugs, Pharm. Res. 6 (1989) 628–632.
[21] N. Barker, J. Hadgraft, Facilitated percutaneous absorption, Acta Pharm. Suecica 20 (1983) 61–62.
[22] C. Valenta, J. Cladera, P. O'Shea, J. Hadgraft, Effect of phloretin on the percutaneous absorption of lignocaine across human skin, J. Pharm. Sci. 90 (2001) 485–492.
[23] C. Valenta, M. Nowak, J. Hadgraft, Influence of phloretin and 6-ketocholestanol on the permeation of progesterone through porcine skin, Int. J. Pharm. 217 (2001) 79–86.
[24] J. Houk, R.H. Guy, Membrane models for skin penetration studies, Chem. Rev. 88 (1988) 455–471.
[25] M. Iervolino, S.L. Raghavan, J. Hadgraft, Membrane penetration enhancement of ibuprofen using supersaturation, Int. J. Pharm. 198 (2000) 229–238.
[26] M.M. Feldstein, I.M. Raigorodskii, A.L. Iordanskii, J. Hadgraft, Modeling of percutaneous drug transport in vitro using skin-imitating carbosil membrane, J. Control. Release 52 (1998) 25–40.
[27] A.S. Michaels, K. Chandrasekaran, J.E. Shaw, Drug permeation through human skin: theory and in vitro experimental measurement, Am. Inst. Chem. Engrs J. 21 (1975) 985–996.
[28] J.A.M. Neelissen, C. Arth, A.H.G.J. Schrijvers, H.E. Junginger, H.E. Bodde, Validation of freeze-drying to visualize percutaneous 3H-estradiol transport: the influence of skin hydration on the efficacy of the method, Skin Pharmacol. Appl. Skin Physiol. 11 (1998) 11–22.
[29] A. Rougier, C. Lotte, P. Corcuff, H.I. Maibach, Relationship between skin permeability and corneocyte size according to anatomic site, age, and sex in man, J. Soc. Cosmet. Chem. 39 (1988) 15–26.
[30] W.J. Albery, J. Hadgraft, Percutaneous absorption: theoretical description, J. Pharm. Pharmacol. 31 (1979) 129–139.
[31] O. Diez-Sales, A. Copovi, V.G. Casabo, M. Herraez, A modelistic approach showing the importance of the stagnant aqueous layers in in vitro diffusion studies, and in vitro–in vivo correlations, Int. J. Pharm. 77 (1991) 1–11.
[32] J. Hadgraft, M. Whitefield, P.H. Rosher, Skin penetration of topical formulations of ibuprofen 5%: an in vitro comparative study, Skin Pharmacol. Appl. Skin Physiol. 16 (2003) 137–142.
[33] K.T. Kierstan, A.E. Beezer, J.C. Mitchell, J. Hadgraft, S.L. Raghavan, A.F. Davis, UV-spectrophotometry study of membrane transport processes with a novel diffusion cell, Int. J. Pharm. 229 (2001) 87–94.
[34] I.T. Degim, W.J. Pugh, J. Hadgraft, Skin permeability data: anomalous results, Int. J. Pharm. 170 (1998) 129–133.

[35] W.J. Pugh, J. Hadgraft, Ab initio prediction of human skin permeability coefficients, Int. J. Pharm. 103 (1994) 163–178.
[36] M.S. Roberts, W.J. Pugh, J. Hadgraft, A.C. Watkinson, Epidermal permeability–penetrant structure relationships. 1. An analysis of methods of predicting penetration of monofunctional solutes from aqueous solutions, Int. J. Pharm. 126 (1995) 219–233.
[37] M.S. Roberts, W.J. Pugh, J. Hadgraft, 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 (1996) 23–32.
[38] W.J. Pugh, M.S. Roberts, J. Hadgraft, 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 (1996) 149–165.
[39] W.J. Pugh, I.T. Degim, J. Hadgraft, 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 (2000) 203–211.
[40] J. du Plessis, W.J. Pugh, A. Judefeind, J. Hadgraft, The effect of the nature of H-bonding groups on diffusion through PDMS membranes saturated with octanol and toluene, Eur. J. Pharm. Sci. 15 (2002) 63–69.
