Chapter 2
Methods for the Measurement of Percutaneous Absorption

In general, the models of skin absorption discussed herein are generated from data published in the literature. These laboratory-based methods may use a variety of methods which could potentially affect the results of the experiments, particularly when used in subsequently developing a mathematical model. Therefore, the aim of this chapter is to discuss the different experimental protocols that are commonly used by researchers in percutaneous absorption in the context of the potential impact such methods of producing a model's inputs might exert upon its output.

Introduction

The vast majority of mathematical estimates of percutaneous absorption use, as their primary input, information on the rate of passage, or permeability, of a chemical across the skin. This is usually the permeability coefficient, k_p, or the more infrequently used (in the context of model development) maximum steady-state flux, J_{max}. In addition, a number of the physicochemical descriptors also modelled are measured experimentally, including measures of lipophilicity (commonly referred to as the octanol–water partition coefficient, log P) and melting point.

While different experiments will output the same general information—k_p or J_{max}—they may derive this information using different experimental protocols. Thus, the nature of the experiment and how it influences our understanding of permeability, not just its application to modelling, is a significant issue. For example, while there may be good reasons for using a range of experimental protocols to determine the permeability of particular penetrants, it is important to understand how this may apply itself to the subsequent—and, we should remember, the very separate—exercise of developing a mathematical model with this data.

The aim of this chapter is to discuss the different experimental protocols that are commonly used by researchers in percutaneous absorption, often to answer very specific experimental questions, contextualising our understanding of where the data used to develop models comes from and how different methods of generating the data might influence the output of models thus derived. It should be noted that

G.P. Moss et al., *Predictive Methods in Percutaneous Absorption*,
DOI 10.1007/978-3-662-47371-9_2

this chapter is not a full description of this field; rather, it highlights the aspects of experimental design that are most relevant for the development of mathematical models. For a comprehensive discussion of this subject, the reader is directed to Bronaugh and Maibach's (1999) or Williams' (2003) excellent texts.

In Vivo and In Vitro Methods: Overview

It should be first commented that the vast majority of mathematical models for percutaneous absorption used data from, and therefore most closely reflect, in vitro laboratory experiments. These are physical experiments that use membranes, which are either mammalian or synthetic in nature, across which the permeation of a chemical is measured experimentally. Such experiments are widely carried out and are an area of substantial interest across a range of industries. They have been used to measure the percutaneous absorption of pharmaceuticals, materials in cosmetic formulations, for toxicology studies and for estimation of risk assessment and occupational exposure of materials used in a variety of industrial applications.

In vitro methods are commonly used prior to in vivo experiments and in some cases (such as for the assessment of new chemical entities) are solely used to provide an indication of potential toxicity prior to any human exposure. Consequently, in vitro models are widely and commonly employed to assess the risks and hazards associated with exposure of human skin to exogenous chemicals.

Classically, in vivo studies have been conducted and provided valuable information on the mechanism of percutaneous absorption. However, these studies were generally non-invasive in that they measured a response in the skin, such as vasodilatation or skin blanching, rather than taking blood samples or punch biopsies of the skin for subsequent analysis. Despite their advantages, such methods are clearly limited in their applicability to other chemicals, particularly those that do not result in a non-invasively measurable physiological change. In addition, the non-invasive monitoring of certain topically applied chemicals, such as cosmetic formulations, may be measured in terms of efficacy by a range of biophysical methods, but such methods generally (with the exception of, for example, patch testing) do not provide any indication of cutaneous toxicity.

The in vivo estimation of percutaneous absorption may be considered appropriate if an established material (such as the drug ibuprofen) is used, and its absorption, distribution, metabolism and elimination are estimated by the analysis of bodily fluids. This, however, is extremely difficult to do for a wide range of potential penetrants—not just for the toxicological reasons mentioned above—but for logistical reasons, particularly the consistent availability of volunteers. It is also potentially unethical, should novel materials or techniques be investigated, such as the use of chemical or physical methods of enhancing absorption (i.e. formulation-based approaches or the use of electrical currents—iontophoresis—to facilitate absorption). In vivo experiments can provide realistic information on the amount of a topically applied chemical that is absorbed into and across the skin and which

becomes bioavailable. However, in the context of the mathematical modelling of percutaneous absorption, the vast majority of models are based on in vitro experiments using excised human skin, as the paucity of in vivo data, and lack of consistent endpoints (i.e. the measurement of a penetrant in a body compartment or the use of a non-invasive clinical response) means that there is insufficient data available in the literature from which a valid model can be constructed. Thus, while not lessening the overall significance in the wider field of percutaneous absorption of in vivo testing, the main focus of this chapter will be on in vitro methods for the measurement of percutaneous absorption. Clearly, in vitro methods are informed by, and attempt to replicate, in vivo methods and it is in that context that the biorelevance of in vitro testing should be considered.

In Vitro Experimental Methods

Preamble

In vitro methods for the characterisation of percutaneous absorption, while ultimately delivering the same outcome, are many and varied in the details of their methods. Selection of the diffusion membrane, type of cell (i.e. the use of either "static" or "flow-through" cell designs, described below), nature of the experiment (e.g. duration, occlusion) and the composition of the phases that sit either side of the diffusion membrane are some of the key parameters that add to the diversity of acceptable experimental protocols from which the data to construct mathematical models is abstracted. The main issues in the experimental design for the measurement of percutaneous absorption are discussed below.

Membrane Selection

In vitro methodologies for the measurement of percutaneous absorption are widely established and validated (Franz 1975; Maibach 1975; Mali 1978; Bronaugh et al. 1982; Barry 1983; Bronaugh and Maibach 1985a, b; Friend 1992). They are used commonly in a variety of research fields as described above. Human skin from various sources, including cosmetic surgery and amputations, has been widely used for the in vitro assessment of percutaneous penetration (Franz 1975; Bronaugh et al. 1982; Barry 1983; Friend 1992). The use of various animal skins is also a commonly accepted constituent of in vitro percutaneous penetration studies. Skin from a wide range of species, including pigs, rats, guinea pigs, monkeys and snakes, among others, has been suggested as a suitable replacement for human skin (Bartek et al. 1972; Marzulli and Maibach 1975; Wester and Maibach 1976; Chow et al. 1978; Wester and Noonan 1980; Itoh et al. 1990; Roberts and Mueller 1990; Sato et al. 1991; Lin et al. 1992; Harada et al. 1993). Generally, skin from the pig and the

rat has found the most widespread use, with the former in particular offering similar barriers to diffusion for the penetration through human skin of a wide range of molecules. Rat or mouse skin may be much more (up to 10 times) permeable than human skin, while pigskin has been claimed to be a better surrogate (Bartek et al. 1972; Chow et al. 1978; Wester and Noonan 1980; Roberts and Mueller 1990; Sato et al. 1991; Lin et al. 1992; Harada et al. 1993). However, rodent skin is still widely used as an in vitro membrane, possibly due to the use of such species more broadly in pharmacological research.

Several researchers have developed artificial skin equivalents, often known as living skin equivalents (LSEs) in an attempt to address some of the issues associated with using animal tissue in place of human skin (such as the lack of similarity in diffusional characteristics or complexity compared to human skin, and the *stratum corneum* in particular). LSEs have been used with some success in skin grafting and in the surgical treatment of burns (Young et al. 1998; Berger et al. 2000; Kremer et al. 2000; Machens et al. 2000; Mizunuma et al. 2000; Yang et al. 2000). Such materials aim to replicate the hydrophilic and hydrophobic balance of human *stratum corneum*, as well as the manifestation of its barrier function in, for example, the control of transepidermal water loss (TEWL) and control of bacterial ingress to the deeper epidermal and dermal tissues.

LSEs have also been used to assess percutaneous absorption. They generally consist of skin membranes which may include reconstituted epidermal cells that have been grown in tissue culture. They were proposed as an alternative to animal skin for in vitro percutaneous permeation studies but have to date failed to gain widespread acceptance. This is due to the reproducibility, cost (particularly when compared to animal tissue and where a large number of replicates of an experiment are required), their lack of robustness compared to human or animal skin (i.e. particularly when a formulation has to be directly applied to the skin, such as a semi-solid in a manner consistent with its clinical or consumer use) and their ability to replicate these tissues in terms of permeability and other physical properties. Several researchers have demonstrated that LSEs can have similar diffusional characteristics to mammalian skin but that they generally overestimate the rate of permeation across the membrane (Pelle et al. 1993; Hager et al. 1994; Horiguchi et al. 1997; Nemecek and Dayan 1999; Ramsamooj et al. 1998; Wang et al. 2000).

Artificial membranes have been used when human or animal skin is difficult to obtain, or where a large number of experiments are to be carried out, particularly with regard to preformulation screening experiments. The most widely used artificial membranes are polydimethylsiloxane (PDMS) and cellulose acetate (porous dialysis tubing) (Kurosaki et al. 1991; Megrab et al. 1995a, b; Stott et al. 2001; van Hal et al. 1996; Esposito et al. 1998; Woolfson et al. 1998; Minghetti et al. 1999). However, these membranes have often been shown to overestimate significantly the flux across skin and their use is significantly limited. For example, Moss et al. (2006) compared the permeability of a series of prodrugs across pigskin and PDMS membranes in vitro. They demonstrated a reasonable relationship for hydrophilic molecules, whereas an increase in hydrophobicity resulted in a significant difference

in permeability, with the PDMS showing significant overestimation of permeability compared to pigskin.

However, while studies using animal skin are experimentally sound and widely used, it should be noted that very few mathematical models of percutaneous absorption are based on animal tissue. This may be due to the relatively early publication of the seminal 1992 paper by Potts and Guy which developed quantitative structure–permeability relationships using human skin—thereafter, the development of a mathematical model based on skin from other species would not have the same significance and would almost appear to be a retrograde or rather redundant step, despite the opportunity to glean, in a comparative sense, important mechanistic information on the relative value of using non-human skin, as has been achieved by comparing mathematical models of human skin to an artificial membrane, polydimethylsiloxane, which also finds widespread use as a substitute for animal tissue in such experiments (Potts and Guy 1992; Geinoz et al. 2002; Moss et al. 2006).

Hence, the use of animal skin, artificial membranes or skin equivalents in place of human skin is limited, but does highlight the issue, particularly in the context of its extensive use in the wider field of percutaneous absorption, the importance of mathematical models in relation to elucidating the mechanism of action of permeants.

Such a discussion may infer that the use of human tissue is optimal for in vitro experiments. However, it is not without its problems. Human skin is usually obtained from skin banks or from tissue donated by the patient as a result of surgery —generally, this can range from cosmetic procedures including face lifts, "tummy tucks" and breast reduction or from medical procedures including amputations. The experimenter therefore has little control over the handling and quality of the skin obtained. For example, surgical procedures and protocols used to remove skin may include the use of alcohol-based disinfectants; while this is clearly essential for the surgical procedure and is a central part of infection control policies, it may affect the permeability in subsequent experiments as the alcohol has the potential to remove *stratum corneum* lipids and potentially affect its barrier properties. Further, the skin may be frozen and stored prior to dispatch and use in experiments, and this may result in damage to, or degradation of, the membrane.

Further, variability of skin was discussed in Chapter One and may be due to gender, age, body site and a number of other factors. Tissue from different body sites and from different patients may, not unreasonably, exhibit different properties, including permeability. In terms of experimental use, such variation is generally minimised by attempting to use tissue that is as similar as possible (i.e. from the same region, or from the same patient, or from a small patient population) or by normalising against a known standard. While there are different implications for the type of experiment being conducted (i.e. a comparative formulation study might require different standards than a toxicological study), all such data from a range of experiments have been collated into data sets and used to develop mathematical models of skin permeability, thus introducing a possible source of variance—or potentially even error—into the models. While this is a significant issue, it should ultimately be considered in the context of tissue availability as discussed above, and

any modellers should be aware of such issues, particularly the history and nature of the tissue, and consider their output accordingly.

The skin used in percutaneous absorption experiments has to be prepared before use. This involves several stages. Firstly, once the skin has been harvested surgically, it is used either fresh or frozen. Keeping human skin frozen at −20 °C has been shown to have no detrimental effect on its permeability for up to 455 days (Harrison et al. 1984). Consequently, with regard to availability of supply and convenience, skin for in vitro permeation studies is frequently used from frozen, being defrosted prior to use. Skin samples are normally defrosted at ambient temperature and gently patted dry before use.