[41] J. du Plessis, W.J. Pugh, A. Judefeind, J. Hadgraft, Physico-chemical determinants of dermal drug delivery: effects of the number and substitution pattern of polar groups, Eur. J. Pharm. Sci. 16 (2002) 107–112.
[42] I.T. Degim, J. Hadgraft, S. Ilbasmis, Y. Ozkan, Prediction of skin penetration using artificial neural network (ANN) modeling, J. Pharm. Sci. 92 (2003) 656–664.
[43] J. Hadgraft, J. Peck, D.G. Williams, W.J. Pugh, G. Allan, Mechanisms of action of skin penetration enhancers/retarders: azone and analogues, Int. J. Pharm. 141 (1996) 17–25.
[44] M. Holtje. Computer modelling of skin barrier lipid layers by molecular dynamics; in: T. Forster (Ed.), Cosmetic Lipids and the Skin Barrier, vol. 24, Marcel Dekker, New York, 2002, pp. 75–96.
[45] J. Hadgraft, Trans(dermal) delivery, present and future perspectives, The Drug Delivery Companies Report Spring/Summer 2003 (PharmaVentures), 2003, pp. 22–27.
[46] T.M. Morgan, B.L. Reed, B.C. Finnin, Enhanced skin permeation of sex hormones with novel topical spray vehicles, J. Pharm. Sci. 87 (1998) 1213–1218.
[47] T.M. Morgan, H.M.M. O'Sullivan, B.L. Reed, B.C. Finnin, Transdermal delivery of estradiol in postmenopausal women with a novel topical aerosol, J. Pharm. Sci. 87 (1998) 1226–1228.
[48] T.M. Morgan, B.L. Reed, B.C. Finnin. Metered-dose transdermal spray; in: M.J. Rathbone, J. Hadgraft, M.S. Roberts (Eds.), Modified-Release Drug Delivery Technology, vol. 126, Marcel Dekker, New York, 2003, pp. 523–531.
[49] J. Hadgraft, Can skin stripping predict local bioavailability, Proceedings of 29th International Symposium on Controlled Release of Bioactive Materials, Controlled Release Society Minneapolis, MN, USA, 2002, pp. 701–702.
[50] M.B. Reddy, A.L. Stinchcomb, R.H. Guy, A.L. Bunge, Determining dermal absorption parameters in vivo from tape strip data, Pharm. Res. 19 (2002) 292–298.
[51] J.E. Harrison, A.C. Watkinson, D.M. Green, J. Hadgraft, K. Brain, The relative effect of Azone® and Transcutol® on permeant diffusivity and solubility in human stratum corneum, Pharm. Res. 13 (1996) 542–546.
[52] M. Dias, J. Hadgraft, S.L. Raghavan, J. Tetteh, The effect of solvent on permeant diffusion through membranes studied using ATR-FTIR and chemometric data analysis, J. Pharm. Sci. 93 (2004) 186–196.
[53] J. Hadgraft, Modulation of the barrier function of the skin, Skin Pharmacol. Appl. Skin Physiol. 14 (2001) 72–81.
[54] J.N. Twist, J.L. Zatz, Influence of solvents on paraben permeation through idealized skin model membranes, J. Soc. Cosmet. Chem. 37 (1986) 429–444.
[55] A.F. Davis, J. Hadgraft, Effect of supersaturation on membrane transport. 1. Hydrocortisone acetate, Int. J. Pharm. 76 (1991) 1–8.
[56] M.A. Pellett, S.L. Raghavan, J. Hadgraft, A.F. Davis. The application of supersaturated systems to percutaneous drug delivery; in: R.H. Guy, J. Hadgraft (Eds.), Transdermal Drug Delivery, vol. 123, Marcel Dekker, New York, 2003, pp. 305–326.
[57] S.L. Raghavan, A. Trividic, A.F. Davis, J. Hadgraft, Crystallization of hydrocortisone acetate: influence of polymers, Int. J. Pharm. 212 (2001) 213–221.
[58] S.L. Raghavan, B. Kiepfer, A.F. Davis, S.G. Kazarian, J. Hadgraft, Membrane transport of hydrocortisone acetate from supersaturated solutions: the role of polymers, Int. J. Pharm. 221 (2001) 95–105.
[59] S.L. Raghavan, A. Schuessel, A.F. Davis, J. Hadgraft, Formation and stabilisation of triclosan colloidal suspensions using supersaturated systems, Int. J. Pharm. 261 (2003) 153–158.
[60] F. Zaman, A.E. Beezer, J.C. Mitchell, Q. Clarkson, J. Elliot, M. Nisbet, A.F. Davis, The stability of benzoyl peroxide formulations determined from isothermal microcalorimetric studies, Int. J. Pharm. 225 (2001) 135–143.
[61] A.E. Beezer, S. Gaisford, A.K. Hills, R.J. Willson, J.C. Mitchell, Pharmaceutical microcalorimetry: applications to long-term stability studies, Int. J. Pharm. 179 (1999) 159–165.
[62] M.A. Pellett, M.S. Roberts, J. Hadgraft, Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique, Int. J. Pharm. 151 (1997) 91–98.
[63] G.M. Golden, J.E. McKie, R.O. Potts, Role of stratum corneum lipid fluidity in transdermal drug flux, J. Pharm. Sci. 76 (1987) 25–28.
[64] V.H.W. Mak, R.O. Potts, R.H. Guy, Oleic acid concentration and effect in stratum corneum determined in vivo by infra red spectroscopy, J. Invest. Dermatol. 90 (1988) 584.
[65] B. Ongpipattanakul, R.R. Burnette, R.O. Potts, M.L. Francoeur, Evidence that oleic acid exists in a separate phase within stratum corneum lipids, Pharm. Res. 8 (1991) 350–354.
[66] M. Walker, J. Hadgraft, Oleic acid—a membrane 'fluidiser' or fluid within the membrane?, Int. J. Pharm. 71 (1991) R1–R4.
[67] J.E. Harrison, P.W. Groundwater, K.R. Brain, J. Hadgraft, Azone® induced fluidity in human stratum corneum. A Fourier transform infrared spectroscopy investigation using the perdeuterated analogue, J. Control. Release 41 (1996) 283–290.
[68] D. Bommannan, R.O. Potts, R.H. Guy, Examination of the effect of ethanol on human stratum corneum in vivo using infrared spectroscopy, J. Control. Release 16 (1991) 299–304.
[69] M.M. Dias, Facilitated percutaneous penetration, PhD Thesis, Cardiff University, 2001.
[70] R.M. Deal, J. Hadgraft, A.E. Beezer, S.E. Burgess, An ATR-FTIR investigation into the effectiveness of conventional light therapy for the treatment of psoriasis, Unpublished data, 2002.
[71] M. Jackson, K. Kim, J. Tetteh, J.R. Mansfield, B. Dolenko, R.L. Somorjai, F.W. Orr, P.H. Watson, H.H. Mantsch, Cancer diagnosis by infrared spectroscopy: methodological aspects, Proceedings of SPIE 3257, 1998, pp. 24–34.
[72] S.G. Kazarian, S.L. Raghavanand, J. Hadgraft, Unpublished data, 2003.
[73] J. Cladera, P. O'Shea, J. Hadgraft, C. Valenta, Influence of molecular dipoles on human skin permeability: use of 6-ketocholestanol to enhance the transdermal delivery of bacitracin, J. Pharm. Sci. 92 (2003) 1018–1027.
[74] M. Dias, J. Hadgraft, P.M. Glover, P.J. McDonald, Stray field magnetic resonance imaging: a preliminary study of skin hydration, J. Phys. D-Appl. Phys. 36 (2003) 364–368.
[75] P.A. Cornwell, B.W. Barry, C.P. Stoddart, J.A. Bouwstra, Wide-angle X-ray diffraction of human stratum corneum: effects of hydration and terpene enhancer treatment, J. Pharm. Pharmacol. 46 (1994) 938–950.

[76] L. Norlen, A. Al-Almoudi, J. Dubochet, A cryotransmission electron microscopy study of skin barrier formation, J. Invest. Dermatol. 120 (2003) 555–560.