The most rudimentary preparation immediately prior to an experiment involves removing the surface hairs, or clipping them to a length where they will not interfere with the experiment by, for example, compromising the seal between the skin surface and the compartment directly above it (ensuring that this is done in a manner that will not compromise the skin barrier), and removing subcutaneous fat normally by the careful application of dissection techniques. Such processing of the skin may also be conducted prior to freezing the skin samples. However, there are instances when specific layers of the skin—most normally, the epidermis or the *stratum corneum*—are isolated from the rest of the tissue and used rather than whole skin.

Next, if the experimental protocol determines that the skin has to be separated in some manner, several methods are available to facilitate this. For example, the use of blistering, either by the application of chemicals or suction, can be used although it may damage the skin and cause issues with membrane integrity. Skin may be processed to the required thickness by the use of a dermatome. There are a number of methods commonly employed for this, and one good example of the difficulties encountered is described by Williams (2003). He reports a technique that involves freezing the predominately aqueous dermal side of the excised skin to a steel plate. This fixes the skin to the steel surface, and once it has thawed slightly, it can be dermatomed to the required thickness.

Alternatively, chemicals may be used to separate either the *stratum corneum* or the entire epidermis from the underlying tissue. Elias et al. (1974) used staphylococcal epidermolytic toxin and ammonia fumes to separate the epidermis from the dermis. A more widely used method involves the application of heat to separate the epidermis from the dermis (Kligman and Christophers 1963). The skin is placed in water at 60 °C for a short duration (usually 45 s to several minutes is recommended), removed, patted dry and the epidermis removed, usually by pinning the skin onto a cork board and peeling the epidermis off.

Use of isolated *stratum corneum* involves separation from the rest of the skin by enzymatic methods, such as the use of a trypsin solution, where the skin sample is placed, *stratum corneum* side upwards, onto a solution of trypsin. Tissue from the viable epidermis and the dermis is removed by this process, and any residue remaining on the skin surface is subsequently gently removed, and the resulting membrane is dried and stored. Such methods are rarely used as the isolated *stratum corneum* is very thin and fragile—also, in a diffusion cell, the fragile *stratum*

corneum may also require a support to maintain integrity of the diffusion cell. Removal of the *stratum corneum* from the underlying viable epidermis may result in tears to the barrier, and therefore, barrier integrity checks need to be carried out before and after permeation experiments using this tissue.

It is more common to prepare the skin by using epidermal tissue which has been separated from the lower layers of the skin by heat. As the *stratum corneum* is the main barrier to percutaneous absorption, the tissues of the viable epidermis, which are predominately aqueous in nature, essentially provide little more than physical and mechanical support and improved flexibility compared to isolated *stratum corneum*. Removal of the dermis from the experimental tissue has little effect in the process of percutaneous absorption as the main clearance of materials absorbed into the skin occurs at the top of the dermis, at its junction with the epidermis, where the majority of the skin's vasculature that clears such permeants resides. Conversely, as the dermis is also a predominately aqueous tissue which, in the context of an in vitro percutaneous absorption study, provides physical and mechanical support rather than a diffusing stratum, it is often included in experiments as its presence will have little effect on the overall process of absorption. However, it may in practice mean that the skin is excessively thick and therefore cumbersome in the diffusion apparatus, and it is often removed, wholly or partially, by the use of a dermatome which results in the use of a consistent thickness of skin.

Williams (2003) elaborates on such aspects of experimental design by considering them in the context of the penetrant's solubility. If the penetrant is hydrophilic (defined as having an octanol–water partition coefficient below 3), the main barrier to diffusion across the skin will reside in the *stratum corneum* and the absence of dermal tissue is not an issue. However, in the case of lipophilic penetrants (defined as having an octanol–water partition coefficient above 3), the lower, predominately aqueous layers of the skin may become significant as they will provide a substantial challenge to the permeation of such materials and, at the very least, an epidermal membrane, rather than dermatomed skin, would be the preferred membrane.

Integrity Testing

The substantial amount of processing of the skin undergoes prior to its use in a diffusion experiment means that it is susceptible to damage, particularly to the fine *stratum corneum* barrier. Thus, assessment of skin integrity is then carried out to determine the viability of the tissue. This usually involves the measurement of a particular property of the skin, or the passage of a "marker" compound through the skin prior to the experiment. Water naturally evaporates from the body and across the skin in a process called transepidermal water loss (TEWL). The rate of evaporation can be measured by sensitive equipment (i.e. typical value 0.5 $\mu L/cm^2/h$; Imhof et al. 2009) and the rates compared to known benchmarks, or the standard deviation can be determined for a batch of skin being used in a particular experiment, or set of experiments, and those samples whose TEWL falls outside the

chosen range can be discarded as being atypical. Measurement of TEWL is non-invasive and does not alter the integrity of the skin by the direct application of chemicals prior to the experiment. TEWL is sensitive to the environmental factors, particularly relative humidity. Similarly, the electrical resistance of skin can be measured by placement of electrodes either side of the skin tissue. As with TEWL measurements, determination of electrical resistance is non-invasive and atypical values, depending on the criteria adopted by researchers, can lead to the rejection of particular samples from the experiment. Another option is to use a chemical to determine the integrity of the skin barrier. Tritiated compounds (^{3}H), including tritiated water or radiolabelled sugars such as glucose, have been used by several researchers to ensure the integrity of the skin barrier for a period of time (usually not more than 6–8 h) prior to an experiment (Bronaugh et al. 1986; Harrison et al. 1984). Skin is usually accepted for use if the permeability coefficient (cm/h) of the penetrant is within a certain range—often, while there is a general consensus as to what constitutes "acceptable", different researchers use comparable but often subjective measurements to remove poor atypical tissue from an experiment. ^{3}H and other radiolabelled compounds do not find widespread use in percutaneous absorption experiments due to the logistics and costs associated with such experiments. With the skin prepared and its integrity checked, the apparatus can now be assembled.

Selection of the Diffusion Cell Apparatus

Fundamentally, the aim of an in vitro percutaneous absorption study is to determine the amount of permeant that passes into and across the skin. This involves the use of a diffusion chamber in which the membrane (normally human or animal skin, or an artificial membrane) separates the two compartments—the donor compartment is where the formulation containing the permeant of interest is introduced at the start of the experiment; and the receptor compartment is the chamber into which the permeant of interest may diffuse, following passage into and across the membrane. In addition to measuring the amount, and rate, of permeant that passes across the membrane and into the receptor phase, the experiment also presents the experimenter with the opportunity to determine how much of the material of interest has passed into the skin and has remained there at the end of the experiment. This is usually achieved by using adhesive tape to remove the *stratum corneum* and by digesting the remaining tissue using acid/solvent mixtures. These samples can then be prepared for quantitative analysis.

Thus, the experiment is essentially a passive diffusion process that is governed by the diffusion gradient across the membrane as well as the experimental protocol and the physicochemical properties of the permeant. Despite various designs, some more complex than others (Fig. 2.1), the fundamentals of the process are very similar—passage of the permeant of interest from the donor chamber to the receptor chamber while maintaining a viable diffusion gradient and avoiding equilibrium

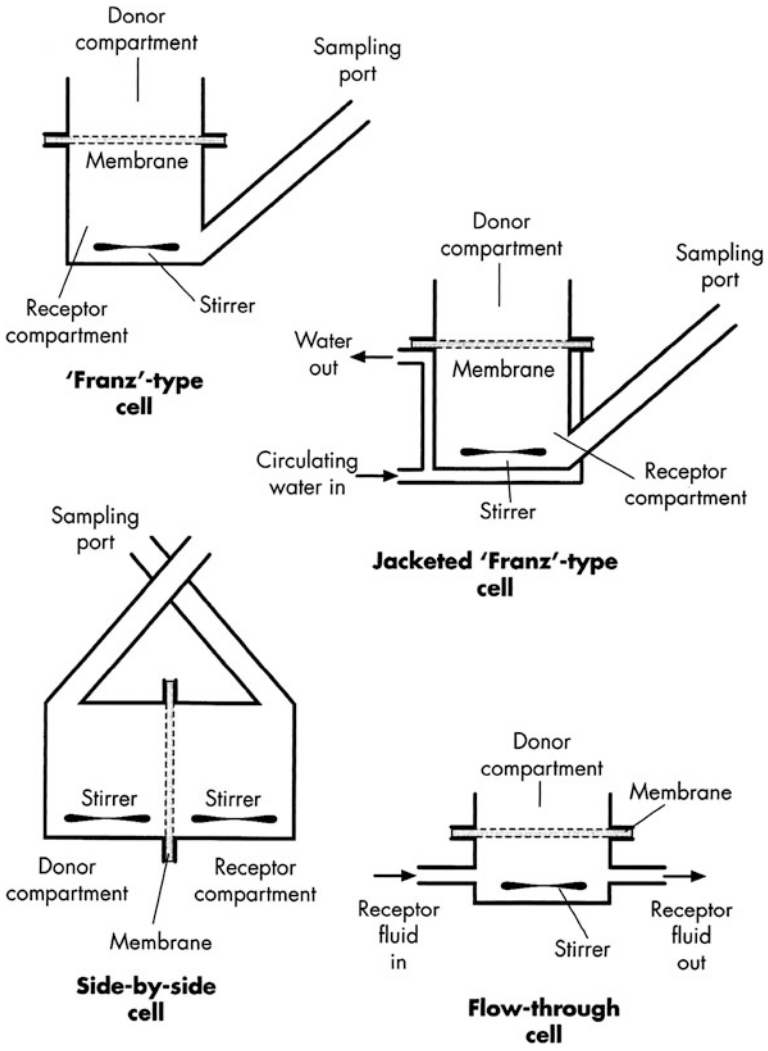

Fig. 2.1 Examples of commonly used diffusion cells (© Williams (2003), used with permission)

between both compartments. The receptor compartment normally has a sampling port—usually a sidearm—which is occluded during the experiment to stop evaporative loss and from which aliquots of the receptor media may be removed (and replaced with fresh receptor fluid) for analysis.

There are two main types of diffusion cells: static and flow-through cells. The vast majority of in vitro percutaneous absorption experiments are conducted using upright static glass diffusion cells, known as Franz or Franz-type cells (Franz 1975). These involve the partition of the permeant into a "static" receptor compartment of a fixed volume that is maintained at a controlled temperature and is continually stirred

throughout the experiment to ensure complete mixing and to avoid the presence of any "dead" zones or diffusion gradients within the receptor compartment. Franz-type diffusion cells are found in a range of designs and sizes (Fig. 2.1) and are therefore very flexible, with particular cells being chosen for particular purposes (e.g. when a different surface area might be required, or when the receptor compartment may need to be varied based on the solubility of a particular permeant). The simple design of the upright cells allows a wide range of formulations to be applied to the skin surface, including solutions, creams, ointments and various other semi-solid materials of a pharmaceutical or cosmetic nature. A variation on the Franz-type cells is the side-by-side cell, which allows both compartments to be stirred at the same time, although the range of formulations suitable for use in such cells is clearly limited.

One of the main perceived limitations of the Franz-type cells is their susceptibility to "sink conditions". As described above, the diffusion process requires a gradient to be established, and maintained, across the membrane that divides the donor and receptor compartments. Thus, in a static diffusion cell, it is important to maintain a concentration gradient such that, normally, the concentration of the permeant of interest is not greater than 10 % of its saturated solution in the receptor compartment (Martin et al. 1983; Anissimov and Roberts 1999; Roberts and Cross 1999; Roberts et al. 1999). Flow-through diffusion cells, often called "Bronaugh cells", offer an alternative approach to the issue of sink conditions and attempt to mimic in vivo conditions by utilising a constantly perfusing receptor compartment, flowing usually at 1–2 ml/h, which aims to mimic the blood flow beneath the skin (Bronaugh et al. 1999a, b). This is normally achieved with the use of a peristaltic pump which is connected to tubing that supplies a compartment beneath the membrane. The receptor fluid flows into a receptacle and is analysed, either offline or, more infrequently, via an online flow-through method of analysis. Either arrangement provides the facility for substantial automation of sample collection. This arrangement ensures that sink conditions are maintained throughout the experiment and that the receptor phase does not have to be replaced should a penetrant be rapidly absorbed. While flow-through cells are more flexible (i.e. in terms of automation), they are more complex and substantially more expensive than Franz cells. Consequently, the use of Franz-type static diffusion cells is more common than the use of flow-through cells. In either case, most experiments use between 6 and 24 cells per experiment in order to ensure reproducibility.