[77] A.C. Watkinson, J. Hadgraft, P.R. Street, R.W. Richards, Neutrons, surfaces, and skin, in: R.O. Potts, R.H. Guy (Eds.), Mechanisms of transdermal drug delivery, Drugs and The Pharmaceutical Sciences, vol. 83, Marcel Dekker, New York, 1997, pp. 231–265.
[78] C.L. Gay, T.M. Murphy, J. Hadgraft, I.W. Kellaway, J.C. Evans, C.C. Rowlands, An electron spin resonance study of skin penetration enhancers, Int. J. Pharm. 49 (1989) 39–45.
[79] D. Lewis, J. Hadgraft, Mixed monolayers of dipalmitoylphosphatidylcholine with Azone or oleic acid at the air–water interface, Int. J. Pharm. 65 (1990) 211–218.
[80] D. Lewis, J. Hadgraft, H.E. Boddé, Mixed monolayer studies of DPPC with *N*-alkyl-aza-cycloheptanones at the air–water interface, in: R.C. Scott, R.H. Guy, J. Hadgraft, H.E. Boddé (Eds.), Prediction of Percutaneous Penetration, IBC Technical Services, 1991, pp. 355–364.
[81] G.M. Golden, D.B. Guzek, R.R. Harris, J.E. McKie, R.O. Potts, Lipid thermotropic transitions in human stratum corneum, J. Invest. Dermatol. 86 (1986) 255–259.
[82] V.L.C. Moffat, R.D. Green, J. Hadgraft, F. Renauld, A. Etienne, J.A. Bouwstra, Functional siloxanes as skin penetration enhancers, Proceedings of the International Symposium on Controlled Release of Bioactive Materials 18, 1991, pp. 536–539.
[83] J. Hadgraft, J.W. Hadgraft, I. Sarkany, The effect of thermodynamic activity on the percutaneous absorption of methyl nicotinate from water glycerol mixtures, J. Pharm. Pharmacol. 25 (1973) 122P–123P.
[84] W.J. Albery, R.H. Guy, J. Hadgraft, Percutaneous absorption—transport in the dermis, Int. J. Pharm. 15 (1983) 125–148.
[85] J. Beastall, R.H. Guy, J. Hadgraft, I. Wilding, The Influence of urea on percutaneous absorption, Pharm. Res. 3 (1986) 294–297.
[86] H.A. Benson, J.C. McElnay, R. Harland, J. Hadgraft, Influence of ultrasound on the percutaneous absorption of nicotinate esters, Pharm. Res. 8 (1991) 204–209.
[87] J. Hadgraft, D. Beutner, H.M. Wolff, In vivo–in vitro comparisons in the transdermal delivery of nitroglycerin, Int. J. Pharm. 89 (1) (1993) R1–R4.
[88] R.H. Guy, J. Hadgraft, Rate control in transdermal drug delivery?, Int. J. Pharm. 82 (1992) R1–R6.
[89] N.J. Evans, R.H. Guy, J. Hadgraft, G.D. Parr, N. Rutter, Transdermal drug delivery to neonates, Int. J. Pharm. 24 (1985) 259–265.
[90] N.J. Evans, N. Rutter, J. Hadgraft, G. Parr, Percutaneous theophylline administration to the preterm neonate, J. Pediatr. 107 (1985) 307–311.
[91] R.H. Guy, J. Hadgraft, Pharmacokinetic interpretation of the plasma-levels of clonidine following transdermal delivery, J. Pharm. Sci. 74 (1985) 1016–1018.
[92] J. Hadgraft, S. Hill, M. Hümpel, L.R. Johnston, L.R. Lever, R. Marks, T.M. Murphy, C. Rapier, Investigations on the percutaneous absorption of the antidepressant rolipram in vitro and in vivo, Pharm. Res. 7 (1990) 1307–1312.
[93] P.G. Green, J. Hadgraft, M. Wolff, Physicochemical aspects of the transdermal delivery of bupranolol, Int. J. Pharm. 55 (1989) 265–269.
[94] R.H. Guy, R.O. Potts, Penetration of industrial chemicals across the skin—a predictive model, Am. J. Ind. Med. 23 (1993) 711–719.