As mentioned above, the cell design can influence the formulation type that can be applied to it. For example, the side-by-side cells, while having the advantage of even stirring and mixing in both donor and receptor compartments, are limited in the type of formulation that can be applied—pharmaceutical dosage forms such as creams, gels, ointments and patches are very difficult to apply in this manner. Thus, the cell design and the protocol used can all affect the outcome—the measurement of percutaneous absorption and the cell design should be considered when considering the uniformity of data that are to be used in the construction of mathematical models of skin absorption.

For example, the manner in which the product is applied to the skin surface is important to consider in model construction, as each individual experiment may require specific amounts, or even frequencies of application, or they may require that the donor phase is occluded, or not occluded—the experimental protocol used is dependent on the nature of the penetrant and the context of the experiment. An experiment might wish to examine the occupational hazard of a chemical being exposed to the skin—in, for example, the context of crop spraying of a pesticide. Therefore, an experimental protocol might use a non-occluded experiment (to mimic the potential of the solvent, the pesticide is delivered in evaporating vehicle) and a fixed, or finite, dose which would represent the amount commonly sprayed and therefore the amount that the skin (per unit area) might be exposed to. Equally, an experiment may wish to determine the "worst-case scenario" for the application of a new chemical to the skin, and, in such circumstances, an occluded experiment may be favoured as this generally increases the permeation of a topically applied chemical (Zhai and Maibach 2001). Such a protocol might also use an excessive amount of chemical—an infinite dose—that aims to maintain the maximum flux of this chemical across the skin, in order to determine permeation in the "worst case". Such an arrangement also finds use when examining the fundamental behaviour of a material and is used to establish the steady-state rate of permeation across the skin. Infinite doses are also used when considering the effects of formulation ingredients that alter the barrier function of skin (penetration enhancers) on the penetration of a chemical (Williams 2003).

The application of an infinite dose to the surface of the skin means that there is little or no significant change to its concentration (in the donor phase) or thermodynamic activity throughout the experiment. However, once permeation commences, the donor phase becomes depleted and this scenario is no longer valid. In a practical sense if the concentration in the receptor does not exceed 10 % of the saturated solubility, an infinite dose can be assumed. Further, supersaturated systems have been employed (e.g. Raghavan et al. 2001; Dias et al. 2003; Raghavan et al. 2003) to resolve this issue; the donor phase is composed of a saturated solution of the penetrant of interest, into which is also added an additional amount of the penetrant, which will be present as a solid. As diffusion into and across the skin commences, the donor phase can maintain the saturated solution should the conditions, including the rate of solubility, permit it to do so (and, therefore, maintain in theory the same concentration and thermodynamic activity) throughout the duration of the experiment.

Conversely, in a finite-dose experiment, a fixed amount of a material is applied to the skin surface and it is expected that it will deplete during the course of the experiment. This may additionally be facilitated by whether or not the donor phase is covered (occluded) during the experiment, as a non-occluded system may result in the evaporation of any solvent which may alter, both positively and negatively, the permeation of the material. Such experiments are very useful in that they allow an estimation of permeation that is more realistic and which more accurately represents the "in-use" performance of a product.

Temperature

The temperature at which an in vitro percutaneous penetration experiment is conducted is normally 37 °C, maintained by the use of circulating water, as discussed above. This results in a temperature of 32 °C at the skin surface. It is important to maintain this temperature throughout the experiment as skin permeability may vary significantly with even small changes in temperature (Chilcott et al. 2005). However, data sets collated for several studies (i.e. Moss et al. 2009; Lam et al. 2010) show that, while the vast majority of experiments that constitute those data sets have been conducted at either 32 or 37 °C, a significant number of experiments were conducted at temperatures of 31–32, 25–31 and 22–30 °C. The implications of this are discussed in later chapters.

Formulation and Solubility Factors

The nature of any formulation applied to the skin must be considered when using the information from an in vitro diffusion cell experiment to construct a mathematical model of permeation. If a chemical applied to the skin, whether it is the permeant of interest or a material formulated to facilitate the delivery of the permeant, alters the permeability of the skin, then this can significantly influence permeability. For example, certain solvents will alter the permeability of the skin barrier. These include water (whose hydration effects are, comparatively, somewhat limited), ethanol, propylene glycol and other organic solvents. These materials may be used to facilitate the delivery of drugs into or across the skin. For example, urea has also been shown to increase the hydration of the *stratum corneum* and increase the onset of erythema (Hellgren and Larsson 1979; Beastall et al. 1988). Urea has also been employed clinically to enhance hydrocortisone penetration from commercially available products, such as Alphaderm® and Calmurid®.

However, in the context of mathematical modelling, most of the data used to construct models come from experiments where formulation, other than solvents, is not considered (this is a limitation that will be discussed in detail later). Generally, the permeability data from which models have been constructed use aqueous or water–ethanol solvents in the donor phase, and few studies have explored formulation matters in detail (Pugh et al. 2005; Ghaforuain et al. 2010a, b; Moss et al. 2011). This also applies to ionisation, which is not generally considered by most models of percutaneous absorption.

The main issue with solvent effects in percutaneous absorption experiments that are relevant to the construction of mathematical models is the use of non-aqueous materials in the receptor compartment of the diffusion apparatus. Ideally, the receptor fluid should mimic as closely as possible the nature of the sink into which the permeant will diffuse when applied topically in vivo—the viable tissues of the skin and the blood supply. Chapter 1 described the structure of the skin and how its

substantial vascularised structure provides a very efficient means of rapidly removing permeants and maintaining an effective diffusion gradient across the skin. Thus, an efficient receptor fluid in an in vitro experiment should similarly aim to maintain sink conditions and while not altering the nature of the membrane. In general, the vast majority of experiments use similar receptor fluids, although significant differences do occur and should be considered, both for their impact on permeation and for their impact on the subsequent, and in most cases unconnected, development of mathematical models. Most receptor fluids are based on buffered aqueous systems (or, in the case of non-ionised species, simple aqueous systems). Such systems are usually buffered at pH 7.4, usually with a phosphate buffer, to mimic physiological pH. However, there are exceptions, such as the use of a receptor phase buffered at pH 5 which is used to measure the permeability of aluminium across human skin (Mistry et al. 2013). This study used a receptor phase buffered at pH 5 as aluminium otherwise forms an insoluble oxide, which is difficult to analyse. Antimicrobial agents are often added to receptor fluids to maintain the integrity of the receptor compartment. If the penetrant of interest is lipophilic, then organic solvents, such as ethanol or propylene glycol, can be used, often in concentrations up to 25 %, with the remainder of the fluid being composed of water or a buffered aqueous system. Other solubilising agents have been used in receptor phases, including various surfactants and protein (i.e. bovine serum albumin). Care should be taken to consider the effect that the receptor compartment, through back-diffusion into the skin, may exert on barrier integrity, hence the suggestion to measure barrier integrity at the beginning and the end of an experiment. Therefore, the selection of a receptor fluid may be experiment-specific as different experiments aim to evaluate the permeability of a range of chemicals. Thus, care should be taken when using such information to construct models.

Detection of the Permeant

Once the receptor compartment fluid has been collected, it will be analysed by a number of commonly used methods. These include "cold" chromatographic methods (predominately high-performance liquid chromatography, HPLC, which is increasingly being coupled with mass spectrometric (MS) methods of analysis) or "hot" radiolabelled methods, usually by ^{14}C or ^{3}H-labelling of the permeant. This latter method is expensive, requires the use of licensed premises and, in the case of ^{3}H-labelled materials, offers the opportunity for tritium exchange between the material of interest and other materials in the analytical sample. HPLC conversely is comparatively inexpensive and widely used as a method of detection in biologically derived samples. In simpler diffusion experiments, such as those where artificial membranes (i.e. PDMS) have been used, UV spectrometry has been employed. While each method has its advantages and disadvantages, they all, if used and validated appropriately, can quantify the permeability of a material of interest such that accurate diffusion profiles can be produced, from which flux and permeability

coefficients can be derived. Flux (as concentration/surface area/time, i.e. mg/cm^2/h) is usually the gradient of the steady-state portion of the diffusion profile. The permeability coefficient, k_p, is, in essence, the concentration-corrected flux and has units of distance and time (i.e. cm/s). Further, the lag time can be determined by extrapolating the steady-state section of the graph to the x-axis (the axis of the diffusion plot on which time is normally plotted). It is this data, mostly k_p but, in some cases flux (i.e. Magnusson et al. 2004), that is used by those constructing mathematical models of percutaneous absorption.

Conclusions

There exists a wide range of potentially conflicting experimental protocols for the determination of percutaneous absorption. All these methods are entirely valid in that they have been developed with specific penetrants in mind and not with the development of mathematical models, the latter activity often being unconnected to the original experiments. Thus, their results should be considered in this context—so too should their use in the development of the mathematical models and their limitations clearly enunciated and understood. For example, as we will see in later chapters, data sets have been compiled, and mathematical models developed, from experimental data that have been collated from different sources, which often use differing experimental protocols.

Ideally, in order to assess the relationships between the different methods, the relative permeabilities through the membranes of the same compounds would be assessed. This is, however, impractical as few comparative data exist for this purpose.

One solution to this problem has been to develop quantitative structure–activity (or permeability) relationships (QSARs, or QSPRs) based on the data that can be compared. QSPRs attempt to relate statistically the experimentally determined percutaneous penetration of a range of exogenous chemicals to known physicochemical parameters. However, such methods have been criticised as they, and their conclusions, are limited by the nature of the data on which they are based. This may include, for example, the lack of such models to consider formulation or ionisation effects. This may limit the scope of such models significantly. However, considered within this context, the models still yield valuable information on the mechanism of percutaneous absorption and, in many cases, allow the permeability of a chemical that is not within the data set but which fits within its "molecular space" to be predicted, provided that the model results in the output of a functional mathematical relationship between the experimentally measured parameter (usually k_p or J_{max}) and the statistically significant physicochemical properties of a penetrant.

Thus, this chapter has highlighted the complexity of a seemingly simple experiment from which permeability data are derived. It follows that the nature of this data, and its experimental origins in particular, should be described and understood when developing models, as the nature of the experimental data that forms the input to the mathematical model can clearly influence its output.

References

Anissimov YG, Roberts MS (1999) Diffusion modelling of percutaneous absorption kinetics. 1. Effects of flow rate, receptor sampling rate and viable epidermal resistance for a constant donor concentration. J Pharm Sci 88:1201–1209

Barry BW (1983) Dermatological formulations: percutaneous absorption. Marcel Dekker, New York

Bartek MJ, LaBudde JA, Maibach HI (1972) Skin permeability in vivo: comparison in rat, rabbit, pig and man. J Invest Dermatol 58:114–123

Beastall JC, Hadgraft J, Washington C (1988) Mechanism of action of Azone® as a percutaneous penetration enhancer: lipid bilayer fluidity and transition temperature effects. Int J Pharm 43:207–213

Berger A, Tanzella U, Machens HG, Liebau J (2000) Use of Integra in burn wounds and unstable scars. Chirurgia 71:558–563

Bronaugh RL, Maibach HI (1985a) Percutaneous Penetration. Marcel Dekker, London

Bronaugh RL, Maibach HI (1985b) Percutaneous-absorption of nitroaromatic compounds—in vivo and in vitro studies in the human and monkey. J Invest Dermatol 84:180–183

Bronaugh RL, Maibach HI (1999) Percutaneous absorption, 3rd edn. Marcel Dekker, Inc. CRC Press, New York

Bronaugh RL, Stewart RF, Congdon ER, Giles AL (1982) Methods for in vitro percutaneous absorption studies I: Comparison with in vivo results. Appl Pharmacol 62:481–488

Bronaugh RL, Hood HL, Kraeling MEK, Yourick JJ (1999) Determination of percutaneous absorption by in vitro techniques. In: Bronaugh RL, Maibach HI (eds.) Percutaneous absorption 3rd (Edn), New York: Marcel Dekker, Inc. CRC Press, pp 229–234

Bronaugh RL, Kraeling MEK, Yourick JJ, Hood HL (1999) Cutaneous metabolism during in vitro percutaneous absorption. In: Bronaugh RL, Maibach HI (eds.) Percutaneous absorption 3rd (Edn), New York: Marcel Dekker, Inc. CRC Press, pp 57–64

Bronaugh RL, Stewart RF, Simon M (1986) Methods for in vitro percutaneous absorption studies. 7. Use of excised human skin. J Pharm Sci 75:1094–1097

Chilcott RP, Barai N, Beezer AE, Brain SL, Brown MB, Bunge AL, Burgess SE, Cross S, Dalton CH, Dias M, Farinha A, Finnin BC, Gallagher SJ, Green DM, Gunt H, Gwyther RL, Heard CM, Jarvis CA, Kamiyama F, Kasting GB, Ley EE, Lim ST, McNaughton GS, Morris A, Nazemi MH, Pellett MA, Du Plessis J, Quan YS, Raghavan SL, Roberts M, Romonchuk W, Roper CS, Schenk D, Simonsen L, Simpson A, Traversa BD, Trottet L, Watkinson A, Wilkinson SC, Williams FM, Yamamoto A, Hadgraft J (2005) Inter- and intra-laboratory variation of in vitro diffusion cell measurements: an international multicenter study using quasi-standardised methods and materials. J Pharm Sci 94:632–638

Chow C, Chow AYK, Downie RH, Buttar HS (1978) Percutaneous absorption of hexachlorophene in rats, guinea pigs and pigs. Toxicology 9:147–154

Dias MMR, Raghavan SL, Pellett MA, Hadgraft J (2003) The effect of beta-cyclodextrins on the permeation of diclofenac from supersaturated solutions. Int J Pharm 263:173–181

Elias PM, Fritsch P, Tappeine G, Mitterma H, Wolff K (1974) Experimental staphylococcal toxic epidermal necrolysis (10) in adult humans and mice. J Lab Clin Med 84:414–424

Esposito E, Zanella C, Cortesi R, Menegatti E, Nastruzzi C (1998) Influence of liposomal formulation parameters on the in vitro absorption of methyl nicotinate. Int J Pharm 172:255–260

Franz TJ (1975) On the relevance of in vitro data. J Invest Dermatol 64:190–195

Friend DR (1992) In vitro skin permeation techniques. J Cont Rel. 18:235–248

Geinoz S, Rey S, Boss G, Bunge AL, Guy RH, Carrupt PA, Reist M, Testa B (2002) Quantitative structure-permeation relationships for solute transport across silicone membranes. Pharm Res 19:1622–1629

Ghafourian T, Samaras EG, Brooks JD, Riviere JE (2010a) Modelling the effect of mixture components on permeation through skin. Int J Pharm 398:28–32

Ghafourian T, Samaras EG, Brooks JD, Riviere JE (2010b) Validated models for predicting skin penetration from different vehicles. Eur J Pharm Sci 41:612–616

Hager DF, Mancuso FA, Nazareno JP, Sharkey JW, Siverly JR (1994) Evaluation of a cultured skin equivalent as a model membrane for iontophoretic transport. J Cont Rel 30:117–123

Harada K, Murakami T, Kawasaki E, Hagashi Y, Yamamoto S, Yata N (1993) In vitro skin permeability to salicyclic acid of human, rodent and shed snake skin. J Pharm Pharmacol 45:414–418

Harrison SM, Barry BW, Dugard PH (1984) Effects of freezing on human skin permeability. J Pharm Pharmacol 36:261–262

Hellgren L, Larsson K (1979) On the effect of urea on human epidermis. Dermatologica 149:289–293

Horiguchi Y, Suzuki S, Toda K, Ueda M, Leigh IM, Imamura S (1997) Three-dimensional cultures of keratinocytes and an application to in vitro amyloid production of cutaneous amyloidosis. Microsc Res Tech 38:387–393

Imhof RE, de Jesus MEP, Xiao P, Ciortea LI, Berg EP (2009) Closed-chamber transepidermal water loss measurement: microclimate, calibration and performance. Int J Cosmet Sci 31:97–118

Itoh T, Xia J, Magavi R, Nishihata T, Rytting JH (1990) Use of shed snake skin as a membrane for in vitro percutaneous penetration studies. Pharm Res 7:1042–1047

Kligman AM, Christophers E (1963) Preparation of isolated sheets of human stratum corneum. Pharmacol Skin 1:138–153

Kremer M, Lang E, Berger AC (2000) Evaluation of dermal-epidermal skin equivalents (“composite-skin”) of human keratinocytes in a collagen-glycosaminoglycan matrix (Integra™ Artificial Skin). Br J Plast Surg 53:459–465

Kuroski Y, Nagahara N, Taniza WA, Nishmura H, Nakayama T, Kimura T (1991) Use of lipid disperse systems in transdermal drug delivery—comparative study of flufenamic acid permeation among rat abdominal skin, silicon rubber membrane and stratum corneum sheet isolated from hamster cheek pouch. Int J Pharm 67:1–9

Lam LT, Sun Y, Davey N, Adams R, Prapopoulou M, Brown MB, Moss GP (2010) The application of feature selection to the development of Gaussian Process models for percutaneous absorption. J Pharm Pharmacol 62:738–749

Lin SY, Hou SJ, Hsu THS, Yeh FL (1992) Comparisons of different animal skins with human skin in drug percutaneous absorption studies. Meth Find Exp Clin Pharmacol 14:645–654

Machens HG, Berger AC, Mailaender P (2000) Bioartificial skin. Cells Tissue Organs 167:88–94

Magnusson BM, Anissimov YG, Cross SE, Roberts MS (2004) Molecular size as the main determinant of solute maximum flux across the skin. J Invest Dermatol 122:993–999

Maibach HI (1975) Animal models in dermatology. Churchill-Livingstone, Edinburgh

Mali JHW (1978) Current problems in dermatology, vol 7. Karger, Basel

Martin A, Swarbrick J, Cammarata A (1983) Physical pharmacy, 3rd edn. Lea & Febinger, Philadelphia

Marzulli FN, Maibach HI (1975) Relevance of animal models: the hexachlorophene story. In: Maibach HI (ed) Animal models in dermatology. Churchill-Livingstone, Edinburgh

Megrab NA, Williams AC, Barry BW (1995a) Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems. Int J Pharm 116:101–112

Megrab NA, Williams AC, Barry BW (1995b) Oestradiol permeation through human skin and silastic membrane: effects of propylene glycol and supersaturation. J Cont Rel 36:277–294

Minghetti P, Casiraghi A, Cilurzo F, Montanari L, Marazzi M, Falcone L, Donati V (1999) Comparison of different membranes with cultures of keratinocytes from man for percutaneous absorption of nitroglycerine. J Pharm Pharmacol 51:673–678

Mistry T, Ajum K, Owen J, Stair J, Wilkinson SC, Staff K, Moss GP (2013) The percutaneous absorption of aluminium from antiperspirant products. In: Brain KR, Chilcott R (eds) Advances in the dermatological sciences. UK, Royal Society of Chemistry, Cambridge, pp 256–264

Mizunuma M, Yanai A, Seno H, Hirabayashi S (2000) Experience in repair utilizing artificial skin for exposed bone surfaces. Eur J Plast Surg 23:305–308

Moss GP, Gullick DR, Cox PA, Alexander C, Ingram MJ, Smart JD, Pugh WJ (2006) Design, synthesis and characterisation of captopril prodrugs for enhanced percutaneous absorption. J Pharm Pharmacol 58:167–177

Moss GP, Sun Y, Davey N, Adams R, Pugh WJ, Brown MB (2009) The application of Gaussian Processes to the prediction of percutaneous absorption. J Pharm Pharmacol 61:1147–1153

Moss GP, Sun Y, Wilkinson SC, Davey N, Adams R, Martin GP, Prapopoulou M, Brown MB (2011) The application and limitations of mathematical modelling in the prediction of permeability across mammalian skin and polydimethylsiloxane membranes. J Pharm Pharmacol 63:1411–1427

Nemecek GM, Dayan AD (1999) Safety evaluation of human living skin equivalents. Tox Pathol 27:101–103

Pelle E, Mammone T, Combatti M, Marenus K, Maes D (1993) UV-induced lipid-peroxidation in living skin equivalents. Clin Res 41:A504

Potts RO, Guy RH (1992) Predicting skin permeability. Pharm Res 9:663–669

Pugh WJ, Wong R, Falson F, Michniak BB, Moss GP (2005) Discriminant analysis as a tool to identify compounds with potential as transdermal enhancers. J Pharm Pharmacol 57:1389–1396

Raghavan SL, Kiepfer B, Davis AF, Kazarian SG, Hadgraft J (2001) Membrane transport of hydrocortisone acetate from supersaturated solutions; the role of polymers. Int J Pharm 221:95–105

Raghavan SL, Schuessel K, Davis A, Hadgraft J (2003) Formation and stabilisation of triclosan colloidal suspensions using supersaturated systems. Int J Pharm 261:153–158

Ramsamooj R, Strande L, Kain MH, Doolin EJ, Hewitt CW (1998) A novel in vitro model for xenorejection and immune mechanisms using bioengineered living skin equivalents. Transplant Proc 30:1087–1088

Roberts MS, Cross SE (1999) A physiological pharmacokinetic model for solute disposition in tissues below a topical application site. Pharm Res 9:1392–1398

Roberts ME, Mueller KR (1990) Comparisons of in vitro nitroglycerin (TNG) flux across Yucatan pig, hairless mouse and human skins. Pharm Res 7:673–676

Roberts MS, Anissimov YG, Gonsalvez RA (1999) Mathematical models in percutaneous absorption. In: Bronaugh RL, Maibach HI (eds.) Percutaneous absorption 3rd (Edn), New York: Marcel Dekker, Inc. CRC Press, pp 3–56

Sato K, Sugibayashi K, Morimoto Y (1991) Species differences in percutaneous absorption of nicorandil. J Pharm Sci 80:104–107

Stott PW, Williams AC, Barry BW (2001) Mechanistic study into the enhanced transdermal permeation of a model β-blocker, propranolol, by fatty acids: a melting point depression effect. Int J Pharm 219:161–176

van Hal D, van Rensen A, de Vringer T, Junginger H, Bouwstra J (1996) Diffusion of estradiol from non-ionic surfactant vesicles through human stratum corneum in vitro. STP Pharma Sci 6:72–78

Wang CK, Nelson CF, Brinkman AM, Miller AC, Hoffler WK (2000) Spontaneous cell sorting of fibroblasts and keratinocytes creates an organotypic human skin equivalent. J Invest Deramtol 114:674–680

Wester RC, Maibach HI (1976) Relationship of total dose and percutaneous absorption in Rhesus monkey and man. J Invest Dermatol. 67:518–520

Wester RC, Noonan PK (1980) Relevance of animal models for percutaneous absorption. Int J Pharm 7:99–110

Williams AC (2003) Transdermal and topical drug delivery. The Pharmaceutical Press, London

Woolfson AD, McCafferty DF, Moss GP (1998) Development and characterisation of a moisture-activated bioadhesive drug delivery system for percutaneous local anaesthesia. Int J Pharm 169:83–94

Yang EK, Yoon HH, Lee DH, Park JK (2000) Assessment of toxic potential of industrial chemicals using a cultured human bioartificial skin model: production of interleukin 1α and hydroxyeicosatetraenoic acids. Skin Pharmacol App Skin Physio 13:246–257

Young CD, Wu JR, Tsou TL (1998) Fabrication and characteristics of polyHEMA artificial skin with improved tensile properties. J Mem Sci. 146:83–93

Zhai HB, Maibach HI (2001) Effects of skin occlusion on percutaneous absorption: an overview. Skin Physiol Appl Skin Pharmacol 14:1–10

http://www.springer.com/978-3-662-47370-2

Predictive Methods in Percutaneous Absorption

Moss, G.; Gullick, D.; Wilkinson, S.

2015, XV, 199 p. 16 illus., 8 illus. in color., Hardcover
ISBN: 978-3-662-47370-2